


A mutation update for the *FLNC* gene in myopathies and cardiomyopathies

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Abstract

Filamin C (*FLNC*) variants are associated with cardiac and muscular phenotypes. Originally, *FLNC* variants were described in myofibrillar myopathy (MFM) patients.

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Later, high-throughput screening in cardiomyopathy cohorts determined a prominent role for *FLNC* in isolated hypertrophic and dilated cardiomyopathies (HCM and DCM). *FLNC* variants are now among the more prevalent causes of genetic DCM. *FLNC*-associated DCM is associated with a malignant clinical course and a high risk of sudden cardiac death. The clinical spectrum of *FLNC* suggests different pathomechanisms related to variant types and their location in the gene. The appropriate functioning of *FLNC* is crucial for structural integrity and cell signaling of the sarcomere. The secondary protein structure of *FLNC* is critical to ensure this function. Truncating variants with subsequent haploinsufficiency are associated with DCM and cardiac arrhythmias. Interference with the dimerization and folding of the protein leads to aggregate formation detrimental for muscle function, as found in HCM and MFM. Variants associated with HCM are predominantly missense variants, which cluster in the ROD2 domain. This domain is important for binding to the sarcomere and to ensure appropriate cell signaling. We here review *FLNC* genotype–phenotype correlations based on available evidence.

KEYWORDS

cardiomyopathy, filamin, *FLNC*, genotype–phenotype correlation, myopathy

1 | BACKGROUND

Similar to other filamins, Filamin C (*FLNC*) is a structural protein which has an actin-binding domain (ABD) composed of two calponin homology (CH) domains, 24 immunoglobulin (Ig) domains divided into a ROD1 and ROD2 subdomain, and a C-terminal dimerization domain (van der Flier & Sonnenberg, 2001). The dimerization of two identical filamin proteins is necessary for appropriate function and occurs via Ig-like domain 24 (Himmel, Van Der Ven, Stocklein, & Furst, 2003). In contrast to filamin A and B, filamin C expression is restricted to striated muscles and localizes around the Z-disc, the sarcolemma, the myotendinous junction, and the intercalated discs (Thompson et al., 2000). Its main role is maintaining the structural integrity of the sarcomere. This is through crosslinking actin filaments and the anchoring of sarcolemmal proteins to the cytoskeleton. The main interactors of *FLNC* are either part of the Z-disc (myotilin, myozenin, myopodin, and calsarcins), signaling molecules (Zhang, Liu, Cheng, Deyoung, & Saltiel, 2007) or sarcolemma-associated proteins (integrin β 1, sarcoglycan delta; Anastasi et al., 2004; Furst et al., 2013; Takada et al., 2001). Proteases such as calpain can regulate the interaction between *FLNC* and the sarcoglycans by cleaving the corresponding binding domains of *FLNC* (Guyon et al., 2003). In addition, *FLNC* interacts with the Xin actin-binding repeat-containing proteins (XIRP) and aciculin to fulfill a function in muscle maintenance (Fujita et al., 2012; Leber et al., 2016; Molt et al., 2014). This interaction is mediated via a unique insertion in Ig-like domain 20, which is absent in the other filamin paralogs. The ROD1 domain (Ig 1–15) is more stretched and lacks interdomain interactions in contrast to the ROD2 domain (Ig 16–23), which is more compact and globularly arranged by

domain pairs. These organizational differences between the domains explain why certain ligands bind exclusively to ROD1 or ROD2.

The *FLNC* gene maps to chromosome 7q32–35 and has two main transcripts, NM_001127487.2 and NM_001458.4. It comprises ~29.5 kb of genomic DNA and is composed of 49 coding exons (Chakarova et al., 2000). The difference between the two transcripts is the presence or absence of exon 31 encoding the hinge region between Ig-like-chroIg-like domains 15 and 16 (Xie, Xu, Davie, & Chung, 1998). The longest transcript, NM_001458.4, encodes a protein with a molecular mass of 291 kDa and 2,725 amino acids, whereas the shorter transcript NM_001127487.2 encodes a slightly shorter protein (287 kDa, 2,692 amino acids) that is assumed to be less flexible. The exact roles of these two isoforms are unknown, but the long *FLNC* isoform is more abundantly expressed during cardiac stress while almost absent in the normal situation (Kong et al., 2010). This could potentially alter the integrity and function of the key sarcomeric structures to cope with increased cell stress. The short isoform is mainly expressed in the normal situation and is 3.5 times higher expressed in skeletal compared with cardiac muscle.

Variants in *FLNC* are traditionally associated with myofibrillar myopathy (MFM; MIM# 609524), but subsequently also with isolated cardiomyopathies (MIM# 617047). To date, most available basic research on *FLNC* has focused on myopathies. Paradoxically, most genetic variants are described in cardiomyopathy patients, as this clinical entity is more prevalent and studies using high-throughput sequencing of *FLNC* are more frequent in cardiomyopathy cohorts. This overview highlights known and novel *FLNC* variants and focuses on specific pathomechanisms important for distinct cardiac or muscular phenotypes.

2 | VARIANTS

All *FLNC* variants are described according to current Human Genome Variation Society mutation nomenclature guidelines based on Genbank accession number NM_001458.4 (longest transcript). Previously reported and novel variants are interpreted and classified using American College of Medical Genetics and Genomics classification recommendations (Richards et al., 2015).

A total of 285 unique variants could be retrieved from the international peer-reviewed literature, the Human Gene Mutation Database and the Leiden Open Variation Database (LOVD; Figure 1). We added 40 novel unique variants, leading to a total of 325 unique variants. All variants were submitted to the LOVD. A clear description of the phenotype associated with the variant was a requirement for this overview. One-hundred variants were excluded from the main analysis, as no clinical information was available (Table S1). Most *FLNC* variants have been reported over the last 5 years, from the time that *FLNC* was recognized as a disease-associated gene in the field of cardiomyopathies. Variant interpretation remains challenging, as the available evidence for pathogenicity is still limited for most types of variants. In general, truncating variants were classified as (likely) pathogenic, and missense variants as a variant of unknown significance (VUS), unless additional evidence from segregation and/or functional experiments was available.

2.1 | Cardiomyopathies

Cardiomyopathies are a heterogeneous group of myocardial diseases associated with mechanical or electrical dysfunction that exhibit inappropriate ventricular hypertrophy or dilatation (Elliott et al., 2008; Maron et al., 2006). In this review, we distinguish between:

1. Dilated cardiomyopathy (DCM): Characterized by the presence of left ventricular dilatation and contractile dysfunction, in the absence of abnormal loading conditions and severe coronary artery disease (Elliott et al., 2008; Maron et al., 2006).
2. Hypertrophic cardiomyopathy (HCM): The presence of increased left ventricular wall thickness that is not solely explained by abnormal loading conditions (Elliott et al., 2008; Maron et al., 2006).
3. Other cardiac diseases, which do not fulfill these criteria for DCM or HCM.

2.1.1 | Dilated cardiomyopathy

Variants predicted to result in a premature stop codon are strongly enriched in DCM, and are classified as (likely) pathogenic, as *FLNC* is highly intolerant for loss-of-function variants (pLI-score = 1; Figures 1 and 2). The prevalence of *FLNC* variants in patients with

Overview *FLNC* variants

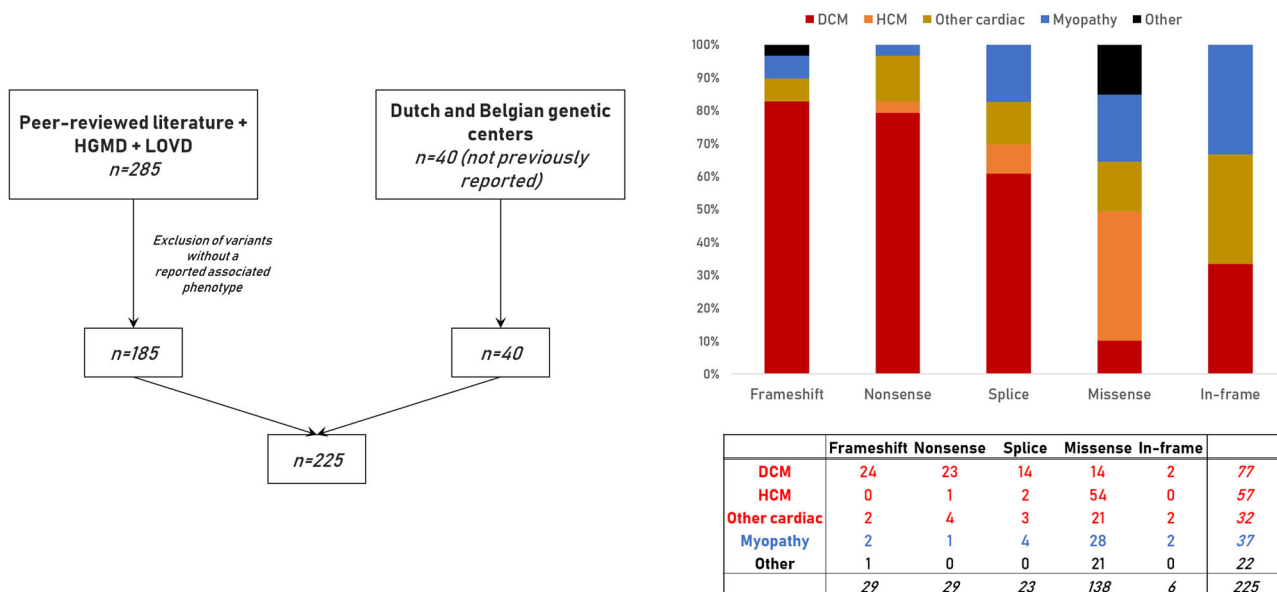


FIGURE 1 Variant selection of all *FLNC* variants and the overview of all variants in association with their phenotype. DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HGMD, Human Gene Mutation Database; LOVD, Leiden Open Variation Database

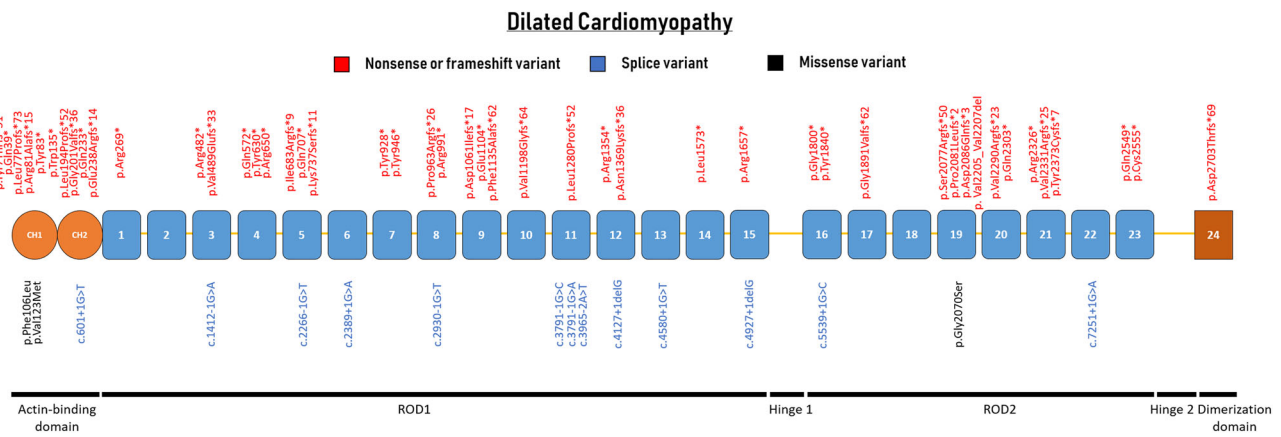


FIGURE 2 Schematic representation of the *FLNC* gene with their protein-coding domains. Numbers inside the boxes refer to the Ig-like domains of filamin C. Above and below the schematic are all unique variants associated with dilated cardiomyopathy. Variants are annotated at the protein level

DCM ranges from 1% to 4.5% (Ader et al., 2019; Begay et al., 2018; Janin et al., 2017; Ortiz-Genga et al., 2016; Table 1).

Three missense variants have been classified as likely pathogenic: p.Phe106Leu, p.Ala123Met, and p.Gly2070Ser. The p.Phe106Leu missense variant occurred on the opposite allele of a nonsense variant (p.Arg991*) in a neonatal patient with DCM (Reinstein et al., 2016). Compound heterozygosity for these *FLNC* variants led to an early-onset phenotype. Heterozygous carriers had not developed DCM by age 40 years. Protein levels were decreased for the p.Phe106Leu variant, and the p.Arg991* was not detectable. The p.Val123Met variant reported in the current study is classified as likely pathogenic, due to the well-investigated p.Val123Ala variant at the same codon in HCM (Valdes-Mas et al., 2014). The p.Gly2070Ser variant is predicted to alter a canonical splice site, although RNA analysis was not performed (Ortiz-Genga et al., 2016). All other missense variants are classified as VUS and are not included in Figure 2, but are listed in Table 1.

2.1.2 | Hypertrophic cardiomyopathy

Missense variants are mainly associated with HCM with a varying prevalence from 1.3% to 8.7% in HCM cohorts (Ader et al., 2019; Cui et al., 2018; Gomez et al., 2017; Valdes-Mas et al., 2014; Figure 1; Table 2). Two studies did not detect an excess of rare missense variants between HCM patients and controls, questioning the importance of *FLNC* missense variants in HCM (Cui et al., 2018; Walsh et al., 2019). Only 13 of the 54 missense variants are supported to be (likely) pathogenic by additional evidence such as functional studies ($n = 4$) and/or segregation ($n = 13$; Ader et al., 2019; Cui et al., 2018; Gomez et al., 2017; Valdes-Mas et al., 2014). Based on current diagnostic classification criteria, all other missense variants would individually be classified as VUS (Table 2). There is a strong clustering of missense variants in the ROD2 domain of the *FLNC*, which is an important domain for cell signaling (Figure 3). Thus,

collectively, missense variants in the ROD2 domain carry an increased likelihood of being pathogenic for HCM.

2.1.3 | Other cardiac phenotypes

FLNC variants have been associated with other cardiac phenotypes such as arrhythmias without detectable structural abnormalities, congenital heart disease, restrictive (RCM), and noncompaction (NCCM) cardiomyopathies (Figure 4; Table 3). The association of *FLNC* with a broad spectrum of cardiac phenotypes shows an important gap in knowledge. Hence, not all reported variants have proven to be causal. There can also be a large overlap between phenotypes: cardiac noncompaction can be a trait observed in other cardiomyopathies and healthy hearts (Hershberger et al., 2018).

One patient with arrhythmogenic bileaflet mitral valve prolapse syndrome (ABiMVPS) was recently reported in association with a truncating variant (p.Trp34*) identified in whole-exome sequencing data (Bains et al., 2019). It was speculated that *FLNC* haploinsufficiency was the underlying arrhythmogenic substrate, which was exacerbated by the mitral valve prolapse. Another recent report described familial sudden cardiac death without signs of cardiomyopathy in association with a truncating variant in *FLNC* (p.Pro2513Glufs*12; Mangum & Ferns, 2019). Both cases highlight the arrhythmogenic potential associated with *FLNC* truncating variants. However, it remains unknown if alternative diagnostic tools such as global longitudinal strain analysis could detect subtle changes in cardiac function. In addition, arrhythmias can also be accompanied by a cardiomyopathy phenotype with right, left, or biventricular involvement called arrhythmogenic cardiomyopathy (ACM). When there is prominent left ventricular involvement, it is difficult to clinically distinguish it from DCM. A recent cohort study in ACM patients found four truncating variants (3.3%; Table 3; Hall et al., 2019).

FLNC variants in RCM and NCCM are less prevalent compared with DCM and HCM, making it difficult to draw any conclusions on the role

TABLE 1 FLNC variants found in individuals with dilated cardiomyopathy (DCM) previously reported and from this study

Exon	c-Notation	p-Notation	Variant type	Domain	Location	Reference	Effect
1	c.19del	p.Tyr7Thrfs*51	Frameshift	ABD	CH1	(Ader et al., 2019)	Likely pathogenic
1	c.115C>T	p.Gln39*	Nonsense	ABD	CH1	(Janin et al., 2017)	Likely pathogenic
1	c.230_234delITCAGC	p.Leu77Profs*73	Frameshift	ABD	CH1	(Janin et al., 2017)	Likely pathogenic
1	c.241delC	p.Arg81Alafs*15	Frameshift	ABD	CH1	(Ortiz-Genga et al., 2016)	Likely pathogenic
1	c.248_265dup	p.Pro88_Arg89InsHisArg LysPheHisPro	In-frame	ABD	CH1	Current study	VUS
1	c.249C>G	p.Tyr83*	Nonsense	ABD	CH1	(Ortiz-Genga et al., 2016)	Likely pathogenic
1	c.318C>G	p.Phe106Leu	Missense	ABD	CH1	(Reinstein et al., 2016)	Likely pathogenic
1	c.328G>A	p.Glu110Lys	Missense	ABD	CH1	Current study	VUS
2	c.367G>A	p.Val123Met	Missense	ABD	CH1	Current study	Likely pathogenic
2	c.404G>A	p.Trp135*	Nonsense	ABD	CH1	LOVD	Likely pathogenic
2	c.581_599del19	p.Leu194Profs*52	Frameshift	ABD	CH2	(Ortiz-Genga et al., 2016)	Likely pathogenic
2	c.601+1G>T	p.?	Splice	ABD	CH2	(Janin et al., 2017)	Likely pathogenic
3	c.602-716_1010delins TGCCCCGGGAGGGGTGC CTCAGTCTCCC TGTCCCTCTG	p.Gly201Valfs*36	Frameshift	ABD	CH2	(Ortiz-Genga et al., 2016)	Likely pathogenic
3	c.697C>T	p.Gln233*	Nonsense	ABD	CH2	Current study	Likely pathogenic
4	c.711del	p.Glu238Argfs*14	Frameshift	ABD	CH2	(Ader et al., 2019)	Likely pathogenic
4	c.805C>T	p.Arg269*	Nonsense	ROD1	Ig-like 1	(Begay et al., 2018)	Likely pathogenic
6	c.970-4A>G	p.?	Splice	ROD1	Ig-like 1	Current study	VUS
9	c.1412-1G>A	p.?	Splice	ROD1	Ig-like 3	(Ader et al., 2019)	Likely pathogenic
9	c.1444C>T	p.Arg482*	Nonsense	ROD1	Ig-like 3	(Tobita et al., 2017)	Likely pathogenic
9	c.1466_1472del	p.Val489Glyfs*33	Frameshift	ROD1	Ig-like 3	Current study	Likely pathogenic
9	c.1466_1473delinsA	p.Val489Glyfs*33	Frameshift	ROD1	Ig-like 3	LOVD	Likely pathogenic
11	c.1714C>T	p.Gln572*	Nonsense	ROD1	Ig-like 4	(Ortiz-Genga et al., 2016)	Likely pathogenic
12	c.1890C>A	p.Tyr630*	Nonsense	ROD1	Ig-like 4	(Janin et al., 2017)	Likely pathogenic
12	c.1948C>T	p.Arg650*	Nonsense	ROD1	Ig-like 4	LOVD	Likely pathogenic

(Continues)

TABLE 1 (Continued)

Exon	c-Notation	p-Notation	Variant type	Domain	Location	Reference	Effect
13	c.2041_2047dup	p.Ile683Argfs*9	Frameshift	ROD1	Ig-like 5	(Ader et al., 2019)	Likely pathogenic
13	c.2119C>T	p.Gln707*	Nonsense	ROD1	Ig-like 5	(Begay et al., 2018)	Likely pathogenic
14	c.2208delT	p.Lys737Serfs*11	Frameshift	ROD1	Ig-like 5	(Ortiz-Genga et al., 2016)	Likely pathogenic
15	c.2266-1G>T	p.?	Splice	ROD1	Ig-like 5	(Janin et al., 2017)	Likely pathogenic
15	c.2389+1G>A	p.?	Splice	ROD1	Ig-like 6	(Nozari et al., 2018)	Likely pathogenic
16	c.2425G>A	p.Val809Met	Missense	ROD1	Ig-like 6	(Janin et al., 2017)	VUS
18	c.2784C>G	p.Tyr928*	Nonsense	ROD1	Ig-like 7	(Ader et al., 2019)	Likely pathogenic
19	c.2838T>A	p.Tyr946*	Nonsense	ROD1	Ig-like 7	LOVD	Likely pathogenic
19	c.2888delC	p.Pro963Argfs*26	Frameshift	ROD1	Ig-like 8	(Ortiz-Genga et al., 2016)	Likely pathogenic
20	c.2930-1G>T	p.?	Splice	ROD1	Ig-like 8	(Begay et al., 2018)	Likely pathogenic
20	c.2971C>T	p.Arg991*	Nonsense	ROD1	Ig-like 8	(Reinstein et al., 2016)	Likely pathogenic
20	c.3180del	p.Asp1061Ilefs*17	Frameshift	ROD1	Ig-like 9	Current study	Likely pathogenic
21	c.3310G>T	p.Glu1104*	Nonsense	ROD1	Ig-like 9	(Janin et al., 2017)	Likely pathogenic
21	c.3380_3402dup23	p.Phe1135Alafs*62	Frameshift	ROD1	Ig-like 9	(Ortiz-Genga et al., 2016)	Likely pathogenic
21	c.3592dup	p.Val1198Glyfs*64	Frameshift	ROD1	Ig-like 10	(Ader et al., 2019)	Likely pathogenic
22	c.3791-1G>A	p.?	Splice	ROD1	Ig-like 11	(Ortiz-Genga et al., 2016)	Likely pathogenic
22	c.3791-1G>C	p.?	Splice	ROD1	Ig-like 11	(Deo et al., 2014)	Likely pathogenic
22	c.3838dup	p.Leu1280Profs*52	Frameshift	ROD1	Ig-like 11	Current study	Likely pathogenic
23	c.3965-2A>T	p.?	Splice	ROD1	Ig-like 11	(Ortiz-Genga et al., 2016)	Likely pathogenic
23	c.4060C>T	p.Arg1354*	Nonsense	ROD1	Ig-like 12	(Janin et al., 2017)	Likely pathogenic
23	c.4106dupA	p.Asn1369Lysfs*36	Frameshift	ROD1	Ig-like 12	(Cuenca et al., 2016)	Likely pathogenic
23	c.4127+1delG	p.?	Splice	ROD1	Ig-like 12	(Ortiz-Genga et al., 2016)	Likely pathogenic
26	c.4580+1G>T	p.?	Splice	ROD1	Ig-like 13	(Ortiz-Genga et al., 2016)	Likely pathogenic
27	c.4700G>A	p.Arg1567Gln	Missense	ROD1	Ig-like 14	(Esslinger et al., 2017)	VUS
27	c.4718T>A	p.Leu1573*	Nonsense	ROD1	Ig-like 14	(Augusto et al., 2019)	Likely pathogenic
28	c.4927+1delG	p.?	Splice	ROD1	Ig-like 15	(Ortiz-Genga et al., 2016)	Likely pathogenic
30	c.4969C>T	p.Arg1657*	Nonsense	ROD1	Ig-like 15	LOVD	Likely pathogenic
30	c.5036C>A	p.Thr1679Lys	Missense	ROD1	Ig-like 15	Current study	VUS

TABLE 1 (Continued)

Exon	c-Notation	p-Notation	Variant type	Domain	Location	Reference	Effect
32	c.5398G>T	p.Gly1800*	Nonsense	ROD2	Ig-like 16	(Ortiz-Genga et al., 2016)	Likely pathogenic
33	c.5520T>A	p.Tyr1840*	Nonsense	ROD2	Ig-like 16	(Chanavat, Janin, & Millat, 2016)	Likely pathogenic
33	c.5539+1G>C	p.?	Splice	ROD2	Ig-like 16	(Ortiz-Genga et al., 2016)	Likely pathogenic
35	c.5672delG	p.Gly1891Valfs*62	Frameshift	ROD2	Ig-like 17	(Begay et al., 2018)	Likely pathogenic
35	c.5791C>T	p.Arg1931Cys	Missense	ROD2	Ig-like 17	Current study	VUS
37	c.6100G>C	p.Gly2034Arg	Missense	ROD2	Ig-like 18	Current study	VUS
37	c.6208G>A	p.Gly2070Ser	Missense	ROD2	Ig-like 19	(Ortiz-Genga et al., 2016)	Likely pathogenic
38	c.6231delT	p.Ser2077Argfs*50	Frameshift	ROD2	Ig-like 19	(Cuenca et al., 2016)	Likely pathogenic
38	c.6240_6259del	p.Pro2081Leufs*2	Frameshift	ROD2	Ig-like 19	(Ortiz-Genga et al., 2016)	Likely pathogenic
38	c.6255_6256del	p.Ile2086Glnfs*3	Frameshift	ROD2	Ig-like 19	Current study	Likely pathogenic
40	c.6518G>A	p.Arg2173His	Missense	ROD2	Intradomain	Current study	VUS
40	c.6614_6622del	p.Val2205_Val2207del	In-frame	ROD2	Intradomain	Current study	Likely pathogenic
41	c.6790G>A	p.Ala2264Thr	Missense	ROD2	Ig-like 20	Current study	VUS
41	c.6864_6867dup	p.Val2290Argfs*23	Frameshift	ROD2	Ig-like 20	Current study	Likely pathogenic
41	c.6877C>T	p.Arg2293Cys	Missense	ROD2	Ig-like 20	Current study	VUS
41	c.6907C>T	p.Gln2303*	Nonsense	ROD2	Ig-like 20	Current study	Likely pathogenic
41	c.6976C>T	p.Arg2326*	Nonsense	ROD2	Ig-like 21	(Ortiz-Genga et al., 2016)	Likely pathogenic
41	c.6989dupG	p.Val2331Argfs*25	Frameshift	ROD2	Ig-like 21	(Janin et al., 2017)	Likely pathogenic
42	c.7118_7119del	p.Tyr2373Cysfs*7	Frameshift	ROD2	Ig-like 21	(Ader et al., 2019)	Likely pathogenic
43	c.7251+1G>A	p.?	Splice	ROD2	Ig-like 22	(Ortiz-Genga et al., 2016)	Likely pathogenic
45	c.7450G>A	p.Gly2484Ser	Missense	ROD2	Ig-like 22	Current study	VUS
46	c.7645C>T	p.Gln2549*	Nonsense	ROD2	Ig-like 23	(Ader et al., 2019)	Likely pathogenic
46	c.7652A>G	p.Asp2551Gly	Missense	ROD2	Ig-like 23	Current study	VUS
46	c.7665T>A	p.Cys2555*	Nonsense	ROD2	Ig-like 23	(Ader et al., 2019)	Likely pathogenic
48	c.8107delG	p.Asp2703Thrfs*69	Frameshift	ROD2	Ig-like 24	(Ortiz-Genga et al., 2016)	Likely pathogenic

Abbreviations: ABD, actin-binding domain; LOVD, Leiden Open Variation Database; VUS, variant of unknown significance.

TABLE 2 *FLNC* variants found in individuals with hypertrophic cardiomyopathy (HCM) previously reported and from this study

Exon	c-Notation	p-Notation	Variant type	Domain	Location	Reference	Effect
1	c.322G>T	p.Glu108*	Nonsense	ABD	CH1	(Valdes-Mas et al., 2014)	Likely pathogenic
2	c.368T>C	p.Val123Ala	Missense	ABD	CH1	(Valdes-Mas et al., 2014)	Likely pathogenic
4	c.743A>T	p.His248Leu	Missense	ABD	CH2	Current study	VUS
4	c.850+4T>G	p.?	Splice	ROD1	Ig-like 1	(Cui et al., 2018)	VUS
5	c.870C>A	p.Asn290Lys	Missense	ROD1	Ig-like 1	(Valdes-Mas et al., 2014)	VUS
6	c.986A>G	p.Asn329Ser	Missense	ROD1	Ig-like 1	(Alejandra Restrepo-Cordoba et al., 2017)	VUS
7	c.1076T>C	p.Ile359Thr	Missense	ROD1	Ig-like 1	(Cui et al., 2018)	VUS
7	c.1132G>T	p.Val378Leu	Missense	ROD1	Ig-like 2	(Cui et al., 2018)	VUS
9	c.1425C>A	p.Asn475Lys	Missense	ROD1	Ig-like 3	Current study	VUS
12	c.1882G>A	p.Val628Met	Missense	ROD1	Ig-like 4	(Cui et al., 2018)	VUS
13	c.2050G>C	p.Val684Leu	Missense	ROD1	Ig-like 5	(Cui et al., 2018)	VUS
13	c.2084G>A	p.Arg695His	Missense	ROD1	Ig-like 5	Current study	VUS
14	c.2170G>A	p.Gly724Ser	Missense	ROD1	Ig-like 5	(Cui et al., 2018)	VUS
15	c.2375G>T	p.Ser792Ile	Missense	ROD1	Ig-like 6	(Jaafar et al., 2016)	VUS
16	c.2450T>C	p.Ile817Thr	Missense	ROD1	Ig-like 6	(Cirino et al., 2017)	VUS
17	c.2587C>T	p.Pro863Ser	Missense	ROD1	Ig-like 7	(Cui et al., 2018)	VUS
18	c.2737G>A	p.Glu913Lys	Missense	ROD1	Ig-like 7	(Cui et al., 2018)	VUS
19	c.2812-4A>G	p.?	Splice	ROD1	Ig-like 7	(Cui et al., 2018)	VUS
21	c.3581C>T	p.Ser1194Leu	Missense	ROD1	Ig-like 10	(Ader et al., 2019)	VUS
21	c.3623C>T	p.Ala1208Val	Missense	ROD1	Ig-like 10	(Cui et al., 2018)	VUS
24	c.4271G>T	p.Gly1424Val	Missense	ROD1	Ig-like 12	(Ader et al., 2019)	Likely pathogenic
27	c.4615G>A	p.Ala1539Thr	Missense	ROD1	Ig-like 14	(Valdes-Mas et al., 2014)	Likely pathogenic
28	c.4795A>G	p.Thr1599Ala	Missense	ROD1	Ig-like 14	(Gomez et al., 2017)	VUS
30	c.5042C>T	p.Thr1681Met	Missense	ROD1	Ig-like 15	(Gomez et al., 2017)	VUS
30	c.5068C>T	p.Leu1690Phe	Missense	ROD1	Ig-like 15	(Gomez et al., 2017)	VUS
30	c.5125C>T	p.Pro1709Ser	Missense	ROD1	Ig-like 15	(Cui et al., 2018)	VUS
30	c.5132C>T	p.Pro1711Leu	Missense	ROD1	Ig-like 15	(Cui et al., 2018)	VUS
36	c.5888C>T	p.Thr1963Met	Missense	ROD2	Ig-like 18	(Cui et al., 2018)	VUS
36	c.5954C>T	p.Ser1985Leu	Missense	ROD2	Ig-like 18	Current study	VUS
36	c.5996G>A	p.Arg1999Gln	Missense	ROD2	Ig-like 18	(Jaafar et al., 2016)	VUS
37	c.6032G>A	p.Gly2011Glu	Missense	ROD2	Ig-like 18	(Ader et al., 2019)	VUS
37	c.6053G>A	p.Arg2018His	Missense	ROD2	Ig-like 18	(Chanavat et al., 2016)	VUS
37	c.6115G>A	p.Gly2039Arg	Missense	ROD2	Ig-like 19	(Ader et al., 2019)	Likely pathogenic
37	c.6134G>A	p.Arg2045Gln	Missense	ROD2	Ig-like 19	(Chanavat et al., 2016)	VUS
37	c.6205G>A	p.Ala2069Thr	Missense	ROD2	Ig-like 19	Current study	VUS
39	c.6398G>A	p.Arg2133His	Missense	ROD2	Intradomain	(Valdes-Mas et al., 2014)	Likely pathogenic
39	c.6397C>T	p.Arg2133Cys	Missense	ROD2	Intradomain	(Cui et al., 2018)	Likely pathogenic
39	c.6419G>A	p.Arg2140Gln	Missense	ROD2	Intradomain	(Gomez et al., 2017)	Likely pathogenic
39	c.6451G>A	p.Gly2151Ser	Missense	ROD2	Intradomain	(Valdes-Mas et al., 2014)	VUS
40	c.6589C>T	p.Arg2197Trp	Missense	ROD2	Intradomain	(Alejandra Restrepo-Cordoba et al., 2017)	VUS

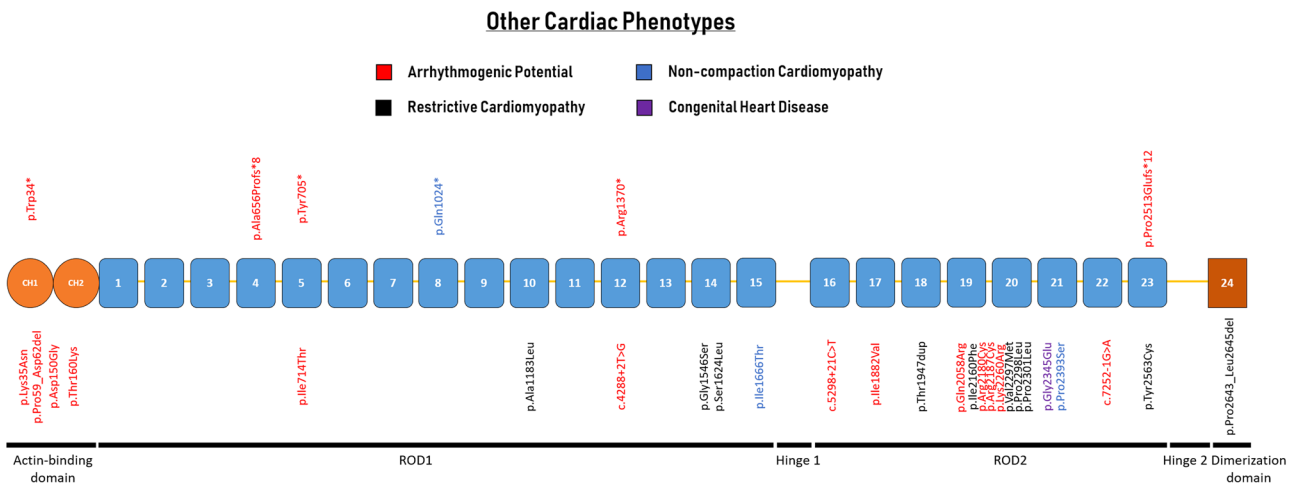


FIGURE 4 Schematic representation of the *FLNC* gene with their protein-coding domains. Numbers inside the boxes refer to the Ig-like domains of filamin C. Above and below the schematic are all unique variants associated with different cardiac phenotypes. Variants are annotated at the protein level

presentation, such as distal and/or proximal myopathy. The more specific diagnosis MFM requires finding protein aggregates in muscle.

The first description of a human phenotype related to *FLNC* was in 2005, when a nonsense variant (p.Trp2710*) was described in a German family with a novel type of autosomal dominant MFM (Vorgerd et al., 2005). The variant was later described in cohorts of varying ethnicity, suggesting codon 2710 to be a mutational hotspot (Kley et al., 2012). *FLNC* variants are mostly associated with a proximal myopathy, with occasional distal involvement (Figure 5; Table 4; van den Bogaart et al., 2017). There are few associations between variant type or location and the corresponding myopathy phenotype or special features such as cardiac involvement. We observed a cluster of missense variants in Ig-like domain 10, a domain which is rarely involved in cardiomyopathies. There was no genotype–phenotype association between variant location, and features of MFM, including tissue protein aggregate formation (Figure 5). Missense, in-frame and nonsense variants are all associated with protein aggregate formation in muscle tissue (Avila-Smirnow et al., 2010; Luan, Hong, Zhang, Wang, & Yuan, 2010; Vorgerd et al., 2005). Only two truncating variants have been reported to cause myopathy. Both are in Ig-like 15, and associated with a form of isolated distal myopathy.

2.3 | Other nonstriated muscle diseases

Twenty-two unique *FLNC* variants have been described in association with noncardiac or muscular phenotypes. These variants are listed in the Supporting Information as they are not the main focus of this overview (Table S2). There is a single large study, which performed high-throughput sequencing in patients with frontotemporal dementia and found a rare *FLNC* missense variant in 3.6% of the patients (Janssens et al., 2015).

3 | BIOLOGICAL RELEVANCE

FLNC variants are associated with a spectrum of cardiac and muscular phenotypes, suggesting that specific variants fall into three pathomechanisms, as previously suggested (Furst et al., 2013):

1. Variants that are predicted to lead to expression of misfolded proteins, which saturate the proteasome and autophagy pathways.
2. Variants, which give a toxic gain-of-function by altering ligand binding properties.
3. Variants causing a premature stop codon and concomitant nonsense-mediated decay (NMD), resulting in haploinsufficiency.

3.1 | Pathomechanisms of *FLNC* variants in cardiomyopathy phenotypes

A landmark paper regarding *FLNC* variants in DCM showed a strong association between truncating variants and this disease (Ortiz-Genga et al., 2016). NMD and subsequent haploinsufficiency were validated for a number of truncating variants as the pathomechanism in *FLNC*-associated DCM. Immunohistochemical analysis showed normal *FLNC* protein in the intercalated discs of patients with DCM. Abnormal *FLNC* protein aggregates in the cytoplasm were not detectable (Ortiz-Genga et al., 2016). The absence of aggregates in the cardiac tissue of patients with truncating *FLNC* variants in the ROD2 domain indicates the lack of an abnormal *FLNC* protein. In addition, western blot analysis in zebrafish models and rat cardiac myoblasts showed the absence of a truncated protein in the truncating variant models (Begay et al., 2016; Reinstein et al., 2016). Haploinsufficiency affects force transduction of striated muscle, specifically in tissues dependent on high-force generation, such as the myocardium.

TABLE 3 FLNC variants found in individuals with a variety of cardiac phenotypes as previously reported and from this study

Exon	c-Notation	p-Notation	Variation type	Domain	Location	Phenotype	Reference	Effect
1	c.102G>A	p.Trp34*	Nonsense	ABD	CH1	ABIMVPS	(Bains et al., 2019)	Likely pathogenic
1	c.105G>C	p.Lys35Asn	Missense	ABD	CH1	ACM/SCD	(Hall et al., 2019)	VUS
1	c.174_185del	p.Pro59_Asp62del	In-frame	ABD	CH1	ACM	(Hall et al., 2019)	VUS
2	c.449A>G	p.Asp150Gly	Missense	ABD	CH1	ACM	Current study	VUS
2	c.479C>A	p.Thr160Lys	Missense	ABD	CH2	ACM/SCD	(Hall et al., 2019)	VUS
12	c.1965_1966delTTG	p.Ala656Profs*8	Frameshift	ROD1	Ig-like 4	ACM/SCD	(Hall et al., 2019)	Likely pathogenic
13	c.2115_2120delTGCCCA	p.Tyr705*	Nonsense	ROD1	Ig-like 5	ACM/SCD	(Hall et al., 2019)	Likely pathogenic
14	c.2141T>C	p.Ile714Thr	Missense	ROD1	Ig-like 5	ACM	(Hall et al., 2019)	VUS
20	c.3070C>T	p.Gln1024*	Nonsense	ROD1	Ig-like 8	NCCM	(Miszalski-Jamka et al., 2017)	Likely pathogenic
21	c.3547_3548delinsCT	p.Ala1183Leu	Missense	ROD1	Ig-like 10	RCM	(Kiselev et al., 2018)	VUS
23	c.4108C>T	p.Arg1370*	Nonsense	ROD1	Ig-like 12	ACM/SCD	(Hall et al., 2019)	Likely pathogenic
24	c.4288+2T>G	p.?	Splice	ROD1	Ig-like 12	ACM/SCD	(Hall et al., 2019)	Likely pathogenic
27	c.4636G>A	p.Gly1546Ser	Missense	ROD1	Ig-like 14	RCM	(Sanoja, Li, Fricker, Kingsmore, & Wallace, 2018)	VUS
28	c.4871C>T	p.Ser1624Leu	Missense	ROD1	Ig-like 14	RCM	(Brodehl et al., 2016)	Likely pathogenic
30	c.4997T>C	p.Ile1666Thr	Missense	ROD1	Ig-like 15	NCCM	(Ader et al., 2019)	VUS
31	c.5298+21C>T	p.?	Splice	ROD2	Ig-like 16	ACM/SCD	(Hall et al., 2019)	VUS
34	c.5644A>G	p.Ile1882Val	Missense	ROD2	Ig-like 17	ACM	(Hall et al., 2019)	VUS
35	c.5839_5841dup	p.Thr1947dup	In-frame	ROD2	Ig-like 18	RCM	(Ader et al., 2019)	VUS
37	c.6173A>G	p.Gln2058Arg	Missense	ROD2	Ig-like 19	ACM	(Hall et al., 2019)	VUS
39	c.6478A>T	p.Ile2160Phe	Missense	ROD2	Intradomain	RCM	(Brodehl et al., 2016)	Likely pathogenic
40	c.6538C>T	p.Arg2180Cys	Missense	ROD2	Intradomain	ACM	Current study	VUS
40	c.6559C>T	p.Arg2187Cys	Missense	ROD2	Intradomain	ACM	Current study	VUS
41	c.6779A>G	p.Lys2260Arg	Missense	ROD2	Ig-like 20	ACM	(Hall et al., 2019)	VUS
41	c.6889G>A	p.Val2297Met	Missense	ROD2	Ig-like 20	RCM	(Tucker et al., 2017)	Likely pathogenic
41	c.6893C>T	p.Pro2298Leu	Missense	ROD2	Ig-like 20	RCM	(Schubert et al., 2018)	Likely pathogenic
41	c.6902C>T	p.Pro2301Leu	Missense	ROD2	Ig-like 20	RCM	(Roldan-Sevilla et al., 2019)	VUS
42	c.7034G>A	p.Gly2345Glu	Missense	ROD2	Ig-like 21	Congenital heart disease	(Kosmicki et al., 2017)	VUS
43	c.7177C>T	p.Pro2393Ser	Missense	ROD2	Ig-like 21	NCCM	Current study	Likely pathogenic

(Continues)

TABLE 3 (Continued)

Exon	c-Notation	p-Notation	Variation type	Domain	Location	Phenotype	Reference	Effect
44	c.7252-1G>A	p.?	Splice	ROD2	Ig-like 22	ACM	(Hall et al., 2019)	Likely pathogenic
45	c.7536_7548del13	p.Pro2513Glufs*12	Frameshift	ROD2	Ig-like 23	Cardiac arrhythmia	(Mangum & Ferns, 2019)	Likely pathogenic
46	c.7688A>G	p.Tyr2563Cys	Missense	ROD2	Ig-like 23	RCM	(Schubert et al., 2018)	Likely pathogenic
47	c.7927_7935del	p.Pro2643_Leu2645del	In-frame	ROD2	Ig-like 24	RCM	(Ader et al., 2019)	VUS

Abbreviations: ABD, actin-binding domain; ABIMVPS, arrhythmic bileaflet mitral valve prolapse syndrome; ACM, arrhythmogenic cardiomyopathy; NCCM, noncompaction cardiomyopathy; RCM, restrictive cardiomyopathy; SCD, sudden cardiac death; VUS, variant of unknown significance.

Both HCM and RCM patients have an enrichment of missense variants causing changes in the secondary protein structure resulting in an abnormal protein. Missense variants are strongly clustered in the short ROD2 domain of the protein in HCM (Figure 3). This region of the protein is important for the interaction between FLNC and the Z-disc. Five missense variants were reported in the intradomain insert (between Ig-like domain 19 and 20), which mediates the specific targeting to the Z-disc. Abnormal protein has been observed within aggregates in the tissue of FLNC-associated HCM and RCM patients in association with marked sarcomeric abnormalities (Kiselev et al., 2018; Valdes-Mas et al., 2014). The progressive accumulation of protein aggregates in the cardiac muscle eventually leads to sarcomeric disarray. Functional studies including the transfection of missense variants in rat cardiac myoblasts confirmed the formation of insoluble filamin C aggregates (Valdes-Mas et al., 2014), although there were differences in the size of aggregates and signal strength on western blots per variant. The overall histopathology of FLNC-associated HCM constituted large nuclei and large fiber diameters, as comparable to established non-FLNC HCM.

3.2 | Pathomechanisms of FLNC variants in myopathy phenotypes with and without protein aggregate formation

The p.Trp2710* variant leads to truncation of Ig-domain 24, which is needed for the formation of FLNC dimers (Voggerd et al., 2005). The mutant messenger RNA (mRNA) is stable and not subject to degradation by NMD, probably because the variant is in the last exon of the gene. Instead, the nonsense variant leads to the formation of protein aggregates of mutated filamin fragments, other known MFM-associated proteins and a number of filamin C binding partners in the skeletal muscle (Kley, Maerkens et al., 2013; Lowe et al., 2007). Interestingly, there is one truncating variant in the last exon described in association with DCM (p.Asp2703Thrfs*69), which is subject to NMD (Ortiz-Genga et al., 2016). This shows that not only the variant type in the last exon is of importance but also the pathomechanism for the subsequent phenotype.

The autophagy pathways which clear protein aggregates in MFM (mainly the “chaperone-assisted selective autophagy” [CASA] pathway) are activated but unable to clear the aggregates, preventing recovery of homeostasis (Kley, van der Ven et al., 2013; Ruparelina, Oorschot, Ramm, & Bryson-Richardson, 2016). More proteins are being associated with the regulation of FLNC proteostasis via autophagy, such as Hspb7 (Mercer, Lin, Cohen-Gould, & Evans, 2018). Consequently, many of the FLNC binding partners are dispersed, which disturbs the stability between the cytoskeleton and the membrane protein complexes, affecting cell signaling. The formation of protein aggregates in combination with FLNC depletion causes myofiber disintegration and muscle weakness, which is aggravated by muscle activity (Chevessier et al., 2015; Kley et al., 2012).

Two missense variants (p.Ala193Thr and p.Met251Thr) in the ABD are associated with a form of isolated distal myopathy without

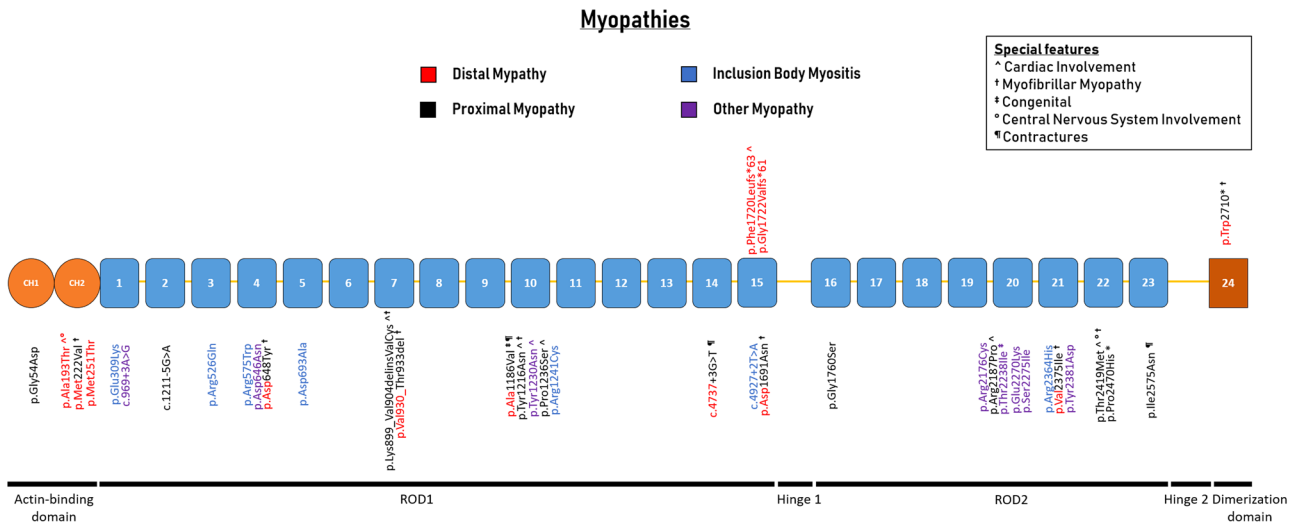


FIGURE 5 Schematic representation of the *FLNC* gene with their protein-coding domains. Numbers inside the boxes refer to the Ig-like domains of filamin C. Above and below the schematic are all unique variants associated with myopathies. Variants are annotated at the protein level

protein aggregate formation (Duff et al., 2011). In contrast to all other reported missense variants in the ABD, these variants are predicted to change from a hydrophobic to an uncharged amino acid. They are predicted to alter intradomain interactions, thereby increasing the binding affinity of filamin C to actin.

A frameshift variant (p.Phe1720Leufs*63) has also been associated with an isolated distal myopathy without protein aggregates (Guergueltecheva et al., 2011). In contrast to p.Trp2710*, this variant occurs in Ig-like domain 15 and activates NMD: analysis of RNA and protein in patients' muscle biopsies showed a 50% decrease in *FLNC* mRNA and protein, implicating haploinsufficiency (Guergueltecheva et al., 2011). When compared with the truncating variants in DCM, this is the only reported frameshift variant in Ig-like domain 15, which is the domain before hinge 1. This hinge is only present in the long isoform of *FLNC* which incorporates exon 31 (Xie et al., 1998), and might be important in the isoform switch during cell stress (Kong et al., 2010). There is no comparable frameshift variant encompassing exon 31 in DCM. The different impact of the frameshift on the two isoforms could be a mechanistic explanation for the clinical variation, although this hypothesis needs further testing to accurately determine the importance of the isoform shift.

4 | ANIMAL MODELS

Animal studies of *FLNC* are performed in (zebra) fish, mice, and *Drosophila* (Figure 6). Shortly after the description of *FLNC* variants in MFM, the first mouse model was developed by the deletion of the last eight exons of *FLNC* (Dalkilic, Schienda, Thompson, & Kunkel, 2006). Homozygous mice died shortly after birth due to respiratory failure. Heterozygous mice had less muscle mass and a decreased number of primary muscle fibers. Their muscles also showed excessive fiber size variation, centrally located nuclei and

a disorganized muscle structure. This suggests a key role for *FLNC* in myogenesis as well as in myofiber structure maintenance.

A Medaka fish (*Orzias latipes*) model was developed to investigate the cardiac and muscular phenotype of *FLNC* variants (Fujita et al., 2012). It contained a nonsense variant resulting in truncation at Ig-15. Despite this variant, these fish had normal myogenesis. However, the myofibrils gradually degenerated and became disorganized, eventually leading to myocardial rupture. This suggests that *FLNC* is mainly involved in muscle structure maintenance instead of myogenesis, partly by affording protection against mechanical stress related to muscle contraction. Fiber dissolution and protein aggregate formation were not described in this model. These characteristics of MFM were observed in a zebrafish (*Danio rerio*) model in which the filamin C-b homolog (*flnbc*) contains a nonsense variant in exon 30 (Ruparelia, Zhao, Currie, & Bryson-Richardson, 2012). A knockdown of the filamin C-a homolog (*flnca*) yielded the same phenotype. However, loss of both homologs leads to a major failure of the muscle fibers. Also here, it was shown that *FLNC* was mainly involved in fiber protection and maintenance rather than fiber specification and myogenesis. Investigation of the cardiac phenotype in fish with a *flnbc* knockdown showed atrium distention and backflow upon contraction (Deo et al., 2014). Optical mapping showed a decrease in ventricular conduction velocity, suggesting alterations in junctional remodeling, and cell-cell coupling. Later studies also showed sarcomere and Z-disc disorganization (Begay et al., 2016). A study using *Drosophila* showed filamin C as an important cohesive element within the Z-disc, where it acts as a bridge between thin filaments and the elastic scaffold protein titin (Gonzalez-Morales, Holenka, & Schock, 2017). The Z-disc requires filamin C to withstand the strong contractile forces acting on the sarcomere.

Other animal models were created to investigate targeted variants by knock-in experiments in mice or overexpression in zebrafish (Chevessier et al., 2015; Kiselev et al., 2018; Ruparelia et al., 2016). Two models were created to investigate the hotspot variant, p.Trp2710* (Chevessier et al., 2015; Ruparelia et al., 2016). Heterozygous mice

TABLE 4 FLNC variants found in individuals with a muscular phenotype as previously reported and from this study

Exon	c-Notation	p-Notation	Variant type	Domain	Location	Phenotype	Reference	Effect
1	c.161G>A	p.Gly54Asp	Missense	ABD	CH1	PM	(Fichna et al., 2018)	VUS
2	c.577G>A	p.Ala193Thr	Missense	ABD	CH2	DM; PM; Car; CNS	(Duff et al., 2011)	Likely pathogenic
3	c.664A>G	p.Met222Val	Missense	ABD	CH2	DM; PM; MFM	(Gemelli et al., 2019)	Likely pathogenic
4	c.752T>C	p.Met251Thr	Missense	ABD	CH2	DM; PM	(Duff et al., 2011)	Likely pathogenic
5	c.925G>A	p.Glu309Lys	Missense	ROD1	Ig-like 1	IBM	(Weihl et al., 2015)	VUS
5	c.969+3A>G	p.?	Splice	ROD1	Ig-like 1	OM	(Dai et al., 2015)	VUS
8	c.1211-5G>A	p.?	Splice	ROD1	Ig-like 2	PM	Current study	VUS
10	c.1577G>A	p.Arg526Gln	Missense	ROD1	Ig-like 3	IBM	(Weihl et al., 2015)	VUS
11	c.1723C>T	p.Arg575Trp	Missense	ROD1	Ig-like 4	IBM	(Weihl et al., 2015)	VUS
12	c.1936G>A	p.Asp646Asn	Missense	ROD1	Ig-like 4	OM	Current study	VUS
12	c.2078A>C	p.Asp648Tyr	Missense	ROD1	Ig-like 4	PM; DM; MFM	(Y. T. Zhang et al., 2018)	Likely pathogenic
13	c.2078A>C	p.Asp693Ala	Missense	ROD1	Ig-like 5	IBM	(Weihl et al., 2015)	VUS
18	c.2695_2712del18insGTTTGT	p.Lys899_Val904delinsValCys	In-frame	ROD1	Ig-like 7	PM; Car; MFM	(Luan et al., 2010)	Likely pathogenic
18	c.2789_2800del12	p.Val930_Thr933del	In-frame	ROD1	Ig-like 7	PM; DM; MFM	(Shatunov et al., 2009)	Likely pathogenic
21	c.3557C>T	p.Ala1186Val	Missense	ROD1	Ig-like 10	PM; DM; CM; Con	(Ghaoui et al., 2015)	Likely pathogenic
21	c.3646T>A	p.Tyr1216Asn	Missense	ROD1	Ig-like 10	PM; Car; MFM	(Avila-Smirnow et al., 2010)	Likely pathogenic
21	c.3688T>A	p.Tyr1230Asn	Missense	ROD1	Ig-like 10	OM; Car	(Vill et al., 2017)	VUS
21	c.3706C>T	p.Pro1236Ser	Missense	ROD1	Ig-like 10	PM; Car	(Yu et al., 2017)	VUS
21	c.3721C>T	p.Arg1241Cys	Missense	ROD1	Ig-like 10	IBM	(Weihl et al., 2015)	VUS
27	c.4737+3G>T	p.?	Splice	ROD1	Ig-like 14	PM; DM; Con	Current study	VUS
28	c.4927+2T>A	p.?	Splice	ROD1	Ig-like 15	OM	(Zenagui et al., 2018)	VUS
30	c.5071G>A	p.Asp1691Asn	Missense	ROD1	Ig-like 15	PM; DM; MFM	(Y. T. Zhang et al., 2018)	Likely pathogenic
30	c.5160delC	p.Phe1720Leufs*63	Frameshift	ROD1	Ig-like 15	DM; Car	(Guergueltcheva et al., 2011)	Likely pathogenic
30	c.5165delG	p.Gly1722Valfs*61	Frameshift	ROD1	Ig-like 15	DM	(Rossi et al., 2017)	Likely pathogenic
31	c.5278G>A	p.Gly1760Ser	Missense	ROD2	Ig-like 16	PM	(Yu et al., 2017)	VUS
40	c.6526C>T	p.Arg2176Cys	Missense	ROD2	Intradomain	IBM	(Cerino et al., 2017)	VUS
40	c.6560G>C	p.Arg2187Pro	Missense	ROD2	Intradomain	PM; Car	Current study	VUS
40	c.6713C>T	p.Thr2238Ile	Missense	ROD2	Intradomain	OM; CM	Current study	VUS

TABLE 4 (Continued)

Exon	c-Notation	p-Notation	Variation type	Domain	Location	Phenotype	Reference	Effect
41	c.6808G>A	p.Glu2270Lys	Missense	ROD2	Ig-like 20	OM	Current study	VUS
41	c.6824G>T	p.Ser2275Ile	Missense	ROD2	Ig-like 20	OM	Current study	VUS
42	c.7091G>A	p.Arg2364His	Missense	ROD2	Ig-like 21	IBM	(Weihl et al., 2015)	VUS
42	c.7123G>A	p.Val2375Ile	Missense	ROD2	Ig-like 21	PM; DM; MFM	(Chen et al., 2019)	VUS
43	c.7141T>G	p.Tyr2381Asp	Missense	ROD2	Ig-like 21	OM	Current study	VUS
44	c.7256C>T	p.Thr2419Met	Missense	ROD2	Ig-like 22	PM; Car; CNS; MFM	(Tasca et al., 2012)	VUS
45	c.7409C>A	p.Pro2470His	Missense	ROD2	Ig-like 22	PM; Car	(Reddy et al., 2017)	VUS
46	c.7724T>A	p.Ile2575Asn	Missense	ROD2	Ig-like 23	PM; Con	Current study	Likely pathogenic
48	c.8130G>A	p.Trp2710*	Nonsense	Dimerization	Ig-like 24	PM; DM; MFM	(Vorgerd et al., 2005)	Likely pathogenic

Abbreviations: ABD, actin-binding domain; Car, cardiac involvement; CNS, congenital myopathy; Con, central nervous system involvement; Con, contractures; DM, distal myopathy; IBM, inclusion body myositis; MFM, diagnosis of myofibrillar myopathy using cardiac tissue; OM, other nonspecified myopathy; PM, proximal myopathy; VUS, variant of unknown significance.

developed muscle weakness and myofibrillar instability (Chevessier et al., 2015). In addition to the classical protein aggregates, they also developed filamin C positive lesions between the Z-discs appearing upon physical exercise. Overexpression of the variant in zebrafish led to the formation of protein aggregates (Ruparelia et al., 2016). In this model, mutant FLNC was localized around the Z-disc and is able to rescue the disintegration phenotype. This led to the hypothesis that it was mainly the aggregates and the sequestration of FLNC away from the Z-disc that cause myofibrillar disintegration. The study further showed that the CASA pathway is impaired, making the cell unable to clear the protein aggregates.

5 | CLINICAL AND DIAGNOSTIC RELEVANCE

Cardiac involvement is a common clinical manifestation in hereditary muscular dystrophies (Hermans et al., 2010). Conversely, muscular problems in cardiomyopathy patients have also been described (Limongelli et al., 2013), showing the strong molecular link between hereditary muscular dystrophies and cardiomyopathies. In line with this, cardiac involvement is not uncommon in FLNC-associated myopathy (Figure 5), but muscle involvement has not been described (yet) at the moment of diagnosis in FLNC-associated cardiomyopathy (Ader et al., 2019). A single patient with DCM developed distal myopathy during follow-up (Ortiz-Genga et al., 2016).

5.1 | Specific clinical characteristics of FLNC-associated cardiomyopathies

Compared with the average clinical course of DCM, FLNC-associated DCM is more malignant, characterized by ventricular arrhythmias, myocardial fibrosis, and a high risk of sudden cardiac death (Ortiz-Genga et al., 2016). The average age of onset is 39.7 ± 14.5 . Considering all available literature at the moment, filaminopathy has a distinct cardiac phenotype. In contrast to arrhythmogenic cardiomyopathies, FLNC-associated DCM is left-dominant in the absence of right ventricular involvement (Augusto et al., 2019; Ortiz-Genga et al., 2016). Diagnostic clues can be inferolateral negative T waves on the electrocardiogram, mild to moderate left ventricular dysfunction and regional dyskinesia. It also has a characteristic ring-like scar pattern in the left ventricle as detected by cardiovascular magnetic resonance imaging (Augusto et al., 2019). The combination of increased myocardial fibrosis, ventricular arrhythmias, and sudden cardiac death are also found in laminopathies, desminopathies, and desmosomal variants (Hasselberg et al., 2017; Lopez-Ayala et al., 2014). In contrast to these forms of genetic DCM, cardiac conduction abnormalities such as an atrioventricular block are uncommon in FLNC-associated DCM while they are common in laminopathies and desminopathies. In addition, desmosomal variants are strongly correlated to isolated or predominant right ventricular

FLNC-associated animal studies

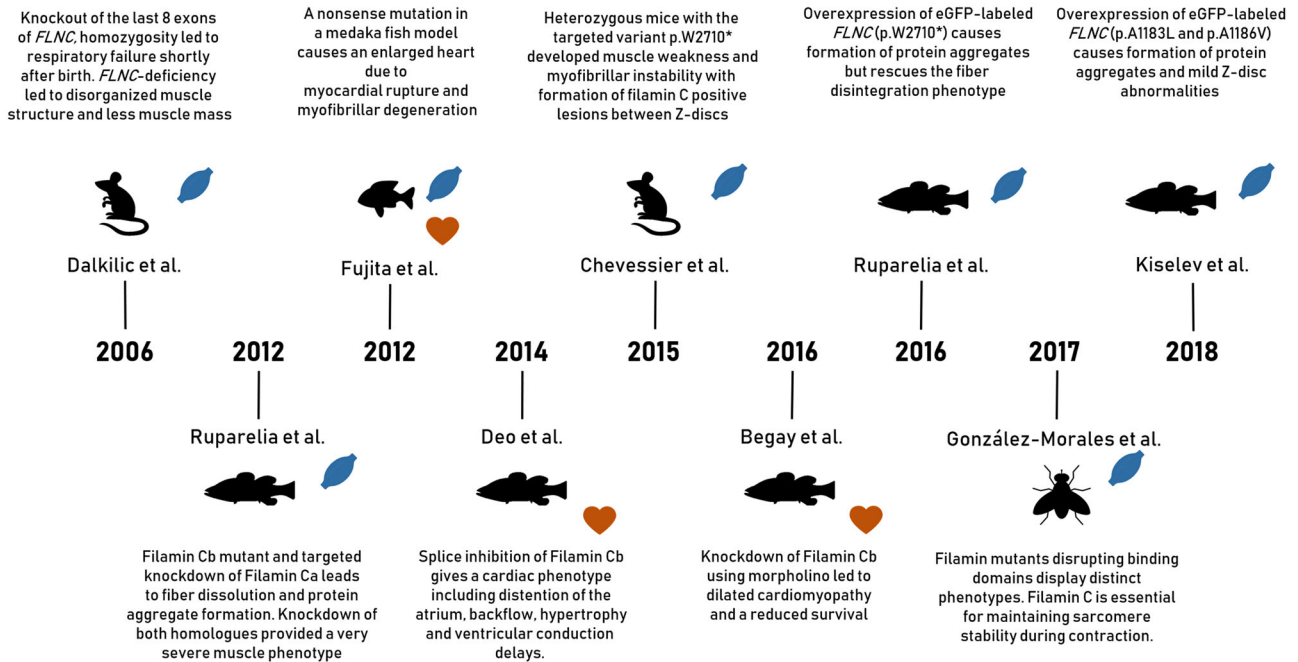


FIGURE 6 A timeline representation of animal models generated to study *FLNC* variants. eGFP indicates enhanced green fluorescent protein

involvement. For patients with DCM, the finding of a truncating *FLNC* is likely causative, and relatives should be screened for this variant (Tayal & Cook, 2016). The finding of a truncating *FLNC* variant in otherwise healthy subjects outside of a familial context is much less clear at the moment, as there is not enough knowledge regarding penetrance, expression, and clinical correlation. Although the prevalence of truncating *FLNC* variants is very low in the general population (<0.01% in gnomAD).

The mean age of onset of HCM in *FLNC* carriers is 35.9 ± 14.8 . In a previous cohort of patients with HCM, it was reported that 34% of the *FLNC* variant carriers had elevated creatine kinase (CK) levels (Valdes-Mas et al., 2014), also in RCM, there were some patients with mildly elevated CK levels (Brodehl et al., 2016). However, this finding is not consistent across different patient cohorts (Ader et al., 2019).

5.2 | Specific clinical characteristics of *FLNC*-associated myopathies

The two classic muscular phenotypes are MFM and distal myopathy (with and without protein aggregates respectively; Furst et al., 2013). Other genes have been associated with these phenotypes, such as *DES*, *LDB3*, and *BAG3* (Hermans et al., 2010). Besides the muscular phenotype, these genes are also associated with isolated cardiomyopathies. Characteristic features of *FLNC*-associated MFM is the symmetrical involvement of proximal muscles in the lower extremities, respiratory weakness

during the disease course, and a specific set of imaging characteristics for muscle involvement (Kley et al., 2012). About one-third of the *FLNC*-MFM showed cardiac involvement. Distal myopathies due to *FLNC* variants are characterized by weakness in the hand and calf muscles with an onset in early adulthood (Furst et al., 2013).

6 | GENOTYPE/PHENOTYPE CORRELATIONS

Genotype–phenotype correlations are currently incomplete, but some patterns are starting to emerge (Figure 7).

There is no clear clustering of DCM variants in any specific region of the gene, partly because most truncating variants are predicted to result in NMD. Truncating *FLNC* variants are strongly associated with DCM and arrhythmogenic potential. Just three out of 55 truncating variants are associated with a muscular phenotype. Two are a frameshift in Ig-like 15 spanning hinge 1 and one is a nonsense variant in Ig-like 24 of the dimerization domain. The different pathomechanism underlying missense and truncating variants partly explains the structural changes at the histological level and the corresponding clinical phenotype. Missense variants are mainly found in ROD2, which is essential for filamin C dimerization and Z-disc interaction. These variants interfere with the secondary protein structure, leading to sarcomere disarray and aggregate formation. These histopathological changes are associated with HCM and RCM phenotypes.

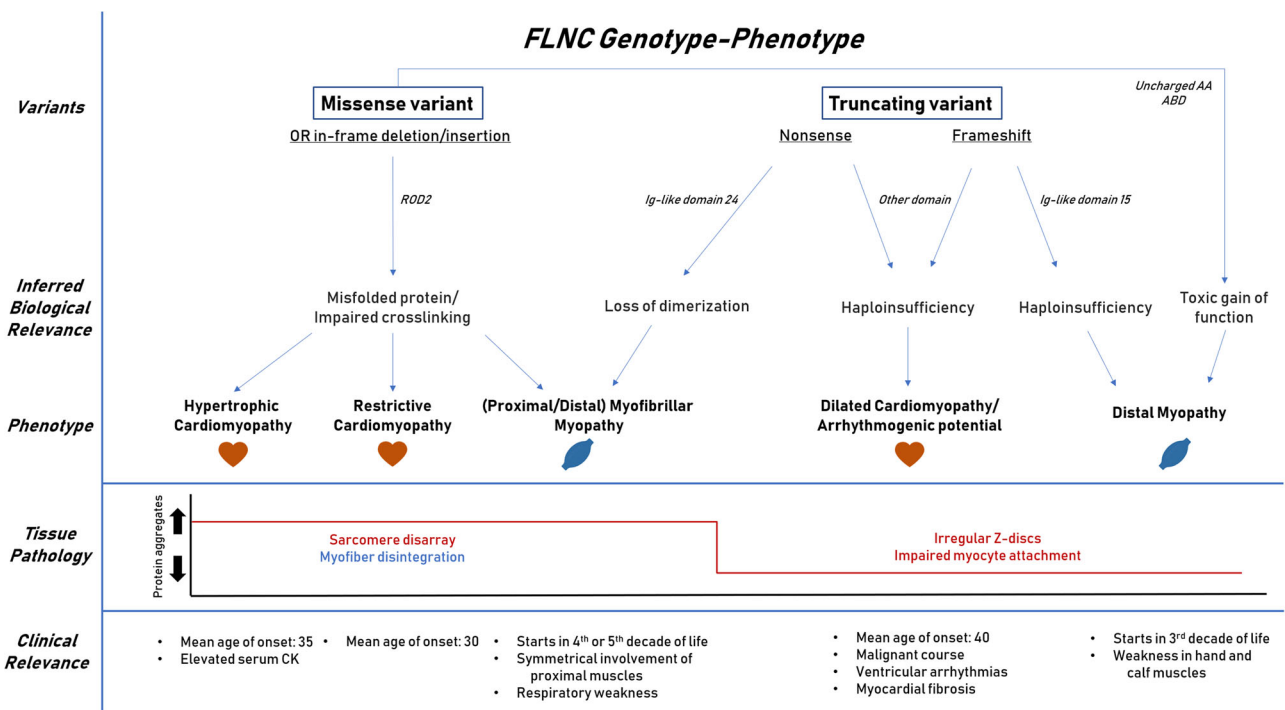


FIGURE 7 A summary how different variants in *FLNC* lead to a variety of disease mechanisms eventually giving a spectrum of clinical entities with the corresponding structural histological changes. These *FLNC*-associated diseases contain specific clinical characteristics compared with other forms of the corresponding disease

1. Truncating variants are found throughout the whole gene, and are expected to invoke haploinsufficiency via NMD. This will lead to Z-disc disarray and weakened cell–cell adhesion with subsequent impaired mechanotransduction. These structural alterations make the heart prone for developing DCM with arrhythmogenesis and promote fibrogenesis contributing to the arrhythmogenesis.
2. Frameshift variants spanning hinge 1 (exon 31) potentially interfere with flexibility for isoform switching. Both described frameshift variants spanning exon 31 are associated with distal myopathy.
3. Missense variants in the ABD that create an uncharged amino acid give a toxic gain-of-function with a stronger actin-binding activity. These variants have been associated with distal myopathy.
4. A nonsense variant in the dimerization domain interferes with the ability to form *FLNC* homodimers, although proteins are still translated. These proteins form aggregates in the skeletal muscle leading to MFM.

7 | FUTURE PROSPECTS

As *FLNC* is now included in many genetic screening panels for muscular and cardiac diseases, the number of variants will increase in the coming years. A better understanding of the molecular alterations due to *FLNC* variants can shed light on potential treatment targets. It can help us in understanding and predicting genotype–phenotype correlations. Appropriate functioning of *FLNC* depends on multiple interactions with other proteins (correlations Mercer et al., 2018). These interactions

could contribute to specific phenotypes. The ROD2 domain encompasses binding sites for the majority of *FLNC* interactors, and is necessary for mechanosensing and muscle maintenance functions. Chaperones such as HspB1 need to bind to *FLNC* to ensure these functions (Collier et al., 2019). The inability of HspB1 to bind to *FLNC* can lead to cardiac dysfunction. This is one example how variants in a specific domain could affect protein interactions and contribute to a specific phenotypes. A field that should be further explored. As an example, we formulated the following research questions:

1. Which factors explain the clinical variety among truncating variants in the *FLNC* gene. For example: Why does p. Phe1720Leufs*63 lead to a distal myopathy while p.Asn1369Lysfs*36 leads to DCM? Does flexibility in isoform switching play a role in this?
2. How does a nonsense variant in Ig-like 24 (p.Trp2710*) lead to an MFM and a frameshift variant in the same region (p.Asp2703Thrfs*69) lead to arrhythmogenic DCM?
3. What are the exact structural protein changes related to missense variants in the ABD and how do they explain the clinical difference between HCM and distal myopathy?
4. Are truncating variants in certain parts of the gene better tolerated clinically and therefore less penetrant?
5. Which molecular pathways are differentially activated due to *FLNC* variants and can these pathways serve as potential therapeutic targets?
6. What is the clinical relevance and outcome of (truncating) *FLNC* variants in the general population?

8 | CONCLUSION

Variants in *FLNC* can lead to myopathies and cardiomyopathies. The difference in phenotypes can be partly explained by the pathomechanism associated with the variant type and location within the gene. As a general rule, interference with the dimerization and folding of the protein leads to aggregate formation, as found in HCM or MFM. Truncating variants with subsequent haploinsufficiency lead to weakened structural adhesion mainly associated with DCM and cardiac arrhythmias.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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