FLNC pathogenic variants in patients with cardiomyopathies

Prevalence and genotype-phenotype correlations

Running Title : *FLNC* variants genotype-phenotype correlation

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Pathogenic variants in *FLNC* encoding filamin C have been firstly reported to cause myopathies, and were recently linked to isolated cardiac phenotypes. Our aim was to estimate the prevalence of *FLNC* pathogenic variants in subtypes of cardiomyopathies and to study the relations between phenotype and genotype.

DNAs from a cohort of 1150 unrelated index-patients with an isolated cardiomyopathy (700 hypertrophic, 300 dilated, 50 restrictive cardiomyopathies, and 100 left ventricle non-compactions) have been sequenced on a custom panel of 51 cardiomyopathy disease-causing genes.

A *FLNC* pathogenic variant was identified in 28 patients corresponding to a prevalence ranging from 1 to 8% depending on the cardiomyopathy subtype. Truncating variants were always identified in patients with dilated cardiomyopathy, while missense or in-frame variants were found in other phenotypes. A personal or family history of sudden cardiac death (SCD) was significantly higher in patients with truncating variants than in patients carrying missense variants (p=0.01). This work reported for the first time a left ventricular non-compaction associated with *FLNC* pathogenic variant.

This work highlights the role of *FLNC* in cardiomyopathies. A correlation between the nature of the variant and the cardiomyopathy subtype was observed as well as with SCD risk.

Key Word: Genotype-phenotype correlation, *FLNC,* cardiomyopathies, next generation sequencing, myopathy,

Introduction:

Filamin C is a homodimeric protein encoded by *FLNC* gene (7q32) containing 47 coding exons (NM_001458.4). Pathogenic variants in the *FLNC* gene have been firstly reported to cause dominant myofibrillar and distal myopathy[1]. More recently, dominant pathogenic variants in *FLNC* have also been linked to the development of isolated cardiac phenotypes, including hypertrophic cardiomyopathies (HCM)[2], restrictive cardiomyopathies (RCM)[3] and dilated cardiomyopathy (DCM)[4]. In non-compaction left ventricle cardiomyopathy, (LVNC), a *FLNC* variant was recently involved but associated with a second variant in *RYR2* gene[5].

Functionally, filamin C is a striated muscle protein that crosslinks sarcoplasmic F-actin and anchors the cell membrane through the sarcoglycan complex with the cytoskeleton and the sarcomere Z-disk[6].The protein is composed of an actin binding domain, 24 Immunoglobulin (Ig) domains divided into ROD1 and ROD2 sub-domains and a C-terminal dimerization domain[7]. Filamin C plays a role in the myofibril maintenance and the myogenesis in cardiac and skeletal muscles[8]. It has many interacting partners at the sarcomere Z-disk such as nexilin, actinine, myopodin and myozenin[9] which confer a role in the sarcomere maintenance and repair after myofibrillar damage[10].

It appears from recent publications that some variants are preferentially associated with myopathies and others with cardiomyopathies [1,2,11]. Only few publications on cohorts with *FLNC* variants are published and the pathophysiology of *FLNC*-related cardiomyopathy is poorly understood. The prevalence of this gene in cardiomyopathies, the nature and location of variants in the protein, as well as their impact on the phenotype are not yet studied. This makes difficult the interpretation of variants and their impact as disease causing on the sub-phenotype of cardiomyopathies. The objective of this retrospective study is to establish the prevalence of pathogenic variants in the *FLNC* gene in the different subtypes of cardiomyopathies and to search for genotype-phenotype associations in the perspective of improving genetic diagnosis and counseling as well as early cardiac management and primary prevention.

Materiel and Methods

Patients and inclusion criteria

In the context of the molecular diagnosis of cardiomyopathies, in the functional unit of Cardiogenetics and Myogenetics at the Pitié-Salpêtrière hospital between 2010 and 2017, a total of 1150 unrelated index patients including 700 hypertrophic cardiomyopathy (HCM), 300 dilated cardiomyopathy (DCM), 50 restrictive cardiomyopathy (RCM) and 100 left ventricle non-compaction (LVNC) patients have been sequenced on a custom panel of 51 cardiomyopathy disease-causing genes, including *FLNC*. According to our molecular strategy, most of HCM and DCM patients were first analyzed on a small panel of major genes (*MYH7, MYBPC3, TNNT2, TNNI3,* and *MYL2, +/- LMNA* gene in case of DCM) before being sequenced on the extensive panel (Figure 1). This restricted panel allowed the molecular diagnosis in 30% of HCM and 10% of DCM and avoided sequencing some of them, particularly in HCM, on the large panel of genes. Genetic screening was done after all the patients have signed an informed written consent for genetic analysis according to the French legislation. Twenty-eight index-patients presenting a unique disease-causing variant of *FLNC* were selected for the rest of the study.

The clinical diagnosis of cardiomyopathy has been established by electrocardiography, echography, and MRI according to usual European recommendations in 14 expert centers in France[12-15].

Sequencing

FLNC gene was sequenced in the context of the molecular diagnosis of inherited cardiomyopathies using a targeted custom panel of 51 genes. The panel (256kb) includes all coding regions and flanking intronic regions (+/- 20 bp) of genes responsible of the diverse morphological subtypes of cardiomyopathies (HCM, DCM, RCM, LVNC and arrhythmogenic right ventricle cardiomyopathies (ARVC)), pediatric phenotypes as well as some cardiomyopathies due to metabolic disorders and/or syndromic cardiomyopathies (*LAMP2, PRKAG2, GLA*) (list of genes in supplementary data).

Patients' DNAs were extracted from peripheral blood with Qiasymphony® (Qiagen, Hilden, Germany) and qualitatively checked using Tape Station DNA genomic array(Agilent, Santa Clara, USA). Custom targeted gene enrichment and DNA library preparation were performed using the NimbleGen EZ choice probes® and Kappa Library preparation kit according to the manufacturer's instructions (Nimblegen®, Roche Diagnostics, Madison, USA).The targeted regions were sequenced using the Illumina MiSeq platform on a 500 cycle Flow Cell (Illumina, Santa Cruz, USA) and MiSeq Software generates FASTQ format files after demultiplexing patients' sequences. Merged single reads and paired-end reads were then aligned on Hg19 human reference genome, using BWA-MEM. Variant calling was performed using the GATK Haplotype Caller programthen annotated using ANNOVAR.

Variant's interpretation

Pathogenicity of variants was determined according to current ACMG guidelines[16] that recommend classifying variants into 5 categories: (1) pathogenic, (2) likely pathogenic, (3) uncertain significance (VUS), (4) likely benign, or (5) benign. A recent publication dedicated to cardiomyopathies recommended the use of a frequency threshold of 0.01% [17]. We evaluated each variant considering several parameters: (i) a frequency threshold < 0.01% in GnomAD database (URL: http://gnomad.broadinstitute.org/), (ii) the in-silico prediction from multiple algorithms (Polyphen2, SIFT, GVGD and Mutation Taster for missense variants and SpliceSiteFinder like®, MaxEntScan®, NNSPLICE®, GeneSplicer® and Human Splicing Finder® for splicing variants), (iii) the location of the variant in the gene and the resulting protein, (iv) a careful review of the literature (HGMD Pro and Pubmed review), (v) functional studies and segregation analyses when available. Additionally, we looked at a personal database of pathogenic variants related to our experience on the molecular diagnosis of cardiomyopathies which could help to confirm the pathogenicity (frequency, familial analyses). In practice, we considered as "pathogenic", a variant with confirmed pathogenicity criteria and already proved as responsible for cardiomyopathies (published with proofs of pathogenicity or functional studies, personal database with segregation analysis) or a novel truncating variant (non-sens, frameshift or splice variant), with a frequency below 0.01% in GnomAD. We considered as "likely pathogenic", unpublished missense variants with a frequency below 0.01% and unknown in our database, located in a functional domain of the protein and with pathogenicity prediction tools mainly (at least 3 out of 4 tools) in favor to a strong effect. Pathogenicity of this likely pathogenic variant is also supported by an informative segregation analysis. Unpublished missense variants with a frequency below 0.01%, unknown in our database, located in a functional domain of the protein and with pathogenicity prediction tools mainly in favor to a strong effect but without segregation analysis available were considered as VUS favor pathogenic. Variants of unknown significance were new missense variants with no evidence for *in silico* predicted deleteriousness and published variants with a frequency over 0.01%.

Detection of copy number variation (CNVs) was performed, after coverage normalization, by computing the ratio of a target's coverage of a given individual over the mean coverage of this target across all patients of the same sequencing run. Their putative frequency was checked with DGV (Database of genomic variants), which collect all large gene rearrangements in a normal population.

Only variants interpreted as **certainly** pathogenic, **likely** pathogenic and VUS favor pathogenic were considered for further clinical study and were confirmed by Sanger sequencing on the initial DNA sample.

Statistical analysis was performed using Student or Fisher test when adapted.

Results:

Clinical pattern

Twenty-eight index patients carrying a probably or certainly pathogenic *FLNC* disease-causing variants were retained for further studies. Among them, 13 were affected with HCM, 10 with DCM, 4 with RCM and 1 by LVNC (Table 1). No clinical skeletal myopathy was noted in these patients and CK levels were normal when available (14/28 patients). Twenty-three patients were Caucasian, 3 from Africa, 1 from Asia and 1 from north Africa.

The 13 HCM patients comprised 10 familial and 3 sporadic patients. Mean age at diagnosis was 33 years (SD 20.4 years), and sex ratio was 5 women for 8 men. Mean interventricular septum thickness was 15.9 ± 3.5mm, mean posterior wall thickness was 15.2±5.4 mm, and mean maximal wall thickness was 16.7 ±2.5 mm. For all patients the ejection fraction of left ventricle was normal. Patient 12 presented an atypical HCM, with maximum wall thickness of 18 mm preserving the interventricular septum at 11 mm and a normal systolic function leading to the diagnosis of HCM with a restrictive profile. However, the patient had a congestive heart failure and there were two relatives with similarly, a HCM with restrictive profile requiring heart transplant. Probably the phenotype in the family a mixed phenotype of HCM and RCM. Regarding ECG parameters in the 13 mutated patients, atrial fibrillation was observed in 3 patients, atrio-ventricular block degree 1 was observed in 2 patients. Complete branch block was observed in 2 patients with HCM. Two patients have been implanted with adefibrillator. Familial history of sudden death before 50 years old was reported in 2 families but no index patient of the cohort deceased froma cardiovascular cause.

In the group of DCM patients (N =10), we reported 6 familial and 4 sporadic cases with a sex ratio of 6 females and 4 males. Mean age at diagnosis of 31 years (SD: 13.7 years). The mean LVEF was 33.6 ± 9.5%. On ECG, two patients presented a moderate short interval PR, two others presented a complete left bundle block branch, one patient presented AF and three had non sustained ventricular tachyarrhythmia (nsTV), no patient presented an AVB. Duringthe follow up, three patients have been hospitalized due to acute heart failure, and three patients suffered from sudden cardiac death (including one patient with confirmed ventricular tachycardia). For patients who died from inaugural sudden cardiac death, data were not fully available.

In the 4 patients with RCM, mean age at diagnosis was 36 years (SD 9.9 years), and sex ratio of 1:1. No significant AVB was observed whereas atrial fibrillationwas observed inallpatientsand ICD was implanted in two patients. No SCD was observed but one patientdeceasedbecause of heart failure (while waiting for heart transplant) at 36 years old, one still alive patient underwent a heart transplantation at 36 years old and one additional patient is still waiting for heart transplant.

Finally, the patient with LVNC was diagnosed at 36 years old, LV EF was 49%, he presented complete right branch block and was implanted with a defibrillator (primary prevention due to nsVT). This case was reported to be sporadic. In this patient abnormal trabeculations on echocardiography and on MRI and criteria were present (on MRI and end-diastolic measurement: non compacted/compacted ratio was 4.2) leading to the diagnosis LVNC.

When considering the whole cohort, 9 patients had atrial fibrillation (32%) and 5 patients had conduction defects including 2 patients with AVB degree 1 and 3 patients with relatively short PR. Five patients had non sustained VT (17.8%) including 2 DCM patients who died from SCD. In 7 cases (25%), sudden cardiac death before 50 years has been reported in family history. Nine patients (32%) have been implanted with automatic defibrillators in primary prevention. Acute heart failure was observed in 7 patients (25%) including one patient was transplanted at 36 years old. Four patients died of cardiac cause including 3 from SCD and 1 from heart failure.

Genetic results:

Prevalence of FLNC disease-causing variants in cardiomyopathies:

The overall prevalence of patients carrying a unique *FLNC* pathogenic variant in our cohort was evaluated from 1 to 8% according to cardiomyopathy subtypes, distributed as following; 1.3% in HCM, 3% in DCM, 8% in RCM and 1% in LVNC.

Molecular genetics

Among the 28 index cases with a pathogenic variant in *FLNC*, 20 index patients from all phenotypes presented a family history with affected relatives, suggesting an autosomal dominant pattern of inheritance and 8 patients without family history were considered as sporadic cases. In ten index cases, we could analyze genetic status of relatives. In RCM, 4 relatives of patients 26 and 27; in DCM, 5 relatives from the 5 families; and in HCM, 3 relatives from 3 families in which segregation analysis was performed are represented in Figure 2. In these families, 10 relatives carrying the variant harbored the same

cardiomyopathy phenotype as the proband. In two DCM families, 2 relatives were presymptomatic carriers but genotyped at a younger age than the age of onset of the disease in the index case (relatives of patient 4 and patient 6) (details in Fig. 2).

The spectrum of *FLNC* variants showed the identification of 28 unique disease-causing variants including 27 novel ones never published before (Table 1 and 2). Among these variants and according to the *FLNC* reference sequence (NM_001458.4), there are 10 null variants consisting in 3 nonsense (stop) variants (p.Tyr928*, p.Cys2555* and p.Gln2549*), 2 intronic variants abolishing the splicing of mRNA (c.1412-1G>A and c.601+1G>T), 5 duplications or deletions leading to a shift in the reading frame (p.Tyr7Thrfs*51, p.Glu238Argfs*14, p.Ile683Argfs*9, p.Val1198Glyfs*64, p.Tyr2373Cysfs*7), one in frame deletion (p.Pro2643_Leu2645del) and one in frame duplication (p.Ile1946_Thr1947dup), and 16 missense variants identified by the residue change (p.Ser1194Leu, p.Gly1424Val, p.Ser1624Leu, p.Ile1666Thr, p.Gly2011Glu, p.Gly2039Arg, p.Arg2140Gln, p.Val2297Met, p.Pro2298Leu, p.Gly2299Ser, p.Arg2318Trp, p.Ile2359Thr, p.Val2375Leu, p.Arg2410Cys, p.Gln2417Pro, and p.Arg2495His).

As the nonsense variants were spanning all along the gene (regions corresponding to actin binding domain, ROD1 and ROD2 domains), missense variants were clustered in the ROD1 and ROD2 domains. The two in-frame deletion and duplication were respectively located in the dimerization domain and the ROD2 domain (Figure 3).

A relation between the genotype and the phenotype was observed both regarding the nature of the variant and its localization in the gene or protein domains. Regarding *the mechanism of the mutation*, all 10 patients presenting with a dilated cardiomyopathy were carriers of a truncating variant while all 13 patients presenting with HCM carried missense variants (p value; 7 10^{-8})(Fig 3). In the 4 patients with restrictive cardiomyopathy, two patients had missense variants; one had an in-frame duplication and the other an in-frame deletion and the only patient with LVNC carried a missense variant. Regarding the risk for SCD, a personal or a familial history of SCD was reported in 7/10 patients with truncating variants (70%) and in 3/16 patients (19%) carrying a missense variants (p=0.01, Fisher Exact Test).

Regarding the variant localization and the subtype of cardiomyopathy: in HCM patients, missense variants are clustered in ROD2 domains (10 variants) then in ROD1 (3 variants). In the 4 patients presenting RCM, 3 variants were located in ROD2 domain, particularly clustered on residue 2297 and 2298, and one in the dimerization domain. The LVNC associated variant was located in ROD2 domain. In patients with DCM, as all variants were null variants disrupting the reading frame, the position of the variant has probably limited

impact, the likely consequence being mRNA decay of the mutated allele. Additionally, no particular association could be observed regarding the rhythm disturbance and the position of the variant in the protein. Nevertheless, in the 9 patients who were implanted with ICD, 7 were mutated in the ROD2 domain. In two patients with restrictive cardiomyopathy, transplanted or awaiting for transplantation, the variants were adjacent, both located in exon 41 (c.6889G>A_p.Val2297Met, and c.6893C>T_ p.Pro2298Leu) in the Ig domain 20.

Discussion

This study was focused on *FLNC*, a gene recently involved in isolated cardiomyopathies. The particularity of our study is to involve all subtypes of cardiomyopathies as well as the analysis of all types of variants (missense and truncating). This cohort of 1150 patients provides new findings regarding the prevalence of this mutated gene in different subtypes of cardiomyopathies as well as some phenotype-genotype relations.

Prevalence

In our cohort composed of various cardiomyopathies, the estimated prevalence of patients carrying a unique *FLNC* variants were ranging from 1% to 8% according to cardiomyopathy subtypes, with about 1-3% in HCM and DCM. In a recent published data[11], in which only truncated variants were considered, the estimated prevalence of *FLNC* was about 3.9% (n=508 patients) in DCM, and 3 % in arrhythmogenic cardiomyopathies (n=219 patients) and 2.2% in RCM (1/45). These observations lead to consider *FLNC* gene as a gene especially prevalent in the DCM[18]. In another publication dedicated to hypertrophic cardiomyopathy, the estimated prevalence on 92 patients was 7.6% and comprised 1 truncating variant and 6 missense variants[2]. These results are in accordance with ours for DCM phenotype, nevertheless the prevalence in RCM patient is higher in our cohort. It could be explained by the fact that we considered truncating as well as non-truncating especially in frame insertion and deletion variants. This relatively high prevalence of *FLNC* in RCM (8%) is a novel finding that extend the spectrum of genes in this particularly severe phenotype. In HCM, the prevalence found in our cohort is lower; this could be due to the size or characteristics of the cohort. In LVNC, this is the first time that a unique pathogenic variant in *FLNC* was found as disease-causing as in the only previous published patient with LVNC, the *FLNC* variant was associated with a *RYR2* variant[5].

Genotype Phenotype correlation

We searched first a putative relation between *the subtype of cardiomyopathy and the mechanism of the mutation and its localization*. In our cohort, all HCM patients were carriers of a missense variant, and all DCM patients carried a truncating variant leading to haploinsufficiency. Our results emphasize a strong correlation between the subtypes of cardiomyopathy and the nature of disease causing variant. As compared with the literature, upon the 59 published variants consistent with our criteria for pathogenicity, 14 variants were associated with a myopathy phenotype *(*6 in distal myopathy and 8 in myofibrillar myopathy)[1] and 45 were associated with an isolated cardiomyopathy (24 variants in DCM, 19 in HCM, 2 in RCM). Among all these published variants, 28 were truncating variants, 29 missense variants, and 2 were deletion or insertion without shift of the reading frame. For this study, we used stringent criteria for classification of variants. Nevertheless, it is important to note that some discrepancy could exist between studies, particularly in gene variants interpretation, especially for missense variants. Similarly to our observation, in HCM patients, among the 19 published variants, 18 were missense variants. Regarding the localization of missense variants in the protein domains, we observed a larger proportion of variants located in the ROD2 domain in HCM patients which is in accordance with published data that revealed a cluster of missense variants in ROD2 domain of filamin in HCM phenotype[2,11,19]. This region is the interaction domain between filamin C and proteins of the Z-disk, as synaptopodin, myozenin, and nexilin and could be an interesting way to understand the mechanism of pathogenicity of these missense variants. The pathophysiological mechanism of missense variants was explored in rat fetal cardiomyocytes and showed that variants located in actin binding domain and dimerization domain lead to filamin aggregates[10]. In this case, the hypothesis was a dominant negative model, however the pathophysiology of this aggregate and the link to HCM development remain unclear. A discrepancy observed, is the variant p.Ser1624Leu identified in an HCM patient in our cohort and in a RCM patient in the publication of Brodhel et al. The patient included in this cohort had a diagnosis of HCM since MWT was 18 mm and normal systolic function. However the patient had a congestive heart failure and there were two relatives with a cardiomyopathy requiring heart transplant. Probably the phenotype in the family a mixed phenotype of HCM and RCM, which presents similarities with the previously published family with (pure) RCM (since no LV hypertrophy was noticed in this publication).

Additionally, in a previous cohort, Valde´s-Mas et al, 2014 found that 34% of the HCM patients carriers of pathogenic variant in FLNC had elevated levels of serum creatine kinase (CK). In this work, all patients have a normal CK level. When presenting a cardiomyopathy, patients were systematically checked for muscle symptoms. A muscular RMI, which could help to detect infra-clinic myopathy, was not systematically performed in this cohort. Two patients presented a transitory CK elevation (patient 21 et 27) but RMI didn't

find any muscle sign. The difference between the cohort of Valde´s-Mas et al is difficult to explain and could be a difference in the cohort recruitment.

However, the implication of missense variants in HCM is questioned and need to be confirmed. Mainly, two publications didn't detect significant excess of rare missense variants between HCM cases and controls [20,21]. One limitation discussed by Walsh is an insufficient size of the cohort[21]. Additionally, in the publication of Cui et al., at least three differences could be noticed: (1) the ethnic origin of the cohort, (2) the threshold of variant frequency and (3) the fact that 33% of their patients were carriers of a second mutation in a major gene which could induce a bias in the expression of the disease[20].

Among the 24 variants reported in DCM, 23 are truncating variants and the only missense variant found was associated with a null variant in a neonatal case of DCM[22]. In this case, the congenital form of the DCM could be due to the cumulative effect of the missense variant associated with the heteroallelic truncating variant in *FLNC.* These finding reinforce the role of *FLNC* null variants in the development of DCM. The pathophysiology of the truncating variants and the development of DCM was explored in zebrafish models and concluded to a haploinsufficiency model[4]. Similarly, in myopathies, the haploinsufficiency mechanism and the RNA decay activation have been confirmed on fish model[8]. In RCM and LVNC cardiomyopathies[3,5], no correlation could be observed due to a small number of patients published. However, it was interesting to notice that, additionally to missense variants, the only variants consisting in in-frame insertion or deletion were associated with RCM.

Secondly, we searched a relationship between the variant type and the *arrhythmia pattern.* In our cohort, 32% of patients were ICD implanted and a familial history of SCD was observed in 25% of cases. A familial or a personal history of SCD was significantly more frequently observed in patients carrying truncating variants than in patients carrying a missense variant suggesting that null or truncating variants may predispose to severe arrhythmias. Additionally, in this cohort, a high number of patients presented a conduction defect (Table 1) respectively 30% in HCM and 40% in DCM suggesting that the mechanism of the variant has no important impact on this clinical feature. In a recent publication on arrhythmogenic cardiomyopathies, it was observed a conduction defect in 12% of the patients, sudden death in 15% of the patients and appropriate ICD Shock in 10%[11]. These finding highlighted the arrhythmogenic pattern of *FLNC* truncating variants cardiomyopathies. Up to now, no large cohort of *FLNC* related cardiomyopathies with a long term follow up has been published, but the initial data in several independent cohorts (this work

and reference 16) support the high risk of SD and conduction defect, that should be carefully evaluated in primary prevention of cardiac death.

Limitations:

This work details the phenotype of limited number of index cases carrying a unique pathogenic variant in *FLNC* gene. Thus, regarding to the penetrance, it seems that the age of onset of all affected patients may varies from young age to adult age. Additionally, the total number of carriers is too small to conclude to the penetrance of theses variants.

In conclusion, we presented in this study the results of the sequencing of a targeted panel of cardiomyopathy genes in a large cohort of index patients with different subtypes of cardiomyopathies. This allowed to evaluate the prevalence of *FLNC* variants in these different groups and then, in mutated patients to exhibit some genotype-phenotype relations. For the first time, a pathogenic variant in *FLNC* was associated with LVNC phenotype. We observed a relation between the nature of the variant and the subtype of cardiomyopathy as well as a clear arrhythmogenic pattern in patients with truncating variants. Our results therefore support the emerging role of *FLNC* in isolated cardiomyopathies and the inclusion of this gene in panels dedicated to sequencing of patients with a cardiomyopathy, whatever the subtype. This data should be taken in consideration for genetic counseling in patient's families.

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Legends to Figures:

Figure 1: Flow chart describing the genetic molecular testing depending of the cardiomyopathy phenotype.

Figure 2: Pedigree tree of 4 families with *FLNC* pathogenic variants for which genetic segregation was possible. Squares indicate males, circles indicate females, slashes indicate deceased individuals, black shading indicates a dilated cardiomyopathy (DCM) phenotype. The arrows indicate the proband. Heterozygous carriers (+/-) and non-carriers (−) of a *FLNC* variant are indicated.

Figure 3: Distribution of *FLNC* variants along the protein in various subtypes of cardiomyopathies according to their nature and location.