

# The role of Z-disc proteins in myopathy and cardiomyopathy

Wadmore, Kirsty; Azad, Amar; Gehmlich, Katja

DOI:

[10.3390/ijms22063058](https://doi.org/10.3390/ijms22063058)

License:

Creative Commons: Attribution (CC BY)

*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Wadmore, K, Azad, A & Gehmlich, K 2021, 'The role of Z-disc proteins in myopathy and cardiomyopathy', *International Journal of Molecular Sciences*, vol. 22, no. 6, 3058, pp. 1-30. <https://doi.org/10.3390/ijms22063058>

[Link to publication on Research at Birmingham portal](#)

## **Publisher Rights Statement:**

Wadmore, K.; Azad, A.J.; Gehmlich, K. The Role of Z-disc Proteins in Myopathy and Cardiomyopathy. *Int. J. Mol. Sci.* 2021, 22, 3058. <https://doi.org/10.3390/ijms22063058>

## **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.



Review

# The Role of Z-disc Proteins in Myopathy and Cardiomyopathy

Kirsty Wadmore <sup>1,†</sup>, Amar J. Azad <sup>1,†</sup> and Katja Gehmlich <sup>1,2,\*</sup>

<sup>1</sup> Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK; KXW047@student.bham.ac.uk (K.W.); A.J.S.Azad@bham.ac.uk (A.J.A.)

<sup>2</sup> Division of Cardiovascular Medicine, Radcliffe Department of Medicine and British Heart Foundation Centre of Research Excellence Oxford, University of Oxford, Oxford OX3 9DU, UK

\* Correspondence: k.gehmlich@bham.ac.uk; Tel.: +44-121-414-8259

† These authors contributed equally.

**Abstract:** The Z-disc acts as a protein-rich structure to tether thin filament in the contractile units, the sarcomeres, of striated muscle cells. Proteins found in the Z-disc are integral for maintaining the architecture of the sarcomere. They also enable it to function as a (bio-mechanical) signalling hub. Numerous proteins interact in the Z-disc to facilitate force transduction and intracellular signalling in both cardiac and skeletal muscle. This review will focus on six key Z-disc proteins:  $\alpha$ -actinin 2, filamin C, myopalladin, myotilin, telethonin and Z-disc alternatively spliced PDZ-motif (ZASP), which have all been linked to myopathies and cardiomyopathies. We will summarise pathogenic variants identified in the six genes coding for these proteins and look at their involvement in myopathy and cardiomyopathy. Listing the Minor Allele Frequency (MAF) of these variants in the Genome Aggregation Database (GnomAD) version 3.1 will help to critically re-evaluate pathogenicity based on variant frequency in normal population cohorts.

**Keywords:**  $\alpha$ -actinin 2; filamin C; myopalladin; myotilin; telethonin; Z-disc alternatively spliced PDZ-motif (ZASP); myopathy; cardiomyopathy; missense variant; truncating variant



**Citation:** Wadmore, K.; Azad, A.J.; Gehmlich, K. The Role of Z-disc Proteins in Myopathy and Cardiomyopathy. *Int. J. Mol. Sci.* **2021**, *22*, 3058. <https://doi.org/10.3390/ijms22063058>

Academic Editors: Yurii Borovikov and Olga Karpicheva

Received: 18 February 2021

Accepted: 11 March 2021

Published: 17 March 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Myopathies (myo—muscle, pathy—suffering) are diseases of the skeletal muscle, which are frequently hereditary. Patients present with a wide variety of clinical phenotypes, which often overlap, but are usually characterised by progressive muscle weakness due to the degeneration of muscle. Myopathies can include proximal and distal myopathy; diseases affecting the proximal muscles (muscles which are close to the centre of the body—shoulder, pelvis, and upper arms) and lower limbs, respectively. Limb-girdle muscular dystrophy (LGMDA) is a proximal muscle disease affecting the proximal limb and girdle muscles (muscles around the hips and shoulders). Patients with such dystrophies present with muscle atrophy, pseudohypertrophy and contractures of the joints, which can consequently lead to impaired or even total loss of movement. Loss of ambulation means patients may become wheelchair-bound. If the diaphragm is affected, lung function might be decreased and their oxygen intake reduced, and they may need to be ventilated. At the histopathological level, myofibrillar myopathies (MFM), which are among the most common skeletal muscle myopathies, commonly display protein aggregation due to a significant level of myofibrillar disorganisation. Characterisation of MFM protein aggregates highlighted the presence of Z-disc proteins such as filamin C, myotilin an Z-disc alternative spliced PDZ-motif (ZASP) just to name a few (reviewed in [1]). Other forms are nemaline myopathy (NM), which is characterised by the presence of ‘nemaline bodies’ and rod-like structures. Though generally classified as diseases of the skeletal muscle, patients with myopathies may additionally present with a cardiac phenotype (‘cardiac involvement’) [2].

Diseases that primarily affect the cardiac muscle are termed cardiomyopathies. They are heterogeneous myocardial disorders affecting the structure and function of the heart

muscle in the absence of coronary artery disease, hypertension, and congenital heart disease [3]. Cardiomyopathies are classified into the following categories: dilated (DCM), hypertrophic (HCM), restrictive (RCM), arrhythmogenic (ACM) cardiomyopathies and left ventricular noncompaction (LVNC). DCM is primarily characterised by a dilation of the left ventricle with poor contraction force (systolic dysfunction). HCM is characterised by the enlargement of cardiac mass (hypertrophy), which is accompanied by impaired relaxation (diastolic dysfunction). HCM is the most common cardiomyopathy with a prevalence of 1:500 [4]. RCM is characterised by a stiffening of the inner ventricular walls, also leading to diastolic dysfunction. ACM is an arrhythmic cardiomyopathy with right, left or biventricular impairment of systolic function. LVNC is characterised by pronounced left ventricle trabeculations. Some cardiomyopathies are associated with a high risk of sudden cardiac death due to ventricular arrhythmias (especially HCM and ACM).

There is phenotypic overlap between these diseases, e.g., ACM can affect primarily the left ventricle, mimicking DCM with arrhythmias [5]. Likewise, both RCM and HCM are characterised by diastolic dysfunction [6]. For a more detailed description of clinical and molecular features of myopathies and cardiomyopathies see excellent reviews [7,8].

The striated muscle cells, myotubes in skeletal muscle and cardiomyocytes in the heart share the general architecture of their contractile apparatus: organised in myofibrils, the smallest contractile units are the sarcomeres, in which actin-based thin filaments and myosin-based thick filaments are assembled. The sliding of these filaments against each other generates force and results in the contraction of the muscle [9]. Thick filaments are anchored by a structure called the M-band [10], while thin filaments are anchored by the Z-disc [11]. Titin, the third filament, connects Z-discs and M-bands by spanning half a sarcomere [12].

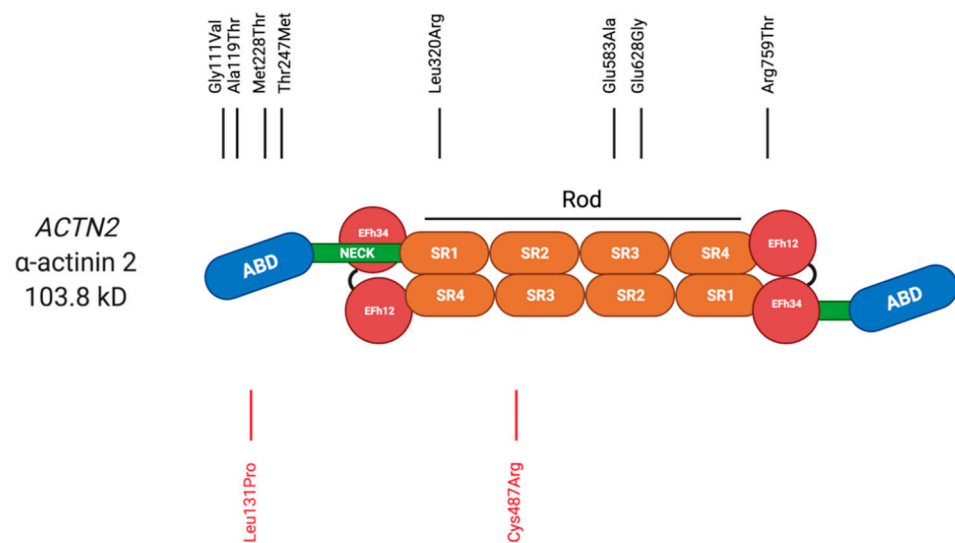
The roles of titin and M-band proteins in striated muscle diseases have been highlighted in excellent reviews, e.g., [10,13]. This review will focus on Z-disc proteins and their role in striated muscle diseases. We have selected Z-disc proteins that are implicated in genetic disease of both skeletal muscle and the heart, or cause myopathy with cardiac involvement. The following text will therefore focus on  $\alpha$ -actinin, filamin C, myopalladin, myotilin, telethonin and ZASP.

## 2. Z-disc Proteins in Myopathy and Cardiomyopathy

### 2.1. $\alpha$ -actinin 2

$\alpha$ -actinin is a protein found in the Z-disc of muscles and is involved in the cross-linking of anti-parallel actin filaments. It also interacts with titin and acts as a protein scaffold for large protein complexes [14]. The protein has four different isoforms (*ACTN1-4*), that are all closely related and perform similar functions, but display different expression profiles across tissues. The binding of non-muscle  $\alpha$ -actinin (*ACTN1* and *ACTN4*) to actin is regulated by the binding of calcium, whilst in muscle isoforms (*ACTN2* and *ACTN3*) binding to actin is calcium insensitive [15]. Instead, muscle  $\alpha$ -actinin is regulated by the binding of phospholipids such as phosphatidylinositol bisphosphate [16]. *ACTN2* (located at chromosome position 1q42-43) is highly expressed in heart and skeletal muscle, while *ACTN3* (located at chromosome position 11q13.2) is exclusively expressed in skeletal muscle.

$\alpha$ -actinin forms antiparallel homodimers [16]. It is comprised of an actin binding domain (ABD), a central rod domain with 4 spectrin repeats, and an EF hand domain (Figure 1). Through its binding to a variety of other signalling molecules, it has an important role in organisation of the cytoskeleton and muscle contraction [14].



**Figure 1.** Schematic representation of  $\alpha$ -actinin shown as a dimer with variants in the *ACTN2* gene that have been previously reported in individuals with myopathy (red) or cardiomyopathy (black). Solid lines represent missense variants. ABD—actin binding domain, EFh—EF hand, SR—spectrin like repeat. Created with [BioRender.com](https://www.biorender.com) (accessed on 7 March 2021). Adapted from [16].

A loss of function study of *ACTN2* in zebrafish embryos using antisense morpholino technology highlighted the importance of  $\alpha$ -actinin 2 in Z-disc lateral alignment in zebrafish cardiogenesis [17]. Depletion of *ACTN2* led to a reduced end-diastolic diameter and overall reduced cardiac function. The loss of function mutants also had reduced cardiomyocyte size and number, leading to reduced ventricular chamber size. This underlines the crucial role of  $\alpha$ -actinin for integrity in the developing heart.

#### 2.1.1. $\alpha$ -actinin 2 in Cardiomyopathy

Several studies report links between variants (*ACTN2*) and HCM. The first description was by Theis et al. [18]. As part of a genetics candidate gene approach, they interrogated 5 genes coding for Z-disc-proteins, including *ACTN2*, in 239 unrelated patients with HCM by polymerase chain reaction (PCR), denaturing high performance liquid chromatography (DHPLC) and direct Sanger DNA sequencing. They identified two pathogenic variants in *ACTN2* (p.Gly111Val and p.Arg759Thr), and also reported *ACTN2* p.Thr495Met. This variant (*ACTN2* p.Thr495Met), was initially thought to be pathogenic, but now appears to be relatively common [19] in normal populations to support a pathogenic role (Table 1). Echocardiography suggested that pathogenic variants in *ACTN2* (as well as other HCM-associated Z-disc genes) resulted in sigmoid septal morphology of cardiac hypertrophy.

A separate study looked at 23 individuals from a 3-generation Australian family that clinically presented with heterogeneous HCM [20]. A genome-wide linkage analysis, combined with candidate gene screening, identified *ACTN2* p. Ala119Thr. The variant was found in all 7 affected members of the HCM family. To further support the link between HCM and pathogenic variants in *ACTN2*, 297 additional probands were screened for variants in *ACTN2*. High-resolution melt analysis of four HCM families identified an additional two *ACTN2* variants (p.Glu583Ala, and p.Glu628Gly).

The *ACTN2* variant p.Met228Thr was identified in an Italian family with atypical HCM and early onset atrial fibrillation (AF) by next generation sequencing [21]. It co-segregated with disease in 11 affected family members. *ACTN2* p.Thr247Met, another variant located in the actin-binding domain, was identified in a patient who presented with familial HCM [22]. A human-induced pluripotent stem cell (hiPSC)-derived cardiomyocyte model for the variant supported its pathogenicity and suggested that the observed prolongation of QT interval in the family could be explained by the reduced interaction of

the *ACTN2* p.Thr247Met variant with the L-type calcium channel. This observation led to the successful treatment of affected family members with L-type calcium channel blocker diltiazem.

In summary, most *ACTN2* identified in HCM patients are located in the actin-binding domain (see Table 1). The crystal structures of two HCM-associated *ACTN2* variants (p.Ala119Thr and p.Gly111Val) located in the actin-binding domain, showed small, yet distinctive changes [23]. The *ACTN2* p.Gly111Val variant reduced thermal stability and impacted tertiary structure of the protein, whilst the *ACTN2* p.Ala119Thr had a more pronounced negative effect on actin binding. The variants showed weaker integration into the Z-discs than wild-type  $\alpha$ -actinin and formed aggregates outside the Z-discs. They further showed reduced ability to leave the Z-disc with reduced fluorescent decay after photoactivation, suggesting that exchange of the protein at Z-discs is reduced in the presence of the pathogenic variants.

Associations of *ACTN2* variants with other types of cardiomyopathies are less strong: *ACTN2* p.Gln9Arg variant was identified in the DCM patient [24]. Despite demonstrating altered binding properties to muscle LIM Protein in vitro and being absent in 200 control individuals, the variant would not pass current criteria of pathogenicity (based on its frequency in normal cohorts, Table 1).

More recently, *ACTN2* p.Leu320Arg was identified by whole-exome sequencing in a Chinese family, who presented with DCM and ventricular tachycardia [25]. 12 family members were followed up and four of them were affected by disease. All four carried had the *ACTN2* p.Leu320Arg variant that co-segregated with disease and was absent in the healthy family members.

A further study associated the *ACTN2* p.Ala119Thr with disease in an Australian family who showed cardiac phenotypic heterogeneity (LVNC or DCM) [26]. Using exome sequencing in two probands, the variant *ACTN2* p.Ala119Thr was ranked the most highly expressed in cardiac tissue according to data derived from RNAseq and found to co-segregate with disease of the four affected family members. Of note, the same change had previously been identified in HCM patients (see above) and it is not understood why it can cause different types of cardiomyopathy in different individuals.

**Table 1.** Variants in the *ACTN2* gene that have been previously reported in individuals with myopathy or cardiomyopathy.

Name Gene	Variant	Het or Homo	Type of Disease	Cardiac or Muscular	* MAF on GnomAD	Location	Ref
$\alpha$ -actinin <i>ACTN2</i>	p.Gln9Arg	Het	DCM	Cardiac	$7.22 \times 10^{-4}$	ABD	[24]
	p.Gly111Val	Het	HCM	Cardiac	<b>Absent</b>	ABD	[18]
	p.Ala119Thr	Het	HCM, LVNC, DCM	Cardiac	<b>Absent</b>	ABD	[20,26]
	p.Leu131Pro	Het	DM	Muscular	<b>Absent</b>	ABD	[27]
	p.Met228Thr	Het	HCM (with AF)	Cardiac	<b>Absent</b>	ABD	[21]
	p.Thr247Met	Het	HCM	Cardiac	<b>Absent</b>	ABD	[22]
	p.Leu320Arg	Het	DCM (with VT)	Cardiac	$7.95 \times 10^{-6}$	SR1	[25]
	p.Cys487Arg	Het	DM	Muscular	<b>Absent</b>	SR2	[27]
	p.Thr495Met	Het	HCM	Cardiac	$2.76 \times 10^{-4}$	SR2	[18]
	p.Glu583Ala	Het	HCM	Cardiac	<b>Absent</b>	SR3	[20]
	p.Glu628Gly	Het	HCM	Cardiac	<b>Absent</b>	SR3	[20]
	p.Arg759Thr	Het	HCM	Cardiac	<b>Absent</b>	EF1	[18]

Abbreviations: ABD, actin-binding domain; AF, atrial fibrillation; DCM, dilated cardiomyopathy; DM, distal myopathy; EF, EF hand; HCM, hypertrophic cardiomyopathy; Het, heterozygous; Homo, homozygous; LVNC, left ventricular noncompaction; SR, spectrin like repeat; VT ventricular tachycardia. \* MAF on GnomAD: The Minor Allele Frequency (MAF) was found on The Genome Aggregation Database (gnomAD) version 3.1. All variants with MAF <  $10^{-4}$  are highlighted in bold.

### 2.1.2. $\alpha$ -actinin in Myopathy

Thus far, only one report performing high-throughput sequencing has linked missense variants in *ACTN2* with autosomal dominant distal myopathy [27]. *ACTN2* p.Cys487Arg was identified in three families and *ACTN2* p.Leu131Pro in a further one. The missense



variants in *ACTN2* co-segregated with the disease in affected family members. Clinically, they showed adult-onset asymmetric distal muscle weakness, e.g., atrophy of the Tibialis anterior muscle, which later progressed to proximal limb muscles.

*ACTN3* p.Arg577X is a common nonsense variant (minor allele frequency, MAF, ~20%) previously described to cause  $\alpha$ -actinin-3 deficiency when individuals are homozygous for the X allele [28]. Hogarth et al. found this genotype to be a modifier of the clinical phenotype of Duchenne muscular dystrophy patients [29]. A double knockout mouse model, lacking both dystrophin and  $\alpha$ -actinin 3, showed reduced muscular strength but was protected from stretch-induced damage with age by an increase in oxidative muscle metabolism.

### 2.1.3. Summary

Pathogenic variants in *ACTN2* are rare and do not significantly contribute to cardiomyopathies in numbers. *ACTN2*'s association with HCM is best documented. Until now, there is only one report linking *ACTN2* to myopathy. In light of  $\alpha$ -actinin2's conservation and documented requirement for cardiac integrity [17], it is likely that most missense variants in *ACTN2* are detrimental to its crucial functions and hence not compatible with life. Nevertheless, the few identified ones are of mechanistic interest.

## 2.2. Filamin C

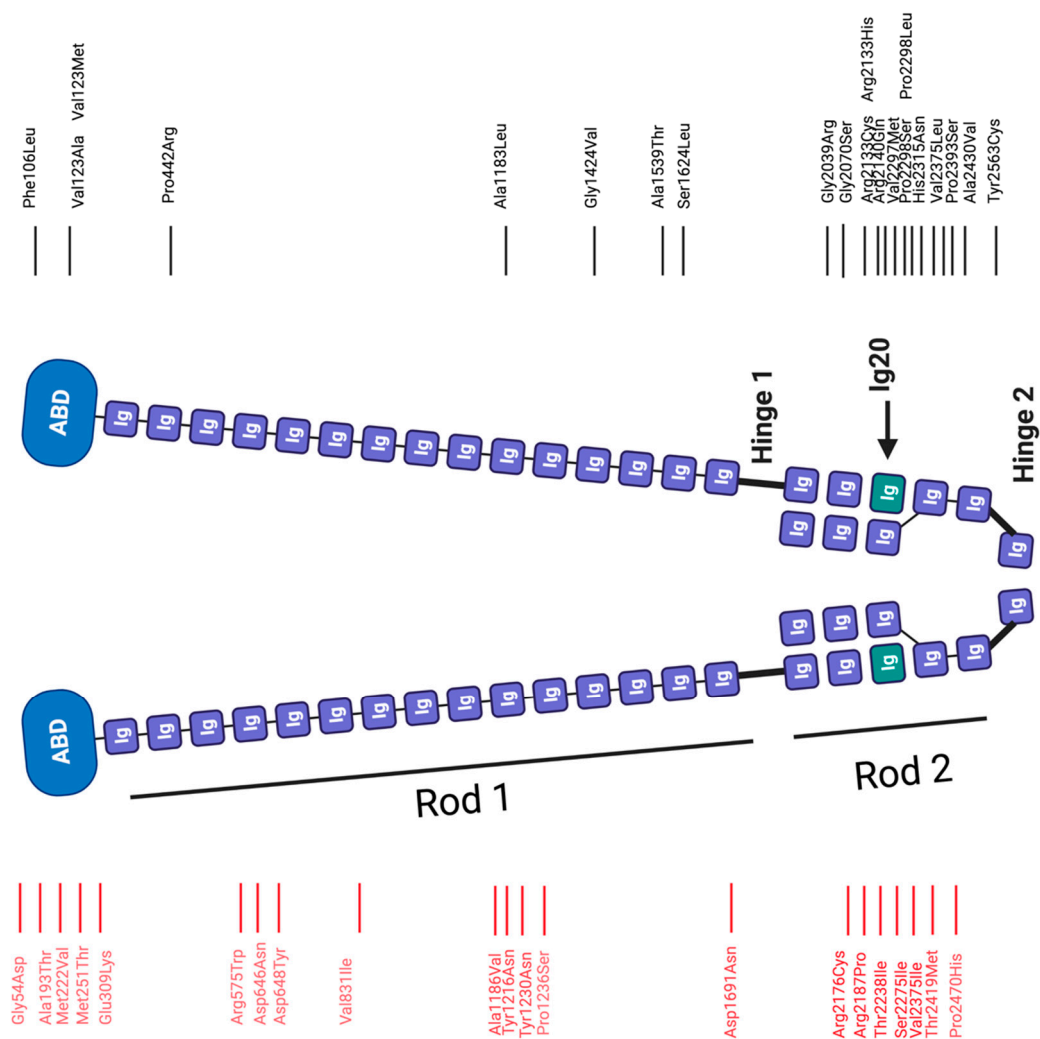
Filamin C is a member of the actin-binding filamin family, consisting of three members: filamin A, B and C. Filamins bind to more than 90 different binding partners and are thought to provide structural stability and to mediate crucial signalling functions. Filamin C is found in the Z-discs and costameres of myotubes and cardiomyocytes and at the intercalated discs of cardiomyocytes, i.e., at load-bearing sites of the cardiac and skeletal muscle cells. It is proposed to play a role in mechanosensing [30–32].

Encoded on chromosome 7q32.1, filamin C consists of an amino-terminal actin binding domain followed by 24 immunoglobulin-like (Ig) domains (Figure 2) [33,34], which are structured into two rod region (Ig1-15 Rod1, Ig16-23 Rod2) [33]. These flexible rod regions regulate the spacing and orientation of F-actin cross-linkages through protein-protein interactions with cytoskeletal, transmembrane and cell signalling proteins [35,36].

Ig20 contains an 82 amino acid insertion unique only to filamin C. It mediates muscle-specific ligand interactions with, e.g., myotilin [37], and is thought to assist in myofibrillogenesis, and presumably plays a role in muscle maintenance [38–40]. These ligand interactions include, but are not necessarily limited to roles such as autophagy control and Z-disc organisation [41,42]. The carboxy-terminal portion of the protein consists of the dimerization domain Ig24, which binds to membrane glycoproteins and allows homodimerization of *FLNC* to form and maintain a network of cross-linking F-actin filaments that link membrane proteins to actin [34,43]. In addition to modulating actin dynamics within the Z-disc, ligand interactions with HSPB7 and calpain 1 around Ig 24 have also been identified [44,45].

In addition, filamin C plays a role in maintaining structural stability of the costameric sarcolemma via interactions with sarcoglycans on the dystrophin-glycoprotein complex [46]. Signalling roles of filamin C includes regulating the morphology and migration of myogenic progenitor cells via binding to cell-matrix adhesion protein migfilin [47]. These processes of the protein can be modulated by phosphorylation events [48,49].

Skeletal muscle functions of filamin C are well established, highlighting its role in chaperone-assisted selective autophagy. However, filamin C's functions within the cardiac muscle are relatively unknown. In part this could be due to early stage lethality in *FLNC* knockout models potentially masking any cardiac implications [50,51]. This has been overcome by an inducible cardiomyocyte-specific *FLNC* knockout mouse model, which displays high rates of lethality along with cardiac defects, including heart dilation, extensive fibrosis and systolic dysfunction [52].



**Figure 2.** Schematic representation of Filamin C shown as a dimer with missense variants in the *FLNC* gene that have been previously reported in individuals with myopathy (red) or cardiomyopathy (black). ABD—actin binding domain, Ig—immuno-globulin like domain. For truncating variants, please refer to Table 2. Created with [BioRender.com](https://www.biorender.com) (accessed on 7 March 2021).

### 2.2.1. Filamin C in Cardiomyopathy

Due to much higher levels of *FLNC* expression in skeletal muscle compared to cardiac muscle (3.5-fold) [53], variants found in this protein have predominantly been associated with neuromuscular conditions such as MFM [43,54–56] (see Section 2.2.2). However, in these cases a cardiac role cannot be excluded since most studies on skeletal muscle-related filaminopathies did not thoroughly characterise cardiac phenotypes (though it is rare for cardiomyopathy patients to have a skeletal phenotype with *FLNC* variants) [54,57,58].

Independent of skeletal muscle involvement, pathogenic *FLNC* variants have been identified in familial HCM, RCM and ACM cases [59,60]. They have also been identified by sequencing in familial DCM, ACM, LVNC, cardiac arrhythmias and arrhythmogenic bileaflet mitral valve prolapse syndrome, with now 101 variants being reported (Table 2) [56,61–63]. Patients carrying *FLNC* variants have a higher risk of sudden cardiac death compared to patients without *FLNC* variants [59,60].

It has been suggested that the subtype of cardiomyopathy is determined by the variant type and site location (Table 2) as seen for other cardiomyopathy-associated genes (e.g., *DES*, *LDB3* [64], *BAG3* [65], *FHL1* [66]) [67]. 95% of *FLNC* variants found in HCM patients are missense variants whilst frameshift, in-frame, nonsense and splice variants resulting in

a loss of function contribute to 81% of DCM-related filaminopathies [68]. Two missense variants have been described in DCM, although one was a case of neonatal DCM resulting in a null variant [69].

In the healthy heart, filamin C localises at the Z-discs of the sarcomeres and at the intercalated discs, where it links actin structures with the cytoskeleton. However, immunohistochemical assessments of cardiomyopathy patients with *FLNC* missense variants reported the loss of filamin C from the intercalated disc [60,67,69]. There is mixed evidence about the role of filamin C aggregation in cardiomyopathy [61,62]. The amino acid change and location of a *FLNC* variant may render it susceptible to unfolding and aggregation or not [59–62,69–72]. Whilst filamin C aggregates have not been observed in DCM patients [61], they have been associated with *FLNC* missense variants (*FLNC* p.Ser1624Leu, p.Ile2160Phe, p.Val2297Met) in patients with HCM, cardiac arrhythmias and RCM [57,58,60,70,73].

Overexpression of a missense *FLNC* variant in the zebrafish model resulted in myofibrillar protein aggregation and of irregular *FLNC* distribution along the Z-disc [67]. Cellular models (rat cardiac myoblasts, H9C2, and mouse C2C12 myoblasts) also exhibited protein aggregation in the presence of HCM-related missense variants, (*FLNC* p.Val123Ala, p.Ala1539Thr, p.Arg2133His and p.Ala2430Val) [59]. In some missense variants (p.Ala1539Thr and p.Arg2133His), actin aggregates were present rather than filamin C aggregates suggesting that these filamin C variants alter folding and result in cytoskeleton rearrangements [59].

The pathogenic RCM-associated variant *FLNC* p.Val2297Met, located in Ig20, was investigated in a stem cell-derived cardiomyocyte model [70]. This variant exhibited reduced fractional shortening and patient-observed relocalisation to suggest it acts as a loss-of-function variant [70].

### 2.2.2. Filamin C in Myopathy

In agreement with the severe skeletal muscle defects observed when *FLNC* is inactivated in mice, *FLNC* has been implicated in human skeletal muscle pathologies [50]. These skeletal myopathies have features of progressive degeneration of the muscle fibres and are characterised by protein aggregation and the presence of rimmed vacuoles in muscle fibres.

In 2005, Vorgerd et al. [54] discovered the first pathogenic *FLNC* variant, *FLNC* p.Trp2710X, in patients with MFM characteristics [54]. This nonsense *FLNC* variant interferes with dimerisation of Ig24, due to the deletion the carboxy-terminal 16 amino acids [74] and has been identified as an ethnically independent variant hotspot [75]. Almost one-third of patients carrying the *FLNC* p.Trp2710X variant also presented with cardiac abnormalities. Patients carrying this variant or the later described deletion variants in Ig7 (*FLNC* p.Val930\_Thr933del, p.Lys899\_Val904del/Val899\_Cys900ins, p.931\_935del [76,77]) exhibited defects in muscle structure and redistribution of filamin C into protein aggregates and Z-disc streaming in animal models [74]. However, in some distal filaminopathy and MFM cases, protein aggregates are absent despite evidence of fiber disintegration leading to muscle weakness. Whether protein aggregation occurs (as in *FLNC* p.Trp2710X [78]) or there is disruption to *FLNC*'s interactions with its binding partners (as in *FLNC* p.Ala193Thr and p.Met251Thr [55]), there is a resultant deficiency of *FLNC* at the Z-disc, which results in Z-disc destabilisation and the consequent fiber disintegration observed in MFM and DM.

Filamin C is a client target of the chaperone-assisted selective autophagy system [79], which identifies and clears misfolded filamin C from the cells and initiates synthesis of new filamin C molecules. It is thought that the variants induce aggregation by disrupting this autophagy clearance mechanism [78,80].

*FLNC* missense and frameshift variants have also been shown to cause distal myopathy—a disease characterised by muscle weakness in the limbs [81]. MFM with lower limb involvement characterised by myofibrillar aggregation was reported in *FLNC* p.Val2375Ile and p.Met222Val [75]. However, the distal and proximal myopathy-related missense



variants in the amino-terminal actin-binding domain of filamin C (*FLNC* p.Ala193Thr, p.Met251Thr) increased filamin C's affinity to F-actin, resulting in F-actin aggregates [55]. As found in the *FLNC* p.Ala193Thr, p.Arg159His and p.Val831Ile variants, filamin C expression is closely linked to neurodegeneration and aberrant filamin C expression is present in neuronal disease such as frontotemporal dementia [56,82].

Guerguelcheva et al. demonstrated reduced muscular *FLNC* transcript levels in the presence of *FLNC* p.Phe1720LeufsX63 via nonsense-mediated mRNA decay [56]. They concluded that *FLNC* haploinsufficiency can result in a specific type of myopathy—distal myopathy with upper limb predominance [56].

### 2.2.3. Summary

Variants found in *FLNC* lead to both skeletal and cardiac myopathies. The genetic location and type of variant can explain the different phenotypes patients present with (Table 2). For instance, *FLNC* variants in the dimerisation region of filamin C leads to the formation of aggregates found in MFM. Although clearly involved in skeletal myopathies, the overlapping phenotypes observed in *FLNC* variants affecting the actin-binding domain clouds the elucidation of any genotype-phenotype relationship [81]. Moreover, it is not current clear why some *FLNC* truncations can cause skeletal muscle defects yet others result in exclusive cardiac phenotypes.

**Table 2.** Variants in the *FLNC* gene that have been previously reported in individuals with myopathy or cardiomyopathy.

Name Gene	Variant	Het or Homo	Type of Disease	Cardiac or Muscular	* MAF on GnomAD	Location	Ref
Filamin C <i>FLNC</i>	p.Tyr7ThrfsX51	Het	DCM	Cardiac	Absent		[83]
	p.Trp34X	Het	ABiMVP	Cardiac	Absent		[84]
	p.Gln39X	Het	DCM	Cardiac	Absent		[71]
	p.Gly54Asp	Het	PM	Muscular	Absent		ABD/CH1 [85]
	p.Leu77ProfsX73	Het	DCM	Cardiac	Absent		[71]
	p.Arg81AlafsX15	Het	DCM	Cardiac	Absent		[61]
	p.Tyr83X	Het	DCM	Cardiac	Absent		[61]
	p.Phe106Leu	Het	DCM	Cardiac	$3.94 \times 10^{-5}$		ABD/CH1 [69]
	p.Glu108X	Het	HCM	Cardiac	Absent		[59]
	p.Val123Ala	Het	HCM	Cardiac	Absent		ABD [59]
	p.Val123Met	Het	DCM	Cardiac	$6.57 \times 10^{-6}$		ABD [68]
	p.Ala193Thr	Het	DM, PM, CNS	Muscular	$6.57 \times 10^{-6}$		ABD/CH1 [55]
	p.Leu194ProfsX52	Het	DCM	Cardiac	Absent		[61]
	p.Gly201ValfsX36	Het	DCM	Cardiac	Absent		[61]
	p.Met222Val	Het	DM, PM, MFM	Muscular	Absent		ABD/CH1 [86]
	p.Gln233X	Het	DCM	Cardiac	Absent		[68]
	p.Glu238ArgfsX14	Het	DCM	Cardiac	Absent		[83]
	p.Met251Thr	Het	DM, PM, CNS	Muscular	Absent		ABD/CH1 [55]
	p.Arg269X	Het	DCM	Cardiac	Absent		[87]
	p.Glu309Lys	Het	IBM	Muscular	$3.94 \times 10^{-5}$		ABD/CH1 [88]
	p.Pro442Arg	Homo	Muscle weakness	Muscular	Absent		ROD1/Ig2 [89]
	p.Arg482X	Het	DCM	Cardiac	Absent		[90]
	p.Val489GlyfsX33	Het	DCM	Cardiac	Absent		[68]
	p.Arg526Gln	Het	IBM	Muscular	$1.58 \times 10^{-3}$		ROD1/Ig3 [88]
	p.Gln572X	Het	DCM	Cardiac	Absent		[61]
	p.Arg575Trp	Het	IBM	Muscular	Absent		ROD1/Ig4 [88]
	p.Tyr630X	Het	DCM	Cardiac	Absent		[71]
	p.Asp646Asn	Het	OM	Muscular	$3.94 \times 10^{-5}$		ROD1/Ig4 [68]
	p.Asp648Tyr	Het	PM, DM, MFM	Muscular	Absent		ROD1/Ig4 [91]
	p.Ala656ProfsX8	Het	ACM/SCD	Cardiac	Absent		[63]
p.Ile683ArgfsX9	Het	DCM	Cardiac	Absent		[83]	
p.Asp693Ala	Het	IBM	Muscular	$2.87 \times 10^{-3}$		ROD1/Ig5 [88]	

Table 2. Cont.

Name Gene	Variant	Het or Homo	Type of Disease	Cardiac or Muscular	* MAF on GnomAD	Location	Ref
Filamin C <i>FLNC</i>	p.Tyr705X	Het	ACM/SCD	Cardiac	Absent		[63]
	p.Gln707X	Het	DCM	Cardiac	Absent		[87]
	p.Lys737SerfsX11	Het	DCM	Cardiac	Absent		[61]
	p.Val831Ile	Het	Pick's Disease	Muscular	$2.63 \times 10^{-5}$	ROD1/Ig6	[82]
	p.Tyr928X	Het	DCM	Cardiac	Absent		[83]
	p.Pro963ArgfsX26	Het	DCM	Cardiac	Absent		[61]
	p.Arg991X	Het	DCM	Cardiac	Absent		[69]
	p.Gln1024X	Het	LVNC	Cardiac	Absent		[92]
	p.Asp1061IlefsX17	Het	DCM	Cardiac	$6.57 \times 10^{-6}$		[68]
	p.Glu1104X	Het	DCM	Cardiac	Absent		[71]
	p.Phe1135AlafsX62	Het	DCM	Cardiac	Absent		[61]
	p.Ala1183Leu	Het	RCM	Cardiac	Absent		ROD1/Ig10 [67]
	p.Ala1186Val	Het	PM, DM, CM	Muscular	Absent		ROD1/Ig10 [93]
	p.Val1198GlyfsX64	Het	DCM	Cardiac	Absent		[83]
	p.Tyr1216Asn	Het	PM, MFM, Car	Muscular	Absent		ROD1/Ig10 [73]
	p.Tyr1230Asn	Het	Myopathy, Car	Muscular	Absent		ROD1/Ig10 [94]
	p.Pro1236Ser	Het	PM, Car	Muscular	Absent		ROD1/Ig10 [95]
	p.Arg1241Cys	Het	IBM	Muscular	$6.94 \times 10^{-3}$		ROD1/Ig10 [88]
	p.Leu1280ProfsX52	Het	DCM	Cardiac	Absent		[68]
	p.Arg1354X	Het	DCM	Cardiac	Absent		[71]
	p.Asn1369LysfsX36	Het	DCM	Cardiac	Absent		[96]
	p.Arg1370X	Het	ACM/SCD	Cardiac	Absent		[63]
	p.Gly1424Val	Het	HCM	Cardiac	$6.57 \times 10^{-6}$		ROD1/Ig12 [83]
	p.Ala1539Thr	Het	HCM	Cardiac	Absent		ROD1/Ig14 [59]
	p.Leu1573X	Het	DCM	Cardiac	Absent		[97]
	p.Ser1624Leu	Het	RCM	Cardiac	Absent		ROD1/Ig14 [60]
	p.Phe1626SerfsX40	Het	DCM/SCD	Cardiac	Absent		[98]
	p.Asp1691Asn	Het	PM, DM, MFM	Muscular	$5.25 \times 10^{-5}$		ROD1/Ig15 [91]
	p.Gly1760Ser	Het	PM	Muscular	$1.12 \times 10^{-4}$		ROD2/Ig16 [95]
	p.Gly1800X	Het	DCM	Cardiac	Absent		[61]
	p.Tyr1840X	Het	DCM	Cardiac	Absent		[99]
	p.Gly1891ValfsX62	Het	DCM	Cardiac	Absent		[87]
	p.Gly2039Arg	Het	HCM	Cardiac	$6.57 \times 10^{-6}$		ROD2/Ig19 [83]
	p.Gly2070Ser	Het	DCM	Cardiac	Absent		ROD2/Ig19 [61]
	p.Ser2077ArgfsX50	Het	DCM	Cardiac	Absent		[96]
	p.Pro2081LeufsX2	Het	DCM	Cardiac	Absent		[61]
	p.Ile2086GlnfsX3	Het	DCM	Cardiac	Absent		[68]
	p.Arg2133Cys	Het	HCM	Cardiac	Absent		ROD2/Intradomain [59]
	p.Arg2133His	Het	HCM	Cardiac	$6.57 \times 10^{-6}$		ROD2/Intradomain [59]
	p.Arg2140Gln	Het	HCM	Cardiac	$6.61 \times 10^{-6}$		ROD2/Intradomain [72]
	p.Ile2160Phe	Het	RCM	Cardiac	Absent		ROD2/Intradomain [60]
	p.Arg2176Cys	Het	IBM	Muscular	Absent		ROD2/Intradomain [100]
p.Arg2187Pro	Het	PM, Car	Muscular	Absent		ROD2/Intradomain [68]	
p.Glu2189X	Het	ARVC	Cardiac	Absent		[101]	
p.Thr2238Ile	Het	Myopathy, CM	Muscular	Absent		ROD2/Intradomain [68]	
p.Glu2270Lys	Het	OM	Muscular	$6.40 \times 10^{-4}$		ROD2/Ig20 [68]	
p.Ser2275Ile	Het	OM	Muscular	Absent		ROD2/Ig20 [68]	
p.Val2290ArgfsX23	Het	DCM	Cardiac	Absent		[68]	
p.Val2297Met	Het	RCM	Cardiac	Absent		ROD2/Ig20 [70]	
p.Pro2298Leu	Het	RCM	Cardiac	$6.57 \times 10^{-6}$		ROD2/Ig20 [102]	
p.Pro2298Ser	Het	HCM	Cardiac	Absent		ROD2/Ig20 [72]	
p.Pro2301Ala	Het	HCM	Cardiac	Absent		ROD2/Ig20 [72]	
p.Gln2303X	Het	DCM	Cardiac	Absent		[68]	
p.His2315Asn	Het	HCM	Cardiac	Absent		ROD2/Ig21 [59]	
p.Arg2326X	Het	DCM	Cardiac	Absent		[61]	
p.Val2331ArgfsX25	Het	DCM	Cardiac	Absent		[71]	

Table 2. Cont.

Name Gene	Variant	Het or Homo	Type of Disease	Cardiac or Muscular	* MAF on GnomAD	Location	Ref
Filamin C <i>FLNC</i>	p.Gly2345Glu	Het	Congenital HD	Cardiac	Absent	ROD2/Ig21	[103]
	p.Arg2364His	Het	IBM	Muscular	$1.65 \times 10^{-3}$	ROD2/Ig21	[68]
	p.Arg2364His	Het	IBM	Muscular	$1.65 \times 10^{-3}$	ROD2/Ig21	[88]
	p.Tyr2373CysfsX7	Het	DCM	Cardiac	Absent		[83]
	p.Val2375Leu	Het	HCM	Cardiac	Absent	ROD2/Ig21	[83]
	p.Val2375Ile	Het	PM, DM, MFM	Muscular	$2.63 \times 10^{-5}$	ROD2/Ig21	[104]
	p.Pro2393Ser	Het	LVNC	Cardiac	Absent	ROD2/Ig21	[68]
	p.Thr2419Met	Het	PM, MFM, Car, CNS	Muscular	$6.57 \times 10^{-5}$	ROD2/Ig22	[105]
	p.Ala2430Val	Het	HCM	Cardiac	$9.20 \times 10^{-5}$	ROD2/Ig-like 22	[59]
	p.Pro2470His	Het	PM,Car	Muscular	Absent	ROD2/Ig22	[106]
	p.Gln2549X	Het	DCM	Cardiac	Absent		[83]
	p.Cys2555X	Het	DCM	Cardiac	Absent		[83]
	p.Tyr2563Cys	Het	RCM	Cardiac	Absent	ROD2/Ig23	[102]
	p.Asp2703ThrfsX69	Het	DCM	Cardiac	$6.58 \times 10^{-6}$		[61]
p.Trp2710X	Het	PM, DM, MFM	Muscular	Absent	ROD2/Ig24	[54]	

Abbreviations: ABD, actin-binding domain; ABiMVP, arrhythmogenic bileaflet mitral valve prolapse; ACM, arrhythmogenic cardiomyopathy; Car, cardiac involvement; CM, congenital myopathy; CNS, central nervous system involvement; DCM, dilated cardiomyopathy; DM, distal myopathy; HCM, hypertrophic cardiomyopathy; HD, heart disease; Het, heterozygous; Homo, homozygous; IBM, inclusion body myositis; MFM, myofibrillar myopathy; OM, other nonspecified myopathy; PM, proximal myopathy; RCM, restrictive cardiomyopathy; SCD, sudden cardiac death. \* MAF on GnomAD: The Minor Allele Frequency (MAF) was found on The Genome Aggregation Database (gnomAD) version 3.1. All variants with  $MAF < 10^{-4}$  are highlighted in bold.

### 2.3. Myopalladin

Myopalladin is a protein found at the sarcomeric Z-discs and I-bands in both cardiac and skeletal muscle cells [107]. Myopalladin contains five Ig domains that are separated by six interdomain insertions (IS) (Figure 3) and IS3 contains a proline-rich region [108]. Within its carboxy-terminus, myopalladin contains binding sites for  $\alpha$ -actinin and nebulin in skeletal muscle, and nebulin in cardiac muscle [70]. These interactions help to tether actin and titin to the Z-disc and therefore have a role in signalling when the muscle is under stress. Myopalladin also contains an amino-terminal domain that binds to the cardiac ankyrin repeat protein (CARP), which is involved in controlling gene expression in muscle [108]. *MYPN*, the gene coding for myopalladin, is located on 10q21.3.

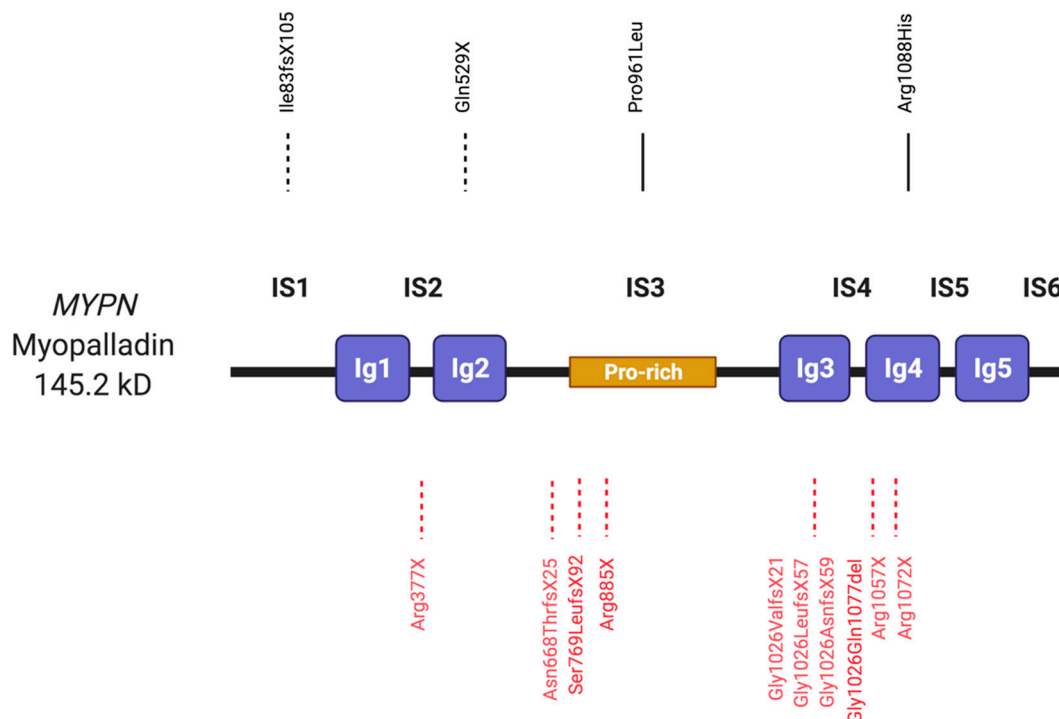
#### 2.3.1. Myopalladin in Cardiomyopathy

A number of missense variants in *MYPN* have been reported in HCM, DCM and RCM patients in several studies [106,108–111] (Table 3). However, only two of them would pass today's scrutiny based on frequency in normal cohorts ( $MAF < 10^{-4}$ ). Only these are discussed here:

*MYPN* p.Pro961Leu was identified in a DCM patient [109]. This variant is located in the third Ig domain, which mediates binding to  $\alpha$ -actinin. Sarcomeric disorganisation was observed in an endomyocardial biopsy of the patient. *MYPN* p.Arg1088His variant was identified in a patient with familial DCM and found to co-segregate with the disease: three further family members were also heterozygous for the variant and clinically affected [111]. Immunofluorescence labelling of explanted heart tissue carrying the *MYPN* p.Arg1088His variant found that the dilated left ventricle of this patient had reduced myopalladin localisation at the Z-disc. In contrast, localisation appeared normal in the right, unaffected ventricle of the patient.

Furthermore, two truncating *MYPN* variants have been associated with cardiomyopathies. After screening and sequencing the coding region of *MYPN* in 114 patients with DCM, an insertion-deletion (*MYPN* p.Ile83fsX105) was identified in a familial case of DCM, co-segregating with disease [111]. Five family members were found to be heterozygous

for the variant; two were clinically affected, two were healthy and one had an unknown clinical status.



**Figure 3.** Schematic representation of Myopalladin shown as a monomer with variants in the *MYPN* gene that have been previously reported in individuals with myopathy (red) or cardiomyopathy (black). Solid lines represent missense variants and dashed lines are truncations. Ig—immuno-globulin like domain, IS—interdomain insertion, Pro-rich—Proline-rich. Created with [BioRender.com](https://www.biorender.com) (accessed on 7 March 2021).

The nonsense mutation *MYPN* p.Gln529X was identified in two siblings who presented with familial RCM [107]. It was predicted to truncate the three Ig repeats and the nebullette and  $\alpha$ -actinin binding domains of myopalladin. Transmission electron microscopy in the siblings explanted hearts after they both received heart transplants, displayed myofibrillar disarray and degeneration. Knock-in mice carrying either a heterozygous or homozygous form of *MYPN* p.Gln526X, homologous to the human *MYPN* p.Gln529X variant, reflected aspects of RCM when investigated by echocardiography, cardiac magnetic resonance imaging and morphohistology [112]. Moreover, gene expression and molecular mechanism studies found that the truncated myopalladin protein translocated to the nucleus and persisted in the heterozygous mice. Western blot analysis showed reduced levels of *Carp*, *Erk1/2*, *Smad2* and *Akt* but increased levels of muscle LIM Protein, *desmin*, *periostin* and *osteopontin*. Altered transcription of these genes appeared to facilitate fibrosis and a reduced hypertrophic response in hearts of the heterozygous mice.

### 2.3.2. Myopalladin in Myopathy

Using a combination of homozygosity mapping and whole exome sequencing, four individuals from four separate families (from 54 screened families) were identified to have biallelic loss-of-function variants in *MYPN* [113]. All patients presented with mild, childhood-to-adult-onset NM; they displayed progressive muscle weakness and walking difficulties when they reached their 40's. Individual 1 possessed one *MYPN* variant (p.Asn668ThrfsX25). Individual 2 had four *MYPN* variants (p.Gly1026ValfsX21, p.Gly1026AsnfsX59, p.Gly1026LeufsX57, p.Gly1026\_Gln1077del) whilst the third individual had one (p.Arg377X). The fourth individual had two variants (p.Arg1057X) and (p.Arg1072X). Immunostaining and Western blot analysis of muscle samples or myotubes

from the patients showed a complete absence of myopalladin in these individuals and supports the variants being loss-of-function.

In a separate study, exome sequencing identified recessive homozygous variants of *MYPN* in three individuals from two families [114]. Family 1 included two siblings who had a nonsense *MYPN* p.Arg885X variant, whilst family 2 had a *MYPN* c.3158 + 1G > A variant affecting exon 16 at the essential donor splice site. All patients presented with slow, progressive congenital cap myopathy. Western blot analysis of muscle extracts from patient 1 revealed full length myopalladin to be significantly decreased compared to control samples. Light and electron microscopy detected myopalladin and  $\alpha$ -actinin-positive caps and rods in the muscle of the patients.

Merlini et al. investigated a family where two members had varying degrees of congenital to adult-onset muscle weakness [115]. Magnetic resonance imaging (MRI) and late gadolinium enhancement revealed cardiac involvement in one of the two siblings, which was attributed to interstitial fibrosis. Neither of them appeared to have features of cap or NM. Analysis using whole exome sequencing discovered a homologous loss-of-function single nucleotide deletion (*MYPN* p.Ser769LeufsTer92) within exon 11 of *MYPN* in the two affected family members. The unaffected parents were heterozygous for the variant whilst an unaffected sibling lacked it completely. Complete absence of full-length myopalladin and the 114kd isoform 2 was documented on Western blotting, in agreement with loss of myopalladin staining from the Z-discs of skeletal muscle on immunofluorescence. Electron microscopy showed that the patient had disrupted and fragmented Z-discs.

### 2.3.3. Summary

*MYPN* variants have been reported in HCM, DCM, and RCM, but only two missense and two nonsense variants could be considered clearly pathogenic (Table 3). The *MYPN* p.Gln529X variant was found to cosegregate with RCM [107] and is supported by animal studies. In contrast to cardiomyopathy-associated heterozygous *MYPN* variants, homozygous or bi-allelic truncating variants in *MYPN* are linked to myopathy, particularly to cap myopathy and NM. Investigation of biopsy material suggested these are loss-of-function variants, however exact disease pathways remain to be elucidated.

**Table 3.** Variants in the *MYPN* gene that have been previously reported in individuals with myopathy or cardiomyopathy.

Name Gene	Variant	Het or Homo	Type of Disease	Cardiac or Muscular	* MAF on GnomAD	Location	Ref
Myopalladin <i>MYPN</i>	p.Tyr20Cys	Het	HCM, DCM	Cardiac	$1.27 \times 10^{-3}$	IS1	[107]
	p.Ile83fsX105	Het	DCM	Cardiac	Absent	IS1	[111]
	p.Arg377X	Homo	NM	Muscular	Absent		[113]
	p.Gln529X	Het	RCM	Cardiac	$4.00 \times 10^{-6}$	Ig2	[107]
	p.Asn668ThrfsX25	Homo	NM	Muscular	Absent		[113]
	p.Ser769LeufsTer92	Homo	Congenital to adult-onset myopathy	Muscular	Absent		[115]
	p.Arg885X	Homo	Congenital cap myopathy	Muscular	$6.58 \times 10^{-6}$		[114]
	p.Arg955Trp	Het	DCM	Cardiac	$4.71 \times 10^{-4}$	Ig3	[109]
	p.Pro961Leu	Het	DCM	Cardiac	Absent	Ig3	[109]
	p.Gly1026ValfsX21	Homo	NM	Muscular	Absent		[113]
	p.Gly1026AsnfsX59	Homo	NM	Muscular	Absent		[113]
	p.Gly1026LeufsX57	Homo	NM	Muscular	Absent		[113]
	p.Gly1026_Gln1077del	Homo	NM	Muscular	Absent		[113]
	p.Arg1057X	Het	NM	Muscular	Absent		[113]
	p.Arg1072X	Het	NM	Muscular	Absent		[113]
	p.Arg1088His	Het	DCM	Cardiac	$3.98 \times 10^{-6}$	Ig4	[111]
	p.Pro1112Leu	Het	HCM, DCM	Cardiac	$3.06 \times 10^{-3}$	Ig4	[111,116]
	p.Val1195Met	Het	DCM	Cardiac	$3.05 \times 10^{-4}$	Ig5	[111]
c.3158 + 1G > A	Homo	Congenital cap myopathy	Muscular	$3.98 \times 10^{-6}$		[114]	

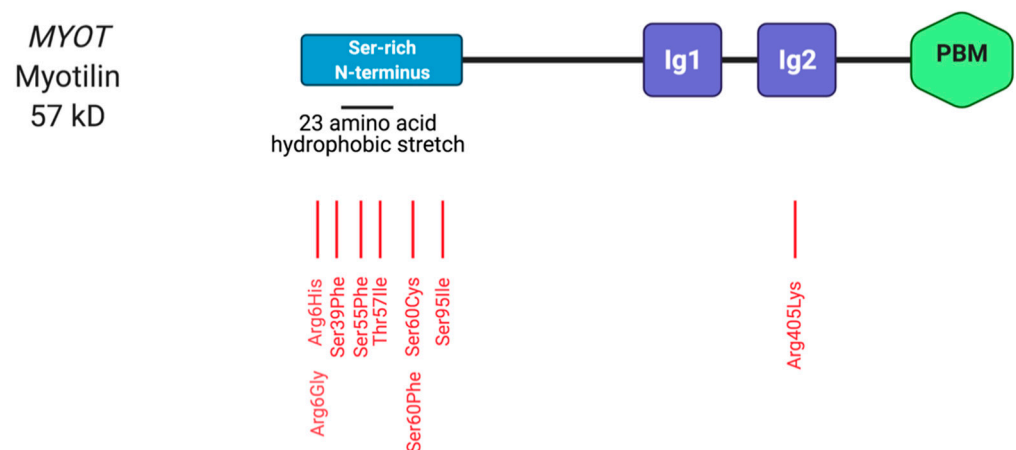
Abbreviations: DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; Het, heterozygous; Homo, homozygous; NM, nemaline myopathy; RCM, restrictive cardiomyopathy. \* MAF on GnomAD: The Minor Allele Frequency (MAF) was found on The Genome Aggregation Database (gnomAD) version 3.1. All variants with  $MAF < 10^{-4}$  are highlighted in bold.



## 2.4. Myotilin

Myotilin is a Z-disc localised thin-filament associated protein found in skeletal muscle, and to a lesser extent in cardiac tissue [117]. Along with palladin and myopalladin, myotilin belongs to a family of the Ig domain-containing actin-binding proteins. This family of sarcomeric proteins are instrumental in sarcomere assembly and maintaining regular muscle structure by binding to  $\alpha$ -actinin and filamins to cross-link actin [108,118].

The 57 kDa protein is composed of 10 exons encoding 498 amino acids and is mapped to chromosome 5q31 [118,119]. The amino-terminus contains a serine-rich region and a 23 amino acid hydrophobic stretch region, while the carboxy-terminus contains a short non-structured tail [118]. The hydrophobic stretch region mediates the localisation of myotilin to the sarcolemmal membrane (Figure 4) [120]. Myotilin is also composed of two Ig domains, which are required for homodimerisation [118]. Myotilin engages in molecular interactions with actin monomers and Z-disc proteins through its Ig domains [121], it binds to  $\alpha$ -actinin, filamins, FATZ/myozenin/calsarcin and Engima proteins (e.g., ZASP) [118,121–124]. Myotilin acts as a stabiliser and anchors actin filaments at the Z-disc, through a myotilin/actin/ $\alpha$ -actinin ternary complex, and thus is fundamentally important for the myofibrillar organisation of the Z-disc and may have roles in sarcomerogenesis [124]. Myotilin also interacts with the MuRF ubiquitin ligases and signalling cascade regulators [125]. Despite these crucial functions, a global inactivation of the *Myot* gene in mice showed no overt phenotype [126].



**Figure 4.** Schematic representation of Myotilin shown as a monomer with variants in the *MYOT* gene that have been previously reported in individuals with myopathy (red). Solid lines represent missense variants. Ig—immuno-globulin like domain, Ser-rich—Serine-rich, PBM—PDZ-binding motif. Created with [BioRender.com](https://www.biorender.com) (accessed on 7 March 2021).

### 2.4.1. Myotilin in Myopathy

*MYOT* variants mainly manifest in myopathies such as LGMD, MFM, distal myopathy and spheroid-body myopathy [127–131] (Table 4). Such diseases caused by pathogenic variants in *MYOT* are present in 10% of MFM patients and have been termed “myotilinopathies” [132]. Myotilinopathy variants usually result in structural changes of the Z-disc and the formation of polymorphous aggregates [122,127,133,134].

Almost all *MYOT* variants have been located in exon 2 in the amino-terminal region, an intrinsically unstructured region [135]. Two separate studies described *MYOT* missense variants affecting the same residue (p.Arg6Gly and p.Arg6His), both causing severe forms of skeletal myopathy, suggesting this residue is a mutational hotspot [136,137]. Further, patients carrying *MYOT* p.Arg6Gly as homozygous variant had a more severe phenotype, suggesting zygosity differences could play a role [137]. Similar to the *MYOT* p.Arg6Gly variant, despite being a dominant-negative variant, a more severe phenotype was reported in patients carrying the homozygous *MYOT* p.Ser60Phe mutation [138]. The only variant

discovered outside of exon 2 is *MYOT* p.Arg405Leu, which is found in exon 9 and affects the second Ig domain and causes LGMD1A [130]. It was suggested that it inhibits myotilin homodimerization thus results in reduced interaction with  $\alpha$ -actinin [130].

Z-disc streaming is reported in myotilinopathies along with the accumulation of dense myotilin and desmin-containing protein aggregates. These aggregates are likely caused by a reduction in protein turnover and myotilin degradation via the ubiquitin proteasome system [139]. However, due to the variants being located away from the binding site regions for  $\alpha$ -actinin and filamin C, Kostan et al. found no evidence of altered actin binding—a causative factor for protein aggregation. Instead, these missense variants may affect the dynamics of Z-disc protein interactions and cause disruption to the maintenance and structural integrity of the Z-discs. Therefore, it was proposed that aggregation may be promoted by pathogenic myotilin variants exposing a hydrophobic cluster since most variants are substitutions from a polar to a hydrophobic residue [122,139,140].

Proteomics analysis on patients with *MYOT* p.Leu36Glu, p.Ser60Cys and p.Ser60Phe found that the Z-disc proteins myotilin, desmin, filamin C and other interacting partners (BAG3, HSPB8, p62) accounted for more than 70% of over-represented proteins in the aggregates indicating that myotilin-related MFM aggregates are structured formations from the Z-disc [141]. Interestingly, filamin C and desmin were more abundant than myotilin.

LGMDA1 patients exhibit disorganised sarcomere striations and Z-disc streaming [122]. This effect is also observed in disease caused by pathogenic variants in other Z-disc proteins, such as telethonin.

Since myotilin aggregation is common in myotilinopathy (like other MFMs), mouse models have been used to better understand the pathological effect. In models overexpressing wildtype myotilin, myofibrillar aggregation was reported along with severe muscle degeneration as found in LGMD1A [126,142]. However, investigating myotilin variants in transgenic mice has proved difficult as many cannot reproduce an in vivo phenotype [143]. Furthermore, zebrafish and murine in vivo electroporation methods have been utilised to characterise the function of myotilin variants [143,144].

#### 2.4.2. Summary

Although the exact pathomechanisms behind myotilinopathies are yet to be completely elucidated, missense variants in *MYOT*, which typically cause MFM via an accumulation of protein aggregates may be due to an impaired protein degradation system linked to Z-disc structural abnormalities [145]. Myotilinopathies have a clear role in myopathy, however patients often present additionally with a cardiac phenotype. For instance, the *MYOT* p.Ser60Phe variant has been described in a case of myopathy with low ejection fraction and cardiomyopathy, while other patients carrying the variant displayed with cardiomyopathy, left bundle branch block and congestive heart failure [127]. Despite this, no isolated cardiomyopathy cases have been attributed to variants in *MYOT*.

**Table 4.** Variants in the *MYOT* gene that have been previously reported in individuals with myopathy or cardiomyopathy.

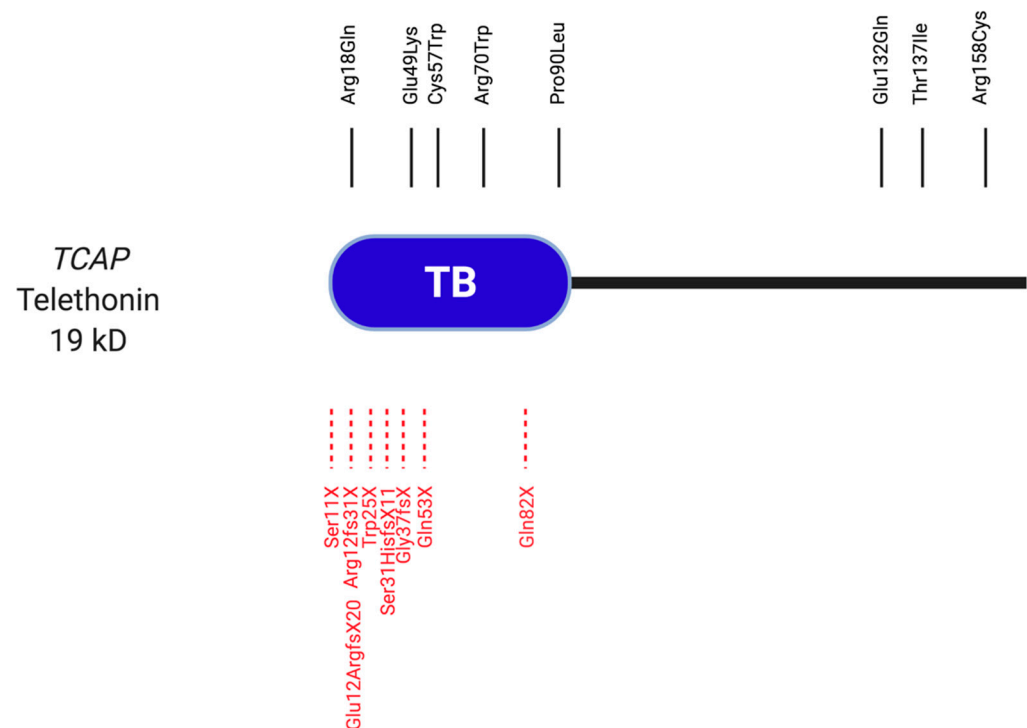
Name Gene	Variant	Het or Homo	Type of Disease	Cardiac or Muscular	* MAF on GnomAD	Location	Ref
Myotilin <i>MYOT</i>	p.Arg6His	Het	LGMD1A	Muscular	$2.63 \times 10^{-5}$	Serine rich N-terminus	[136]
	p.Arg6Gly	Homo	Severe MFM	Muscular	Absent	Serine rich N-terminus	[137]
	p.Ser39Phe	Het	Spheroid body myopathy	Muscular	Absent	Serine rich N-terminus	[133]
	p.Ser55Phe	Het	LGMD1A	Muscular	Absent	Serine rich N-terminus	[120]
	p.Thr57Ile	Het	LGMD1A	Muscular	Absent	Serine rich N-terminus	[122]
	p.Ser60Cys	Het	LGMD1A	Muscular	Absent	Serine rich N-terminus	[127]
	p.Ser60Phe	Het	LGMD1A	Muscular	$1.31 \times 10^{-5}$	Serine rich N-terminus	[127]
	p.Ser95Ile	Het	LGMD1A	Muscular	Absent	Serine rich N-terminus	[127]
	p.Arg405Lys	Het	LGMD1A	Muscular	Absent	Ig2	[130]

Abbreviations: Het, heterozygous; Homo, homozygous; LGMD, limb-girdle muscular dystrophies; MFM, myofibrillar myopathy; N-terminus, amino-terminus. \* MAF on GnomAD: The Minor Allele Frequency (MAF) was found on The Genome Aggregation Database (gnomAD) version 3.1. All variants with MAF  $< 10^{-4}$  are highlighted in bold.

## 2.5. Telethonin

Telethonin (also called TCAP) is found in both human heart and skeletal muscle and is especially abundant in skeletal muscle [146]. It is one of the Z-disc proteins that help to form the stable sarcomere structure and is also required for sarcomerogenesis [147].

Telethonin has been found to interact with titin and FATZ in the sarcomere. The first 140 amino acids of telethonin interact with the Ig domains Z1 and Z2 in the amino-terminal repeat region of titin [148] (Figure 5), leading to a tertiary complex of telethonin with titin and muscle LIM protein; this complex was suggested as a mechanical stretch sensor [149]. Additionally, telethonin interacts with calsarcin-1, which has a role in tethering the phosphatase calcineurin to the Z-disc [150]. Telethonin is encoded by the gene *TCAP*, which is located on chromosome 17q12.



**Figure 5.** Schematic representation of Telethonin shown as a monomer with variants in the *TCAP* gene that have been previously reported in individuals with myopathy (red) or cardiomyopathy (black). Solid lines represent missense variants and dashed lines are truncations. TB—titin binding domain. Created with [BioRender.com](https://www.biorender.com) (accessed on 7 March 2021).

*Tcap* knock-out mice do not display a cardiac phenotype under normal conditions [149]. However, upon biomechanical stress, telethonin deficiency leads to enhanced p53 levels and promotes apoptosis and cell death, suggesting initiation of heart failure development [149].

### 2.5.1. Telethonin in Cardiomyopathy

Hayashi et al. analysed *TCAP* in 346 patients with HCM and 136 patients with DCM and identified the *TCAP* variant p.Thr137Ile in a HCM patient, and *TCAP* p.Glu132Gln in a patient with DCM [150]. Binding assays suggested that the HCM-associated variant reduced the telethonin's binding to Z-disc proteins calsarcin-1 and titin, while the DCM variant additionally impacted binding to muscle LIM protein. However, also *TCAP* p.Arg153His showed alterations in binding properties, despite the fact that it is relatively common to be pathogenic (Table 5).

Two further *TCAP* variants (p.Arg70Trp and p.Pro90Leu) were discovered in a cohort of 389 HCM patients [151]. They also identified *TCAP* p.Glu13del, which is now assumed not to be pathogenic due to its frequency in normal cohorts [152,153].

Moreover, a novel *TCAP* p.Cys57Trp variant was identified in a small family who presented with HCM [154]. The variant co-segregated with disease in affected family members and 11 common HCM disease genes were excluded. The variant was found to have a very low population frequency and was highly conserved across different species. Seven bioinformatic tools suggested that the variant was likely to be pathogenic and therefore damaging [154]. The variant was located in the muscle LIM Protein interacting domain.

Bidirectional sequencing of six genes from samples of 313 patients with idiopathic DCM, revealed three *TCAP* variants from 3 different probands [155]. Two of these variants were found to have a possible disease association (*TCAP* p.Arg18Gln and p.Glu49Lys).

Hirtle-Lewis et al. interrogated *TCAP* by Sanger sequencing to analyse DNA from 96 patients with DCM and 184 control samples [156]. *TCAP* p.Arg158Cys was thought to be of clinical significance and due to the damaging nature of the change.

13 unrelated patients, who underwent heart transplantation due to end-stage DCM, were screened for genetic variants in 15 candidate genes using PCR and next-generation sequencing (NGS). *TCAP* p.Glu105Gln was identified in the cohort, but its frequency in normal cohorts makes a pathogenic role doubtful [157].

In summary, a small number of missense variants in *TCAP* have been associated with either DCM or HCM, but not all variants initially identified would withstand more recent scrutiny based on their frequencies in normal cohort (e.g., *TCAP* p. Glu13del). Altered binding properties to interactors such as muscle LIM Protein, titin and caldesmon-1 have been suggested as modes of action, but all conclusions so far are based on in vitro experiments only.

### 2.5.2. Telethonin in Myopathy

Two *TCAP* variants were identified in patients from three families who presented with LGMD 2G. Their symptoms were variable and included distal muscle weakness in the legs, difficulty walking and severe calf hypertrophy [158]. One truncating variant *TCAP* p.Gln53X was found to be homozygous in two of the families, but heterozygous in another. A second variant was found to segregate with the third affected family and led to the deletion of two guanine nucleotides (in the genomic sequence these were nt 637–640). This variant was predicted to lead to a frameshift mutation resulting in a premature stop codon (*TCAP* p.Gly37fsX).

The homozygous variant *TCAP* p.Trp25X.IA was identified in a 27-year-old woman who presented with LGMD 2G [159]. Analysis of a muscle biopsy using immunohistochemistry (IHC) and Western blotting, showed a complete lack of telethonin in the skeletal muscle in the patient.

A cohort study of 300 patients with autosomal recessive LGMD identified eight patients with LGMD 2G, who either had no or reduced telethonin protein levels [160]. Clinical features were predominantly progressive proximo-distal muscle weakness, scapular winging and calf hypertrophy. Genetic analysis in the eight patients revealed a novel *TCAP* variant *TCAP* p.Ser11X and the previously reported one *TCAP* p.Arg12fs31X [161].

Barresi et al. described a male 49-year-old who presented with progressive LGMD. IHC using an antibody directed at the aa 58–167 (carboxy-terminal end) of telethonin, revealed no signal, and sequencing identified the homozygous *TCAP* p.Gln82X variant [162]. However, when an antibody against the full-length protein was used, the truncated telethonin protein was detected by Western blotting, although the signal appeared weaker than for the control. A muscle biopsy from the patient revealed co-localisation of the truncated protein with filamin C at the Z-discs, suggesting the truncated telethonin variant can still be incorporated into the sarcomere.

Another case study described a 35-year-old female Turkish patient with LGMD 2G [163]. A muscle biopsy revealed myopathic muscle with atrophy of the fibres and complete absence of telethonin in Western blotting. Using NGS, the novel homozygous frame-shift variant in *TCAP* (p.Ser31HisfsX11) was identified, resulting in truncation

and/or nonsense mediated decay of the telethonin protein [163]. The same variant was detected by NGS in a 37-year-old Greek female LGMD 2G patient [164]. Western blot analysis revealed a complete lack of telethonin. Single nucleotide polymorphism analysis of a 10 Mb genomic region containing the *TCAP* gene, found a shared homozygous haplotype of the Greek and Turkish patients [164]. This suggests the possibility of a founder effect for that region of the Mediterranean for the *TCAP* variant described.

18 individuals from a minority of Bulgarian Muslims were found to be homozygous for *TCAP* p.Trp25X [165]. The authors looked at 100 new-borns in the area and found the heterozygous carrier rate of p.Trp25X to be 2%, meaning the expected homozygous patient number would be 1 in 2500 births in the region. Clinical symptoms included weakness in proximal muscle in the legs, supported by an MRI, which also progressed to the upper limbs. Similar to the case study presented by [159], Western blot analysis showed complete absence of the telethonin protein.

Yee et al. [166] identified one novel splice variant (*TCAP* c.110 + 5G > A) and one previously reported variant (*TCAP* p.Glu12ArgfsX20) [167] in a Chinese cohort. The proband of one family was homozygous for the splice variant c.110 + 5G > A, whilst three members of a second family were heterozygous for both variants. The proband from a third family was found to be homozygous for *TCAP* p.Glu12ArgfsX20. Clinical features, muscle MRI and muscle histopathology were used to support the diagnosis and indicate a genotype-phenotype relationship.

### 2.5.3. Summary

Several studies report a link between variations in *TCAP* and HCM and DCM. Some of these variants affect conserved residues and either have a low allele frequency or do not appear in GnomAD. They are therefore considered as (potentially) pathogenic. Further co-segregation and functional studies are needed to support these findings and understand the mechanisms of pathogenicity. However, many of the initially reported variants have an allele frequency higher than  $10^{-4}$  and hence appear relatively frequently in the general population for disease causing variants (Table 5).

A number of studies indicate a causative role of homozygous *TCAP* variants resulting in premature stop codons in LGMD 2G. Analyses of skeletal muscle biopsies suggest lack of functional telethonin protein [159–168]. However, findings depend on the epitopes of the antibodies used, so it is currently not clear how much nonsense mediate decay contributes to the disease mechanism. The variants described have low allele frequencies or are absent in GnomAD, suggesting they do contribute to pathogenicity (Table 5). Mechanistic studies on *TCAP* knockout mice support a role of *TCAP* in LGMD [168].

### 2.6. ZASP/Cypher

ZASP is a member of the enigma family, which are structural Z-disc proteins with signalling potential. The murine homologue of ZASP is called Cypher, when discussing literature pertaining to both human and murine isoforms, the name ZASP will be used. ZASP is a cytoskeletal protein, which is highly important in maintaining Z-disc structural and muscular integrity by forming multiprotein complexes binding to  $\alpha$ -actinin and could be involved in signalling roles via interactions with protein kinase C [169].

ZASP is encoded by the *LDB3* gene on chromosome 10q22.3-10q23.2 and consists of 16 exons encoding six distinct isoforms, which are highly expressed in human striated muscles [170]. There is evidence of alternative splicing: ZASP1, ZASP5 and ZASP8 possess exon 4 and are predominantly expressed in cardiac tissue, whereas the ZASP isoforms that have exon 6 (ZASP2 and ZASP6) are more highly expressed in skeletal muscle [171].



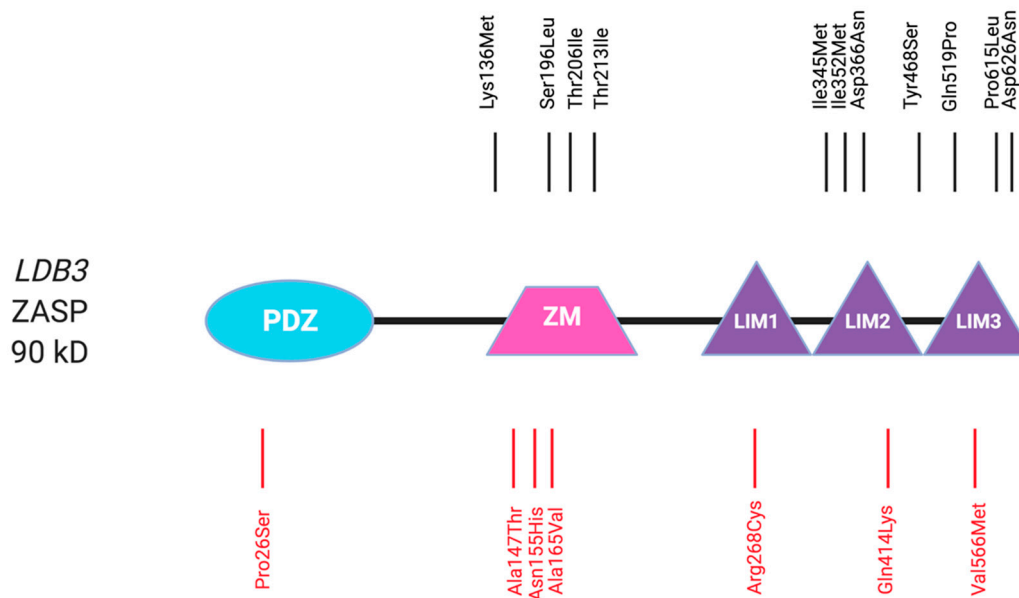
**Table 5.** Variants in the *TCAP* gene that have been previously reported in individuals with myopathy or cardiomyopathy.

Name	Variant	Het or Homo	Type of Disease	Cardiac or Muscular	* MAF on GnomAD	Location	Ref
Telethonin <i>TCAP</i>	p.Ser11X	Homo	LGMD2G	Muscular	$6.57 \times 10^{-6}$		[160]
	p.Arg12fs31X	Homo	LGMD2G	Muscular	Absent		[161]
	p.Glu12ArgfsX20	Homo	LGMD2G	Muscular	$1.31 \times 10^{-5}$		[166]
	p.Glu13del	Het	HCM	Cardiac	$9.99 \times 10^{-4}$	Titin-binding	[151]
	p.Arg18Gln	Het	DCM	Cardiac	$2.39 \times 10^{-5}$	Titin-binding	[155]
	p.Trp25X	Homo	LGMD2G	Muscular	$6.57 \times 10^{-6}$		[159]
	p.Ser31HisfsX11	Homo	LGMD2G	Muscular	Absent		[163]
	p.Gly37fsX	Het	LGMD2G	Muscular	Absent		[158]
	p.Glu49LyS	Het	DCM	Cardiac	$3.70 \times 10^{-5}$	Titin-binding	[155]
	p.Gln53X	Homo/Het	LGMD2G	Muscular	$1.31 \times 10^{-5}$		[158]
	p.Cys57Trp	Het	HCM	Cardiac	$2.20 \times 10^{-5}$	Titin-binding	[154]
	p.Arg70Trp	Het	HCM	Cardiac	$4.62 \times 10^{-5}$	Titin-binding	[151]
	p.Gln82X	Homo	LGMD2G	Muscular	Absent		[162]
	p.Pro90Leu	Het	HCM	Cardiac	$4.10 \times 10^{-6}$	linker	[151]
	p.Glu105Gln	Het	DCM	Cardiac	$5.10 \times 10^{-4}$	linker	[157]
	p.Glu132Gln	Het	DCM	Cardiac	$4.01 \times 10^{-6}$	C-terminus	[150]
	p.Thr137Ile	Het	HCM	Cardiac	Absent	C-terminus	[150]
	p.Arg153His	Het	HCM	Cardiac	$2.37 \times 10^{-4}$	C-terminus	[150]
	p.Arg158Cys	Het	DCM	Cardiac	Absent	C-terminus	[156]
	c.110 + 5G > A	Homo	LGMDR7	Muscular	Absent		[167]

Abbreviations: DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; Het, heterozygous; Homo, homozygous; LGMD, limb-girdle muscular dystrophies. \* MAF on GnomAD: The Minor Allele Frequency (MAF) was found on The Genome Aggregation Database (gnomAD) version 3.1. All variants with  $MAF < 10^{-4}$  are highlighted in bold.

Being a member of the enigma family, ZASP is composed of an amino-terminal PDZ motif and between 0 and 3 LIM domains at the carboxy-terminus (Figure 6), hence the alternative name: LIM domain-binding protein 3 (*LDB3*). Both PDZ and LIM domains are involved in protein-protein interactions [172,173]. Along with other PDZ-LIM domain proteins, actinin-associated LIM protein and carboxy-terminal LIM protein, ZASP has a conserved region named the ZASP-like motif (ZM), found between the PDZ and LIM domains. This motif is present in both cardiac-specific and skeletal muscle predominant ZASP isoforms, mediates binding to actin and can mediate self-interaction of ZASP proteins [174–176]. ZASP interacts with protein kinase C,  $\alpha$ -actinin, myotilin, telethonin and FATZ/calsarcin/myozenin in striated muscle and plays roles in myofibrillogenesis and assembling protein complexes and integrin adhesion sites within the muscle Z-disc [124]. ZASP also binds to Ankrd2 and p53 in a trimeric complex, which may be involved in signalling, regulation of gene expression and muscle differentiation [171].

Ablation of Cypher in a mouse model resulted in neonatal lethality after birth exhibiting severe congenital myopathy phenotypes and DCM with heart failure [169]. Further to this, in Cypher knockout mice, Z-disc abnormalities such as disorganisation and fragmentation were present in both skeletal and cardiac muscle after birth inferring that Cypher is an important player in the maintenance of the Z-disc during contraction [169]. The cardiac phenotype was later supported by a zebrafish morpholino knockdown of Cypher, which resulted in DCM phenotypes [177].



**Figure 6.** Schematic representation of ZASP shown as a monomer with variants in the *LDB3* gene that have been previously reported in individuals with myopathy (red) or cardiomyopathy (black). Solid lines represent missense variants. LIM—Lin-11 Isl-1 Mec-3 (LIM) domain, PDZ—PDZ domain, ZM—ZASP-like motif. Created with [BioRender.com](https://www.biorender.com) (accessed on 7 March 2021).

### 2.6.1. ZASP in Cardiomyopathy

*LDB3* variants were first described in patients with familial and sporadic DCM, LVNC, HCM and ACM [18,178–180]. Due to the alternative splicing of ZASP and their resulting tissue-specific isoform expression, the exon location of each variant tends to specify the myopathy type (cardiac/skeletal) [172]. For instance, patients with variants found in the cardiac-specific exon 4 (*LDB3* p.Thr213Ile and p.Ser196Leu) present with DCM and LVNC. This exon encodes the LIM domain of ZASP, therefore variants may affect cardiac function by altering the binding affinity of ZASP to its binding partners [18,181]. However, a biophysical characterisation of *LDB3* p.Ala157Thr, p.Ala165Thr and p.Arg268Cys found that these ZM-domain variants did not affect the affinity of ZASP to G-actin [182].

HCM patients have also been found to carry *LDB3* variants, which localise between exon 10 and 13 (*LDB3* p.Ser196Leu, p.Asp366Asn, p.Tyr468Ser and p.Pro615Leu) [18,178]. DCM variants in exon 4 and 10 showed impaired binding to phosphoglucomutase, yet variants in exon 6, which is not limited to the heart, had no effect on this interaction [183].

Further, it was documented that the DCM-associated *LDB3* p.Asp117Asn variant affects interactions within the actin cytoskeletal network in vitro, while the *LDB3* p.Asp626Asn variant increases its binding affinity to protein kinase C [179]. While there are suggestions that ZASP variants may result in observed patient phenotypes via altered protein kinase C binding affinity, the underlying molecular mechanisms are yet to be elucidated.

### 2.6.2. ZASP in Myopathy

As mentioned previously, animal models found that ablation of Cypher results in a severe skeletal muscle defects [169]. This is also true in humans. Pathogenic variants of the human *LDB3* gene are reported in skeletal myopathies such body myositis [18,178,184]. Carriers of skeletal muscle-affecting *LDB3* variants usually present with muscle weakness more distally than proximally [185]. The first identified *LDB3* variants with direct skeletal muscle involvement were heterozygous missense variants (Exon 6: *LDB3* p.Ala147Thr, p.Ala165Val. Exon 9: *LDB3* p.Arg268Cys) found in 11 MFM patients. Since then, less than 50 patients have been identified with ZASP-MFM [132,184,186–190]. Like in cardiomyopathy, the loci may determine the disease outcome: skeletal muscle myopathies dominate when the variant is located in exon 6 (MFM: *LDB3* p.Ala165Val and p.Ala147Thr)

though a cardiac phenotype has also been reported (DCM/LVNC: *LDB3* p.Asp117Asn and p.Lys136Met) [178,181,186].

Out of these *LDB3* p.Ala147Thr and p.Ala165Val are by far the most common variants found in ZASP-MFM. This variant hotspot (also containing the *LDB3* p.Asn155His variant [189]) is located in the actin-binding ZM domain. In the presence of these variants, the actin-binding domain is dysfunctional and causes actin disruption along the Z-disc, affecting Z-disc and myofibril integrity [176,190].

These patients displayed sarcolemmal invaginations and aggregates containing ZASP, ubiquitin, p62 and LC3 as well as the accumulation of autophagic vacuoles, inferring that aggregation and autophagy are potentially additional features of zaspopathy [190].

Human myotonic dystrophy patients often present with mis-spliced ZASP isoforms in their skeletal muscle [191]. A *Drosophila* model of this disease found splicing abnormalities in the *Drosophila* orthologue of Cypher along with Z-disc abnormalities suggesting that irregular Cypher splicing may be a cause of human myotonic dystrophy [192].

### 2.6.3. Summary

Variants of *LDB3* have been associated with both cardiomyopathies and MFM. In particular, autosomal dominant missense variants of the *LDB3* gene are linked with ZASP-related MFM [193]. These variants have been reported to disrupt the Z-disc and result in increased myofibrillar aggregation. Variants were found to primarily affect ZASP in the PDZ and LIM domains.

### 3. Conclusions and Future Directions

As demonstrated in this review, the Z-disc proteins  $\alpha$ -actinin, filamin C, myopalladin, myotilin, telethonin and ZASP do not only play important roles in anchoring actin-filaments and relaying signals at the Z-disc signalling hub, but they are also required for the integrity of skeletal muscle and the heart. Pathogenic variants in all six proteins can cause myopathies and/or cardiomyopathies (summarised in Tables 1–6).

**Table 6.** Variants in the *LDB3* gene that have been previously reported in individuals with myopathy or cardiomyopathy.

Name Gene	Variant	Het or Homo	Type of Disease	Cardiac or Muscular	* MAF on GnomAD	Location	Ref
ZASP <i>LDB3</i>	p.Pro26Ser	Het	MFM	Muscular	Absent	PDZ motif	[190]
	p.Asp117Asn	Het	DCM, LVNC	Cardiac	$7.10 \times 10^{-3}$	ZM motif	[178]
	p.Lys136Met	Het	DCM, LVNC	Cardiac	Absent	ZM motif	[178]
	p.Ala147Thr	Het	MFM	Muscular	Absent	ZM motif	[186]
	p.Asn155His	Het	Distal MFM	Muscular	Absent	ZM motif	[186]
	p.Ala165Val	Het	MFM, DCM, Markesbery	Both	Absent	ZM motif	[189]
	p.Ala171Thr	Het	DCM	Cardiac	$1.51 \times 10^{-4}$	ZM motif	[171]
	p.Ser189Leu	Het	DCM	Cardiac	$5.19 \times 10^{-4}$	ZM motif	[178]
	p.Ser196Leu	Het	DCM, LVNC, HCM	Cardiac	$6.57 \times 10^{-6}$	ZM motif	[178]
	p.Thr206Ile	Het	DCM	Cardiac	$6.57 \times 10^{-6}$	ZM motif	[178]
	p.Thr213Ile	Het	DCM, LVNC	Cardiac	Absent	ZM motif	[178]
	p.Ala222Thr	Het	MFM	Muscular	$3.48 \times 10^{-4}$	ZM motif	[88]
	p.Arg268Cys	Het	MFM	Muscular	$4.61 \times 10^{-5}$	LIM domain	[186]
	p.Ile345Met	Het	DCM	Cardiac	Absent	LIM domain	[178]
	p.Ile352Met	Het	DCM	Cardiac	Absent	LIM domain	[178]
	p.Asp366Asn	Het	HCM	Cardiac	Absent	LIM domain	[18]
	p.Gln414Lys	Het	MFM	Muscular	$4.60 \times 10^{-5}$	LIM domain	[88]
	p.Tyr468Ser	Het	HCM	Cardiac	Absent	LIM domain	[18]
	p.Gln519Pro	Het	HCM	Cardiac	Absent	LIM domain	[18]
	p.Val566Met	Het	Distal dominant weakness	Muscular	$2.63 \times 10^{-5}$	LIM domain	[184]
p.Pro615Leu	Het	HCM	Cardiac	Absent	LIM domain	[18]	
p.Asp626Asn	Het	DCM	Cardiac	Absent	LIM domain	[179]	

Abbreviations: DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; Het, heterozygous; Homo, homozygous; LVNC, left ventricular noncompaction; MFM, myofibrillar myopathy. \* MAF on GnomAD: The Minor Allele Frequency (MAF) was found on The Genome Aggregation Database (gnomAD) version 3.1. All variants with MAF  $< 10^{-4}$  are highlighted in bold.

In the last decade, the use of high-throughput sequencing techniques has revolutionised clinical genetics in several ways: geneticists now have a better understanding of genomic variation in normal cohorts [194], which helps to evaluate the pathogenicity of variants identified in patients based on their frequency. For cardiomyopathy variants, which are rare genetic diseases, a threshold of MAF  $< 10^{-4}$  is now consensus [19]. Based on this, a critical review of many, especially missense variants, which were identified before the application of high-throughput sequencing, would now consider them relatively frequent to be pathogenic (see Tables 1–6). Moreover, high-throughput sequencing allows the cost-efficient interrogation of gene panels or whole exomes and may help to identify potentially pathogenic variants in patients. However, an overwhelming challenge in the clinical practice is the evaluation of these variants, especially of missense ones. Co-segregation studies in families are valuable and bioinformatics tools can provide additional support.

For several genes, especially *FLNC*, it is not clear why some truncating or missense variants cause pure myopathy while others cause cardiomyopathy without skeletal muscle involvement. It is tempting to speculate that there are functional differences between the variants. Future functional studies will hopefully be able to shed light on this and application of exciting novel technologies will facilitate such studies: induced pluripotent stem cells allow the generation of human cardiac and skeletal muscle cells from patients [195]. Moreover, genome-engineering allows the precise manipulation of genomes, both in animal models and induced pluripotent stem cells [196]. Hence, researchers will be able to generate accurate models of human disease and interrogate them for open questions in the field. This will help to develop a deeper understanding of pathogenic mechanisms and thereby facilitate the development of novel therapeutic approaches to benefit the patients.

**Author Contributions:** Conceptualization, K.G.; writing—original draft preparation, K.W., A.J.A. and K.G.; writing—review and editing, K.G.; visualization, K.W. and A.J.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** The authors acknowledge support by the British Heart Foundation (BHF) [FS/12/40/29712, PG/15/113/31944, PG/19/45/34419]; the Oxford BHF Centre of Research Excellence [RE/13/1/30181]; the Wellcome Trust [201543/B/16/Z], the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3R, NC/T001747/1); and the Medical Research Council [MR/V009540/1]. The Institute of Cardiovascular Sciences, University of Birmingham, has received an Accelerator Award by the British Heart Foundation [AA/18/2/34218].

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data sharing not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Ferrer, I.; Olivé, M. Molecular pathology of myofibrillar myopathies. *Expert Rev. Mol. Med.* **2008**, *10*, 1–21. [[CrossRef](#)] [[PubMed](#)]
2. Finsterer, J.; Stöllberger, C. Cardiac involvement in primary myopathies. *Cardiology* **2000**, *94*, 1–11. [[CrossRef](#)] [[PubMed](#)]
3. Elliott, P.; Andersson, B.; Arbustini, E.; Bilinska, Z.; Cecchi, F.; Charron, P.; Dubourg, O.; Kühl, U.; Maisch, B.; McKenna, W.J.; et al. Classification of the cardiomyopathies: A position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur. Heart. J.* **2008**, *29*, 270–276. [[CrossRef](#)] [[PubMed](#)]
4. Hershberger, R.E.; Hedges, D.J.; Morales, A. Dilated cardiomyopathy: The complexity of a diverse genetic architecture. *Nat. Rev. Cardiol.* **2013**, *10*, 531–547. [[CrossRef](#)] [[PubMed](#)]
5. Zegkos, T.; Panagiotidis, T.; Parcharidou, D.; Efthimiadis, G. Emerging concepts in arrhythmogenic dilated cardiomyopathy. *Heart Fail. Rev.* **2020**. [[CrossRef](#)]
6. Zaleta-Rivera, K.; Dainis, A.; Ribeiro, A.J.S.; Cordero, P.; Rubio, G.; Shang, C.; Liu, J.; Finsterbach, T.; Parikh, V.N.; Sutton, S.; et al. Allele-Specific Silencing Ameliorates Restrictive Cardiomyopathy Attributable to a Human Myosin Regulatory Light Chain Mutation. *Circulation* **2019**, *140*, 765–778. [[CrossRef](#)] [[PubMed](#)]
7. Watkins, H.; Ashrafian, H.; Redwood, C. Inherited cardiomyopathies. *N. Engl. J. Med.* **2011**, *364*, 1643–1656. [[CrossRef](#)]
8. Shieh, P.B. Muscular dystrophies and other genetic myopathies. *Neurol. Clin.* **2013**, *31*, 1009–1029. [[CrossRef](#)]

9. Rassier, D.E. Sarcomere mechanics in striated muscles: From molecules to sarcomeres to cells. *Am. J. Physiol. Cell Physiol.* **2017**, *313*, C134–C145. [[CrossRef](#)]
10. Lange, S.; Pinotsis, N.; Agarkova, I.; Ehler, E. The M-band: The underestimated part of the sarcomere. *Biochim. Biophys. Acta. Mol. Cell Res.* **2020**, *1867*, 118440. [[CrossRef](#)]
11. Luther, P.K. The vertebrate muscle Z-disc: Sarcomere anchor for structure and signalling. *J. Muscle Res. Cell Motil.* **2009**, *30*, 171–185. [[CrossRef](#)]
12. Azad, A.; Poloni, G.; Sontayananon, N.; Jiang, H.; Gehmlich, K. The giant titin: How to evaluate its role in cardiomyopathies. *J. Muscle Res. Cell Motil.* **2019**, *40*, 159–167. [[CrossRef](#)] [[PubMed](#)]
13. Chauveau, C.; Rowell, J.; Ferreira, A. A rising titan: TTN review and mutation update. *Hum. Mutat.* **2014**, *35*, 1046–1059. [[CrossRef](#)] [[PubMed](#)]
14. Sjöblom, B.; Salmazo, A.; Djinović-Carugo, K. Alpha-actinin structure and regulation. *Cell Mol. Life Sci.* **2008**, *65*, 2688–2701. [[CrossRef](#)]
15. Persechini, A.; Moncrief, N.D.; Kretsinger, R.H. The EF-hand family of calcium-modulated proteins. *Trends Neurosci.* **1989**, *12*, 462–467. [[CrossRef](#)]
16. de Almeida Ribeiro, E.; Pinotsis, N.; Ghisleni, A.; Salmazo, A.; Konarev, P.V.; Kostan, J.; Sjöblom, B.; Schreiner, C.; Polyansky, A.A.; Gkougkoulia, E.A.; et al. The structure and regulation of human muscle  $\alpha$ -actinin. *Cell* **2014**, *159*, 1447–1460. [[CrossRef](#)]
17. Yang, J.; Xu, X.  $\alpha$ -Actinin2 is required for the lateral alignment of Z discs and ventricular chamber enlargement during zebrafish cardiogenesis. *FASEB J.* **2012**, *26*, 4230–4242. [[CrossRef](#)]
18. Theis, J.L.; Bos, J.M.; Bartleson, V.B.; Will, M.L.; Binder, J.; Vatta, M.; Towbin, J.A.; Gersh, B.J.; Ommen, S.R.; Ackerman, M.J. Echocardiographic-determined septal morphology in Z-disc hypertrophic cardiomyopathy. *Biochem. Biophys. Res. Commun.* **2006**, *351*, 896–902. [[CrossRef](#)]
19. Walsh, R.; Thomson, K.L.; Ware, J.S.; Funke, B.H.; Woodley, J.; McGuire, K.J.; Mazzarotto, F.; Blair, E.; Seller, A.; Taylor, J.C.; et al. Reassessment of Mendelian gene pathogenicity using 7855 cardiomyopathy cases and 60,706 reference samples. *Genet. Med.* **2017**, *19*, 192–203. [[CrossRef](#)] [[PubMed](#)]
20. Chiu, C.; Bagnall, R.D.; Ingles, J.; Yeates, L.; Kennerson, M.; Donald, J.A.; Jormakka, M.; Lind, J.M.; Semsarian, C. Mutations in alpha-actinin-2 cause hypertrophic cardiomyopathy: A genome-wide analysis. *J. Am. Coll. Cardiol.* **2010**, *55*, 1127–1135. [[CrossRef](#)] [[PubMed](#)]
21. Girolami, F.; Iascone, M.; Tomberli, B.; Bardi, S.; Benelli, M.; Marseglia, G.; Pescucci, C.; Pezzoli, L.; Sana, M.E.; Basso, C.; et al. Novel  $\alpha$ -actinin 2 variant associated with familial hypertrophic cardiomyopathy and juvenile atrial arrhythmias: A massively parallel sequencing study. *Circ. Cardiovasc. Genet.* **2014**, *7*, 741–750. [[CrossRef](#)]
22. Prondzynski, M.; Lemoine, M.D.; Zech, A.T.; Horváth, A.; Di Mauro, V.; Koivumäki, J.T.; Kresin, N.; Busch, J.; Krause, T.; Krämer, E.; et al. Disease modeling of a mutation in  $\alpha$ -actinin 2 guides clinical therapy in hypertrophic cardiomyopathy. *EMBO Mol. Med.* **2019**, *11*, e11115. [[CrossRef](#)]
23. Haywood, N.J.; Wolny, M.; Rogers, B.; Trinh, C.H.; Shuping, Y.; Edwards, T.A.; Peckham, M. Hypertrophic cardiomyopathy mutations in the calponin-homology domain of ACTN2 affect actin binding and cardiomyocyte Z-disc incorporation. *Biochem. J.* **2016**, *473*, 2485–2493. [[CrossRef](#)]
24. Mohapatra, B.; Jimenez, S.; Lin, J.H.; Bowles, K.R.; Coveler, K.J.; Marx, J.G.; Chrisco, M.A.; Murphy, R.T.; Lurie, P.R.; Schwartz, R.J.; et al. Mutations in the muscle LIM protein and alpha-actinin-2 genes in dilated cardiomyopathy and endocardial fibroelastosis. *Mol. Genet. Metab.* **2003**, *80*, 207–215. [[CrossRef](#)]
25. Fan, L.L.; Huang, H.; Jin, J.Y.; Li, J.J.; Chen, Y.Q.; Xiang, R. Whole-Exome Sequencing Identifies a Novel Mutation (p.L320R) of Alpha-Actinin 2 in a Chinese Family with Dilated Cardiomyopathy and Ventricular Tachycardia. *Cytogenet. Genome Res.* **2019**, *157*, 148–152. [[CrossRef](#)] [[PubMed](#)]
26. Bagnall, R.D.; Molloy, L.K.; Kalman, J.M.; Semsarian, C. Exome sequencing identifies a mutation in the ACTN2 gene in a family with idiopathic ventricular fibrillation, left ventricular noncompaction, and sudden death. *BMC Med. Genet.* **2014**, *15*, 99. [[CrossRef](#)] [[PubMed](#)]
27. Savarese, M.; Palmio, J.; Poza, J.J.; Weinberg, J.; Olive, M.; Cobo, A.M.; Vihola, A.; Jonson, P.H.; Sarparanta, J.; García-Bragado, F.; et al. Actininopathy: A new muscular dystrophy caused by ACTN2 dominant mutations. *Ann. Neurol.* **2019**, *85*, 899–906. [[CrossRef](#)] [[PubMed](#)]
28. North, K.N.; Yang, N.; Wattanasirichaigoon, D.; Mills, M.; Easteal, S.; Beggs, A.H. A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. *Nat. Genet.* **1999**, *21*, 353–354. [[CrossRef](#)]
29. Hogarth, M.W.; Garton, F.C.; Houweling, P.J.; Tukiainen, T.; Lek, M.; Macarthur, D.G.; Seto, J.T.; Quinlan, K.G.; Yang, N.; Head, S.I.; et al. Analysis of the ACTN3 heterozygous genotype suggests that  $\alpha$ -actinin-3 controls sarcomeric composition and muscle function in a dose-dependent fashion. *Hum. Mol. Genet.* **2016**, *25*, 866–877. [[CrossRef](#)] [[PubMed](#)]
30. Feng, Y.; Walsh, C.A. The many faces of filamin: A versatile molecular scaffold for cell motility and signalling. *Nat. Cell Biol.* **2004**. [[CrossRef](#)] [[PubMed](#)]
31. Razinia, Z.; Mäkelä, T.; Yläne, J.; Calderwood, D.A. Filamins in Mechanosensing and Signaling. *Annu. Rev. Biophys.* **2012**. [[CrossRef](#)] [[PubMed](#)]
32. Thompson, T.G.; Chan, Y.M.; Hack, A.A.; Brosius, M.; Rajala, M.; Lidov, H.G.; McNally, E.M.; Watkins, S.; Kunkel, L.M. Filamin 2 (FLN2): A muscle-specific sarcoglycan interacting protein. *J. Cell Biol.* **2000**, *148*, 115–126. [[CrossRef](#)] [[PubMed](#)]



33. Gorlin, J.B.; Yamin, R.; Egan, S.; Stewart, M.; Stossel, T.P.; Kwiatkowski, D.J.; Hartwig, J.H. Human endothelial actin-binding protein (ABP-280, nonmuscle filamin): A molecular leaf spring. *J. Cell Biol.* **1990**, *111*, 1089–1105. [[CrossRef](#)] [[PubMed](#)]
34. Himmel, M.; Van der Ven, P.F.M.; Stöcklein, W.; Fürst, D.O. The limits of promiscuity: Isoform-specific dimerization of filamins. *Biochemistry* **2003**, *42*, 430–439. [[CrossRef](#)]
35. Stossel, T.P.; Condeelis, J.; Cooley, L.; Hartwig, J.H.; Noegel, A.; Schleicher, M.; Shapiro, S.S. Filamins as integrators of cell mechanics and signalling. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 138–145. [[CrossRef](#)]
36. Popowicz, G.M.; Schleicher, M.; Noegel, A.A.; Holak, T.A. Filamins: Promiscuous organizers of the cytoskeleton. *Trends Biochem. Sci.* **2006**, *31*, 411–419. [[CrossRef](#)]
37. Van Der Ven, P.F.M.; Wiesner, S.; Salmikangas, P.; Auerbach, D.; Himmel, M.; Kempa, S.; Hayeß, K.; Pacholsky, D.; Taivainen, A.; Schröder, R.; et al. Indications for a novel muscular dystrophy pathway:  $\gamma$ -Filamin, the muscle-specific filamin isoform, interacts with myotilin. *J. Cell Biol.* **2000**, *151*, 235–247. [[CrossRef](#)]
38. Chen, H.; Chandrasekar, S.; Sheetz, M.P.; Stossel, T.P.; Nakamura, F.; Yan, J. Mechanical perturbation of filamin A immunoglobulin repeats 20–21 reveals potential non-equilibrium mechanochemical partner binding function. *Sci. Rep.* **2013**, *3*, 1–6. [[CrossRef](#)]
39. Molt, S.; Bührdel, J.B.; Yakovlev, S.; Schein, P.; Orfanos, Z.; Kirfel, G.; Winter, L.; Wiche, G.; Van Der Ven, P.F.M.; Rottbauer, W.; et al. Aciculin interacts with filamin C and Xin and is essential for myofibril assembly, remodeling and maintenance. *J. Cell Biol.* **2014**, *127*, 3578–3592. [[CrossRef](#)]
40. Seppälä, J.; Tossavainen, H.; Rodic, N.; Permi, P.; Pentikäinen, U.; Ylännä, J.; Kursula, P. Flexible structure of peptide-bound filamin a mechanosensor domain pair 20–21. *PLoS ONE* **2015**, *10*, e0136969. [[CrossRef](#)]
41. Ruparelia, A.A.; Zhao, M.; Currie, P.D.; Bryson-Richardson, R.J. Characterization and investigation of zebrafish models of filamin-related myofibrillar myopathy. *Hum. Mol. Genet.* **2012**, *21*, 4073–4083. [[CrossRef](#)] [[PubMed](#)]
42. Leber, Y.; Ruparelia, A.A.; Kirfel, G.; van der Ven, P.F.M.; Hoffmann, B.; Merkel, R.; Bryson-Richardson, R.J.; Fürst, D.O. Filamin C is a highly dynamic protein associated with fast repair of myofibrillar microdamage. *Hum. Mol. Genet.* **2016**, *25*, 2776–2788. [[CrossRef](#)]
43. Fürst, D.O.; Goldfarb, L.G.; Kley, R.A.; Vorgerd, M.; Olivé, M.; Van Der Ven, P.F.M. Filamin C-related myopathies: Pathology and mechanisms. *Acta Neuropathol.* **2013**, *125*, 33–46. [[CrossRef](#)] [[PubMed](#)]
44. Juo, L.Y.; Liao, W.C.; Shih, Y.L.; Yang, B.Y.; Liu, A.B.; Yan, Y.T. HSPB7 interacts with dimerized FLNC and its absence results in progressive myopathy in skeletal muscles. *J. Cell Sci.* **2016**, *129*, 1661–1670. [[CrossRef](#)] [[PubMed](#)]
45. Raynaud, F.; Fabrice, R.; Jond-Necand, C.; Carole, J.N.; Marcilhac, A.; Anne, M.; Fürst, D.; Dieter, F.; Benyamin, Y.; Yves, B. Calpain 1-gamma filamin interaction in muscle cells: A possible in situ regulation by PKC-alpha. *Int. J. Biochem. Cell Biol.* **2006**, *38*, 404–413. [[CrossRef](#)] [[PubMed](#)]
46. Zhang, M.; Liu, J.; Cheng, A.; DeYoung, S.M.; Saltiel, A.R. Identification of CAP as a costameric protein that interacts with filamin C. *Mol. Biol. Cell.* **2007**, *18*, 4731–4740. [[CrossRef](#)]
47. Tu, Y.; Wu, S.; Shi, X.; Chen, K.; Wu, C. Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell* **2003**, *113*, 37–47. [[CrossRef](#)]
48. Sequea, D.A.; Sharma, N.; Arias, E.B.; Cartee, G.D. Greater filamin C, GSK3 $\alpha$ , and GSK3 $\beta$  serine phosphorylation in insulin-stimulated isolated skeletal muscles of calorie restricted 24 month-old rats. *Mech. Ageing Dev.* **2013**, *134*, 60–63. [[CrossRef](#)]
49. Reimann, L.; Wiese, H.; Leber, Y.; Schwable, A.N.; Fricke, A.L.; Rohland, A.; Knapp, B.; Peikert, C.D.; Drepper, F.; Van Der Ven, P.F.M.; et al. Myofibrillar Z-discs are a protein phosphorylation hot spot with protein kinase C (PKC $\alpha$ ) modulating protein dynamics. *Mol. Cell. Proteom.* **2017**, *16*, 346–367. [[CrossRef](#)]
50. Dalkilic, I.; Schienda, J.; Thompson, T.G.; Kunkel, L.M. Loss of FilaminC (FLNc) Results in Severe Defects in Myogenesis and Myotube Structure. *Mol. Cell Biol.* **2006**, *26*, 6522–6534. [[CrossRef](#)]
51. O'Brien, S.P.; Seipel, K.; Medley, Q.G.; Bronson, R.; Segal, R.; Streuli, M. Skeletal muscle deformity and neuroanl disorder in Trio exchange factor-deficient mouse embryos. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12047–12078. [[CrossRef](#)]
52. Zhou, Y.; Chen, Z.; Zhang, L.; Zhu, M.; Tan, C.; Zhou, X.; Evans, S.M.; Fang, X.; Feng, W.; Chen, J. Loss of Filamin C Is Catastrophic for Heart Function. *Circulation* **2020**, *141*, 869–871. [[CrossRef](#)]
53. Arndt, V.; Dick, N.; Tawo, R.; Dreiseidler, M.; Wenzel, D.; Hesse, M.; Fürst, D.O.; Saftig, P.; Saint, R.; Fleischmann, B.K.; et al. Chaperone-Assisted Selective Autophagy Is Essential for Muscle Maintenance. *Curr. Biol.* **2010**, *141*, 868–871. [[CrossRef](#)] [[PubMed](#)]
54. Vorgerd, M.; Van Der Ven, P.F.M.; Bruchertseifer, V.; Löwe, T.; Kley, R.A.; Schröder, R.; Lochmüller, H.; Himmel, M.; Koehler, K.; Fürst, D.O.; et al. A mutation in the dimerization domain of filamin c causes a novel type of autosomal dominant myofibrillar myopathy. *Am. J. Hum. Genet.* **2005**, *77*, 297–304. [[CrossRef](#)] [[PubMed](#)]
55. Duff, R.M.; Tay, V.; Hackman, P.; Ravenscroft, G.; McLean, C.; Kennedy, P.; Steinbach, A.; Schöffler, W.; Van Der Ven, P.F.M.; Fürst, D.O.; et al. Mutations in the N-terminal actin-binding domain of filamin C cause a distal myopathy. *Am. J. Hum. Genet.* **2011**, *88*, 729–740. [[CrossRef](#)] [[PubMed](#)]
56. Guergueltcheva, V.; Peeters, K.; Baets, J.; Ceuterick-De Groote, C.; Martin, J.J.; Suls, A.; De Vriendt, E.; Mihaylova, V.; Chamova, T.; Almeida-Souza, L.; et al. Distal myopathy with upper limb predominance caused by filamin C haploinsufficiency. *Neurology* **2011**, *77*, 2105–2114. [[CrossRef](#)] [[PubMed](#)]

57. Kley, R.A.; Hellenbroich, Y.; Van Der Ven, P.F.M.; Fürst, D.O.; Huebner, A.; Bruchertseifer, V.; Peters, S.A.; Heyer, C.M.; Kirschner, J.; Schröder, R.; et al. Clinical and morphological phenotype of the filamin myopathy: A study of 31 German patients. *Brain* **2007**, *130*, 3250–3264. [[CrossRef](#)] [[PubMed](#)]
58. Cui, H.; Wang, J.; Zhang, C.; Wu, G.; Zhu, C.; Tang, B.; Zou, Y.; Huang, X.; Hui, R.; Song, L.; et al. Mutation profile of FLNC gene and its prognostic relevance in patients with hypertrophic cardiomyopathy. *Mol. Genet. Genomic. Med.* **2018**, *6*, 1104–1113. [[CrossRef](#)]
59. Valdés-Mas, R.; Gutiérrez-Fernández, A.; Gómez, J.; Coto, E.; Astudillo, A.; Puente, D.A.; Reguero, J.R.; Álvarez, V.; Morís, C.; León, D.; et al. Mutations in filamin C cause a new form of familial hypertrophic cardiomyopathy. *Nat. Commun.* **2014**, *5*, 1–9. [[CrossRef](#)]
60. Brodehl, A.; Ferrier, R.A.; Hamilton, S.J.; Greenway, S.C.; Brundler, M.A.; Yu, W.; Gibson, W.T.; McKinnon, M.L.; McGillivray, B.; Alvarez, N.; et al. Mutations in FLNC are Associated with Familial Restrictive Cardiomyopathy. *Hum. Mutat.* **2016**, *37*, 269–279. [[CrossRef](#)]
61. Ortiz-Genga, M.F.; Cuenca, S.; Dal Ferro, M.; Zorio, E.; Salgado-Aranda, R.; Climent, V.; Padrón-Barthe, L.; Duro-Aguado, I.; Jiménez-Jáimez, J.; Hidalgo-Olivares, V.M.; et al. Truncating FLNC Mutations Are Associated With High-Risk Dilated and Arrhythmogenic Cardiomyopathies. *J. Am. Coll. Cardiol.* **2016**, *68*, 2440–2451. [[CrossRef](#)]
62. Begay, R.L.; Tharp, C.A.; Martin, A.; Graw, S.L.; Sinagra, G.; Miani, D.; Sweet, M.E.; Slavov, D.B.; Stafford, N.; Zeller, M.J.; et al. FLNC Gene Splice Mutations Cause Dilated Cardiomyopathy. *J. Am. Coll. Cardiol. Basic Trans. Sci.* **2016**, *1*, 344–359. [[CrossRef](#)]
63. Hall, C.L.; Akhtar, M.M.; Sabater-Molina, M.; Futema, M.; Asimaki, A.; Protonotarios, A.; Dalageorgou, C.; Pittman, A.M.; Suarez, M.P.; Aguilera, B.; et al. Filamin C variants are associated with a distinctive clinical and immunohistochemical arrhythmogenic cardiomyopathy phenotype. *Int. J. Cardiol.* **2019**, *307*, 101–108. [[CrossRef](#)]
64. Schreiber, K.H.; Kennedy, B.K. When lamins go bad: Nuclear structure and disease. *Cell* **2013**, *152*, 1365–1375. [[CrossRef](#)] [[PubMed](#)]
65. Konersman, C.G.; Bordini, B.J.; Scharer, G.; Lawlor, M.W.; Zangwill, S.; Southern, J.F.; Amos, L.; Geddes, G.C.; Kliegman, R.; Collins, M.P. BAG3 myofibrillar myopathy presenting with cardiomyopathy. *Neuromuscul. Disord.* **2015**, *25*, 554–562. [[CrossRef](#)] [[PubMed](#)]
66. Hartmannova, H.; Kubanek, M.; Sramko, M.; Piherova, L.; Noskova, L.; Hodanova, K.; Stranecky, V.; Pristoupilova, A.; Sovova, J.; Marek, T.; et al. Isolated X-linked hypertrophic cardiomyopathy caused by a novel mutation of the four-and-a-half LIM domain 1 gene. *Circ. Cardiovasc. Genet.* **2013**, *6*, 542–551. [[CrossRef](#)] [[PubMed](#)]
67. Kiselev, A.; Vaz, R.; Knyazeva, A.; Khudiakov, A.; Tarnovskaya, S.; Liu, J.; Sergushichev, A.; Kazakov, S.; Frishman, D.; Smolina, N.; et al. De novo mutations in FLNC leading to early-onset restrictive cardiomyopathy and congenital myopathy. *Hum. Mutat.* **2018**, *39*, 1161–1172. [[CrossRef](#)] [[PubMed](#)]
68. Verdonschot, J.A.J.; Vanhoutte, E.K.; Claes, G.R.F.; Helderma-van den Eenden, A.T.J.M.; Hoeijmakers, J.G.J.; Hellebrekers, D.M.E.I.; de Haan, A.; Christiaans, I.; Lekanne Deprez, R.H.; Boen, H.M.; et al. A mutation update for the FLNC gene in myopathies and cardiomyopathies. *Hum. Mutat.* **2020**, *41*, 1091–1111. [[CrossRef](#)] [[PubMed](#)]
69. Reinstein, E.; Gutierrez-Fernandez, A.; Tzur, S.; Bormans, C.; Marcu, S.; Tayeb-Fligelman, E.; Vinkler, C.; Raas-Rothschild, A.; Irge, D.; Landau, M.; et al. Congenital dilated cardiomyopathy caused by biallelic mutations in Filamin C. *Eur. J. Hum. Genet.* **2016**, *24*, 1792–1796. [[CrossRef](#)] [[PubMed](#)]
70. Tucker, N.R.; McLellan, M.A.; Hu, D.; Ye, J.; Parsons, V.A.; Mills, R.W.; Clauss, S.; Dolmatova, E.; Shea, M.A.; Milan, D.J.; et al. Novel Mutation in FLNC (Filamin C) Causes Familial Restrictive Cardiomyopathy. *Circ. Cardiovasc. Genet.* **2017**, *10*, e001780. [[CrossRef](#)]
71. Janin, A.; N'Guyen, K.; Habib, G.; Dauphin, C.; Chanavat, V.; Bouvagnet, P.; Eschalier, R.; Streichenberger, N.; Chevalier, P.; Millat, G. Truncating mutations on myofibrillar myopathies causing genes as prevalent molecular explanations on patients with dilated cardiomyopathy. *Clin. Genet.* **2017**, *92*, 616–623. [[CrossRef](#)]
72. Gómez, J.; Lorca, R.; Reguero, J.R.; Morís, C.; Martín, M.; Tranche, S.; Alonso, B.; Iglesias, S.; Alvarez, V.; Díaz-Molina, B.; et al. Screening of the Filamin C Gene in a Large Cohort of Hypertrophic Cardiomyopathy Patients. *Circ. Cardiovasc. Genet.* **2017**, *10*, e001584. [[CrossRef](#)]
73. Avila-Smirnow, D.; Gueneau, L.; Batonnet-Pichon, S.; Delort, F.; Bécane, H.M.; Claeys, K.; Beuvin, M.; Goudeau, B.; Jais, J.P.; Nelson, I.; et al. Cardiac arrhythmia and late-onset muscle weakness caused by a myofibrillar myopathy with unusual histopathological features due to a novel missense mutation in FLNC. *Rev. Neurol.* **2016**, *172*, 594–606. [[CrossRef](#)] [[PubMed](#)]
74. Schuld, J.; Orfanos, Z.; Chevessier, F.; Eggers, B.; Heil, L.; Uszkoreit, J.; Unger, A.; Kirfel, G.; Van Der Ven, P.F.M.; Marcus, K.; et al. Homozygous expression of the myofibrillar myopathy-associated p.W2710X filamin C variant reveals major pathomechanisms of sarcomeric lesion formation. *Acta Neuropathol. Commun.* **2020**, *8*. [[CrossRef](#)] [[PubMed](#)]
75. Kley, R.A.; Serdaroglu-Oflazer, P.; Leber, Y.; Odgerel, Z.; Van Der Ven, P.F.M.; Olivé, M.; Ferrer, I.; Onipe, A.; Mihaylov, M.; Bilbao, J.M.; et al. Pathophysiology of protein aggregation and extended phenotyping in filaminopathy. *Brain* **2012**, *135*, 2642–2660. [[CrossRef](#)] [[PubMed](#)]
76. Luan, X.; Hong, D.; Zhang, W.; Wang, Z.; Yuan, Y. A novel heterozygous deletion-insertion mutation (2695-2712 del/GTTTGT ins) in exon 18 of the filamin C gene causes filaminopathy in a large Chinese family. *Neuromuscul. Disord.* **2010**, *19*, 324–329. [[CrossRef](#)] [[PubMed](#)]

77. Miao, J.; Su, F.F.; Liu, X.M.; Wei, X.J.; Yuan, Y.; Yu, X.F. A case report: A heterozygous deletion (2791\_2805 del) in exon 18 of the filamin C gene causing filamin C-related myofibrillar myopathies in a Chinese family. *BMC Neurol.* **2018**, *18*. [[CrossRef](#)]
78. Ruparelia, A.A.; Oorschot, V.; Ramm, G.; Bryson-Richardson, R.J. FLNC myofibrillar myopathy results from impaired autophagy and protein insufficiency. *Hum. Mol. Genet.* **2016**, *25*, 2131–2142. [[CrossRef](#)] [[PubMed](#)]
79. Ulbricht, A.; Gehlert, S.; Leciejewski, B.; Schiffer, T.; Bloch, W.; Höhfeld, J. Induction and adaptation of chaperone-assisted selective autophagy CASA in response to resistance exercise in human skeletal muscle. *Autophagy* **2015**, *11*, 538–546. [[CrossRef](#)] [[PubMed](#)]
80. Kley, R.A.; Van Der Ven, P.F.M.; Olivé, M.; Höhfeld, J.; Goldfarb, L.G.; Fürst, D.O.; Vorgerd, M. Impairment of protein degradation in myofibrillar myopathy caused by FLNC/filamin C mutations. *Autophagy* **2013**, *9*, 422–423. [[CrossRef](#)]
81. Van den Bogaart, F.J.A.; Claeys, K.G.; Kley, R.A.; Kusters, B.; Schrading, S.; Kamsteeg, E.J.; Voermans, N.C. Widening the spectrum of filamin-C myopathy: Predominantly proximal myopathy due to the p.A193T mutation in the actin-binding domain of FLNC. *Neuromuscul. Disord.* **2017**, *27*, 73–77. [[CrossRef](#)] [[PubMed](#)]
82. Janssens, J.; Philtjens, S.; Kleinberger, G.; Van Mossevelde, S.; van der Zee, J.; Cacace, R.; Engelborghs, S.; Sieben, A.; Banzhaf-Strathmann, J.; Dillen, L.; et al. Investigating the role of filamin C in Belgian patients with frontotemporal dementia linked to GRN deficiency in FTLT-DTP brains. *Acta Neuropathol. Commun.* **2015**, *3*, 68. [[CrossRef](#)]
83. Ader, F.; De Groote, P.; Réant, P.; Rooryck-Thambo, C.; Dupin-Deguine, D.; Rambaud, C.; Khraiche, D.; Perret, C.; Prunty, J.F.; Mathieu-Dramard, M.; et al. FLNC pathogenic variants in patients with cardiomyopathies: Prevalence and genotype-phenotype correlations. *Clin. Genet.* **2019**, *96*, 317–329. [[CrossRef](#)] [[PubMed](#)]
84. Bains, S.; Tester, D.J.; Asirvatham, S.J.; Noseworthy, P.A.; Ackerman, M.J.; Giudicessi, J.R. A Novel Truncating Variant in FLNC-Encoded Filamin C May Serve as a Proarrhythmic Genetic Substrate for Arrhythmogenic Bileaflet Mitral Valve Prolapse Syndrome. *Mayo Clin. Proc.* **2019**, *94*, 906–913. [[CrossRef](#)] [[PubMed](#)]
85. Fichna, J.P.; Macias, A.; Piechota, M.; Korostyński, M.; Potulska-Chromik, A.; Redowicz, M.J.; Zekanowski, C. Whole-exome sequencing identifies novel pathogenic mutations and putative phenotype-influencing variants in Polish limb-girdle muscular dystrophy patients. *Hum. Genom.* **2018**, *12*, 34. [[CrossRef](#)] [[PubMed](#)]
86. Gemelli, C.; Prada, V.; Fiorillo, C.; Fabbri, S.; Maggi, L.; Geroldi, A.; Gibertini, S.; Mandich, P.; Trevisan, L.; Fossa, P.; et al. A novel mutation in the N-terminal acting-binding domain of Filamin C protein causing a distal myofibrillar myopathy. *J. Neurol. Sci.* **2019**, *398*, 75–78. [[CrossRef](#)]
87. Begay, R.L.; Graw, S.L.; Sinagra, G.; Asimaki, A.; Rowland, T.J.; Slavov, D.B.; Gowan, K.; Jones, K.L.; Brun, F.; Merlo, M.; et al. Filamin C Truncation Mutations Are Associated With Arrhythmogenic Dilated Cardiomyopathy and Changes in the Cell-Cell Adhesion Structures. *JACC Clin. Electrophysiol.* **2018**, *4*, 504–514. [[CrossRef](#)]
88. Wehl, C.C.; Baloh, R.H.; Lee, Y.; Chou, T.F.; Pittman, S.K.; Lopate, G.; Allred, P.; Jockel-Balsarotti, J.; Pestronk, A.; Harms, M.B. Targeted sequencing and identification of genetic variants in sporadic inclusion body myositis. *Neuromuscul. Disord.* **2015**, *25*, 289–296. [[CrossRef](#)]
89. Kölbel, H.; Roos, A.; van der Ven, P.F.M.; Evangelista, T.; Nolte, K.; Johnson, K.; Töpf, A.; Wilson, M.; Kress, W.; Sickmann, A.; et al. First clinical and myopathological description of a myofibrillar myopathy with congenital onset and homozygous mutation in FLNC. *Hum. Mutat.* **2020**, *41*, 1600–1614. [[CrossRef](#)]
90. Tobita, T.; Nomura, S.; Morita, H.; Ko, T.; Fujita, T.; Toko, H.; Uto, K.; Hagiwara, N.; Aburatani, H.; Komuro, I. Identification of MYLK3 mutations in familial dilated cardiomyopathy. *Sci. Rep.* **2017**, *7*, 17495. [[CrossRef](#)]
91. Zhang, Y.T.; Pu, C.Q.; Ban, R.; Liu, H.X.; Shi, Q.; Lu, X.H. Clinical, Pathological, and Genetic Features of Two Chinese Cases with Filamin C Myopathy. *Chin. Med. J. (Engl)* **2018**, *131*, 2986–2988. [[CrossRef](#)]
92. Miszalski-Jamka, K.; Jefferies, J.L.; Mazur, W.; Głowacki, J.; Hu, J.; Lazar, M.; Gibbs, R.A.; Liczko, J.; Kłyś, J.; Venner, E.; et al. Novel Genetic Triggers and Genotype-Phenotype Correlations in Patients With Left Ventricular Noncompaction. *Circ. Cardiovasc. Genet.* **2017**, *10*, e001763. [[CrossRef](#)]
93. Ghaoui, R.; Cooper, S.T.; Lek, M.; Jones, K.; Corbett, A.; Reddel, S.W.; Needham, M.; Liang, C.; Waddell, L.B.; Nicholson, G.; et al. Use of Whole-Exome Sequencing for Diagnosis of Limb-Girdle Muscular Dystrophy: Outcomes and Lessons Learned. *JAMA Neurol.* **2015**, *72*, 1424–1432. [[CrossRef](#)]
94. Vill, K.; Blaschek, A.; Gläser, D.; Kuhn, M.; Haack, T.; Alhaddad, B.; Wagner, M.; Kovacs-Nagy, R.; Tacke, M.; Gerstl, L.; et al. Early-Onset Myopathies: Clinical Findings, Prevalence of Subgroups and Diagnostic Approach in a Single Neuromuscular Referral Center in Germany. *J. Neuromuscul. Dis.* **2017**, *4*, 315–325. [[CrossRef](#)]
95. Yu, M.; Zheng, Y.; Jin, S.; Gang, Q.; Wang, Q.; Yu, P.; Lv, H.; Zhang, W.; Yuan, Y.; Wang, Z. Mutational spectrum of Chinese LGMD patients by targeted next-generation sequencing. *PLoS ONE* **2017**, *12*, e0175343. [[CrossRef](#)] [[PubMed](#)]
96. Cuenca, S.; Ruiz-Cano, M.J.; Gimeno-Blanes, J.R.; Jurado, A.; Salas, C.; Gomez-Diaz, I.; Padron-Barthe, L.; Grillo, J.J.; Vilches, C.; Segovia, J.; et al. Genetic basis of familial dilated cardiomyopathy patients undergoing heart transplantation. *J. Heart Lung Transplant.* **2016**, *35*, 625–635. [[CrossRef](#)] [[PubMed](#)]
97. Augusto, J.B.; Eiros, R.; Nakou, E.; Moura-Ferreira, S.; Treibel, T.A.; Captur, G.; Akhtar, M.M.; Protonotarios, A.; Gossios, T.D.; Savvatis, K.; et al. Dilated cardiomyopathy and arrhythmogenic left ventricular cardiomyopathy: A comprehensive genotype-imaging phenotype study. *Eur. Heart J. Cardiovasc. Imaging* **2020**, *21*, 326–336. [[CrossRef](#)] [[PubMed](#)]



98. Sveinbjornsson, G.; Olafsdottir, E.F.; Thorolfsdottir, R.B.; Davidsson, O.B.; Helgadottir, A.; Jonasdottir, A.; Bjornsson, E.; Jensson, B.O.; Arnadottir, G.A.; Kristinsdottir, H.; et al. Variants in NKX2-5 and FLNC Cause Dilated Cardiomyopathy and Sudden Cardiac Death. *Circ. Genom. Precis. Med.* **2018**, *11*, e002151. [[CrossRef](#)]
99. Chanavat, V.; Janin, A.; Millat, G. A fast and cost-effective molecular diagnostic tool for genetic diseases involved in sudden cardiac death. *Clin. Chim. Acta* **2016**, *453*, 80–85. [[CrossRef](#)]
100. Cerino, M.; Gorokhova, S.; Laforet, P.; Ben Yaou, R.; Salort-Campana, E.; Pouget, J.; Attarian, S.; Eymard, B.; Deleuze, J.F.; Boland, A.; et al. Genetic Characterization of a French Cohort of GNE-mutation negative inclusion body myopathy patients with exome sequencing. *Muscle Nerve* **2017**, *56*, 993–997. [[CrossRef](#)] [[PubMed](#)]
101. Brun, F.; Gigli, M.; Graw, S.L.; Judge, D.P.; Merlo, M.; Murray, B.; Calkins, H.; Sinagra, G.; Taylor, M.R.; Mestroni, L. Truncations cause arrhythmogenic right ventricular cardiomyopathy. *J. Med. Genet.* **2020**, *57*, 254–257. [[CrossRef](#)]
102. Schubert, J.; Tariq, M.; Geddes, G.; Kindel, S.; Miller, E.M.; Ware, S.M. Novel pathogenic variants in filamin C identified in pediatric restrictive cardiomyopathy. *Hum. Mutat.* **2018**, *39*, 2083–2096. [[CrossRef](#)] [[PubMed](#)]
103. Jin, S.C.; Homsy, J.; Zaidi, S.; Lu, Q.; Morton, S.; DePalma, S.R.; Zeng, X.; Qi, H.; Chang, W.; Sierant, M.C.; et al. Contribution of rare inherited and de novo variants in 2,871 congenital heart disease probands. *Nat. Genet.* **2017**, *49*, 1593–1601. [[CrossRef](#)] [[PubMed](#)]
104. Chen, J.; Wu, J.; Han, C.; Li, Y.; Guo, Y.; Tong, X. A mutation in the filamin c gene causes myofibrillar myopathy with lower motor neuron syndrome: A case report. *BMC Neurol.* **2019**, *19*. [[CrossRef](#)] [[PubMed](#)]
105. Tasca, G.; Odgerel, Z.; Monforte, M.; Aurino, S.; Clarke, N.F.; Waddell, L.B.; Udd, B.; Ricci, E.; Goldfarb, L.G. Novel FLNC mutation in a patient with myofibrillar myopathy in combination with late-onset cerebellar ataxia. *Muscle Nerve* **2012**, *46*, 275–282. [[CrossRef](#)] [[PubMed](#)]
106. Reddy, H.M.; Cho, K.A.; Lek, M.; Estrella, E.; Valkanas, E.; Jones, M.D.; Mitsuhashi, S.; Darras, B.T.; Amato, A.A.; Lidov, H.G.; et al. The sensitivity of exome sequencing in identifying pathogenic mutations for LGMD in the United States. *J. Hum. Genet.* **2017**, *62*, 243–252. [[CrossRef](#)]
107. Purevjav, E.; Arimura, T.; Augustin, S.; Huby, A.C.; Takagi, K.; Nunoda, S.; Kearney, D.L.; Taylor, M.D.; Terasaki, F.; Bos, J.M.; et al. Molecular basis for clinical heterogeneity in inherited cardiomyopathies due to myopalladin mutations. *Hum. Mol. Genet.* **2012**, *21*, 2039–2053. [[CrossRef](#)]
108. Bang, M.L.; Mudry, R.E.; McElhinny, A.S.; Trombitás, K.; Geach, A.J.; Yamasaki, R.; Sorimachi, H.; Granzier, H.; Gregorio, C.C.; Labeit, S. Myopalladin, a novel 145-kilodalton sarcomeric protein with multiple roles in Z-disc and I-band protein assemblies. *J. Cell Biol.* **2001**, *153*, 413–428. [[CrossRef](#)]
109. Meyer, T.; Ruppert, V.; Ackermann, S.; Richter, A.; Perrot, A.; Sperling, S.R.; Posch, M.G.; Maisch, B.; Pankuweit, S.; Failure, G.C.N.H. Novel mutations in the sarcomeric protein myopalladin in patients with dilated cardiomyopathy. *Eur. J. Hum. Genet.* **2013**, *21*, 294–300. [[CrossRef](#)]
110. Mastroianno, S.; Palumbo, P.; Castellana, S.; Leone, M.P.; Massaro, R.; Potenza, D.R.; Mazza, T.; Russo, A.; Castori, M.; Carella, M.; et al. Double missense mutations in cardiac myosin-binding protein C and myopalladin genes: A case report with diffuse coronary disease, complete atrioventricular block, and progression to dilated cardiomyopathy. *Ann. Noninvasive Electrocardiol.* **2020**, *25*, e12687. [[CrossRef](#)]
111. Duboscq-Bidot, L.; Xu, P.; Charron, P.; Neyroud, N.; Dilanian, G.; Millaire, A.; Bors, V.; Komajda, M.; Villard, E. Mutations in the Z-band protein myopalladin gene and idiopathic dilated cardiomyopathy. *Cardiovasc. Res.* **2008**, *77*, 118–125. [[CrossRef](#)]
112. Huby, A.C.; Mendsaikhan, U.; Takagi, K.; Martherus, R.; Wansapura, J.; Gong, N.; Osinska, H.; James, J.F.; Kramer, K.; Saito, K.; et al. Disturbance in Z-disk mechanosensitive proteins induced by a persistent mutant myopalladin causes familial restrictive cardiomyopathy. *J. Am. Coll. Cardiol.* **2014**, *64*, 2765–2776. [[CrossRef](#)]
113. Miyatake, S.; Mitsuhashi, S.; Hayashi, Y.K.; Purevjav, E.; Nishikawa, A.; Koshimizu, E.; Suzuki, M.; Yatabe, K.; Tanaka, Y.; Ogata, K.; et al. Biallelic Mutations in MYPN, Encoding Myopalladin, Are Associated with Childhood-Onset, Slowly Progressive Nemaline Myopathy. *Am. J. Hum. Genet.* **2017**, *100*, 169–178. [[CrossRef](#)]
114. Lornage, X.; Malfatti, E.; Chéraud, C.; Schneider, R.; Biancalana, V.; Cuisset, J.M.; Garibaldi, M.; Eymard, B.; Fardeau, M.; Boland, A.; et al. Recessive MYPN mutations cause cap myopathy with occasional nemaline rods. *Ann. Neurol.* **2017**, *81*, 467–473. [[CrossRef](#)]
115. Merlini, L.; Sabatelli, P.; Antoniel, M.; Carinci, V.; Niro, F.; Monetti, G.; Torella, A.; Giugliano, T.; Faldini, C.; Nigro, V. Congenital myopathy with hanging big toe due to homozygous myopalladin (MYPN) mutation. *Skelet Muscle* **2019**, *9*, 14. [[CrossRef](#)]
116. Bagnall, R.D.; Yeates, L.; Semsarian, C. Analysis of the Z-disc genes PDLIM3 and MYPN in patients with hypertrophic cardiomyopathy. *Int. J. Cardiol.* **2010**, *145*, 601–602. [[CrossRef](#)] [[PubMed](#)]
117. Dube, D.K.; Wang, J.; Pellenz, C.; Fan, Y.; Dube, S.; Han, M.; Linask, K.; Sanger, J.W. Expression of myotilin during chicken development. *Anat. Rec. (Hoboken)* **2014**, *297*, 1596–1603. [[CrossRef](#)] [[PubMed](#)]
118. Salmikangas, P.; Mykkänen, O.M.; Grönholm, M.; Heiska, L.; Kere, J.; Carpén, O. Myotilin, a novel sarcomeric protein with two Ig-like domains, is encoded by a candidate gene for limb-girdle muscular dystrophy. *Hum. Mol. Genet.* **1999**, *19*, 3068–3079. [[CrossRef](#)] [[PubMed](#)]
119. Yamaoka, L.H.; Westbrook, C.A.; Speer, M.C.; Gilchrist, J.M.; Jabs, E.W.; Schweins, E.G.; Stajich, J.M.; Gaskell, P.C.; Roses, A.D.; Pericak-Vance, M.A. Development of a microsatellite genetic map spanning 5q31-q33 and subsequent placement of the LGMD1A locus between D5S178 and IL9. *Neuromuscul. Disord.* **1994**, *4*, 471–475. [[CrossRef](#)]

120. Hauser, M.A.; Conde, C.B.; Kowaljow, V.; Zeppa, G.; Taratuto, A.L.; Torian, U.M.; Vance, J.; Pericak-Vance, M.A.; Speer, M.C.; Rosa, A.L. myotilin mutation found in second pedigree with LGMD1A. *Am. J. Hum. Genet.* **2002**, *71*. [[CrossRef](#)]
121. Salmikangas, P.; van der Ven, P.F.M.; Lalowski, M.; Taivainen, A.; Zhao, F.; Suila, H.; Schröder, R.; Lappalainen, P.; Fürst, D.O.; Carpén, O. Myotilin, the limb-girdle muscular dystrophy 1A (LGMD1A) protein, cross-links actin filaments and controls sarcomere assembly. *Hum. Mol. Genet.* **2003**, *12*, 189–230. [[CrossRef](#)]
122. Hauser, M.A. Myotilin is mutated in limb girdle muscular dystrophy 1A. *Hum. Mol. Genet.* **2000**, *9*, 2141–2147. [[CrossRef](#)]
123. Gontier, Y.; Taivainen, A.; Fontao, L.; Sonnenberg, A.; van der Flier, A.; Carpen, O.; Faulkner, G.; Borradori, L. The Z-disc proteins myotilin and FATZ-1 interact with each other and are connected to the sarcolemma via muscle-specific filamins. *J. Cell Sci.* **2005**, *118*, 3739–3749. [[CrossRef](#)]
124. Von Nandelstadh, P.; Ismail, M.; Gardin, C.; Suila, H.; Zara, I.; Belgrano, A.; Valle, G.; Carpen, O.; Faulkner, G. A Class III PDZ Binding Motif in the Myotilin and FATZ Families Binds Enigma Family Proteins: A Common Link for Z-Disk Myopathies. *Mol. Cell. Biol.* **2009**, *29*, 822–834. [[CrossRef](#)]
125. Witt, S.H.; Granzier, H.; Witt, C.C.; Labeit, S. MURF-1 and MURF-2 target a specific subset of myofibrillar proteins redundantly: Towards understanding MURF-dependent muscle ubiquitination. *J. Mol. Biol.* **2005**, *350*, 713–722. [[CrossRef](#)] [[PubMed](#)]
126. Moza, M.; Mologni, L.; Trokovic, R.; Faulkner, G.; Partanen, J.; Carpén, O. Targeted Deletion of the Muscular Dystrophy Gene myotilin Does Not Perturb Muscle Structure or Function in Mice. *Mol. Cell. Biol.* **2007**, *27*, 244–252. [[CrossRef](#)]
127. Selcen, D.; Engel, A.G. Mutations in myotilin cause myofibrillar myopathy. *Neurology* **2004**, *62*, 1363–1371. [[CrossRef](#)]
128. Von Nandelstadh, P.; Grönholm, M.; Moza, M.; Lamberg, A.; Savilahti, H.; Carpén, O. Actin-organising properties of the muscular dystrophy protein myotilin. *Exp. Cell Res.* **2005**, *310*, 131–139. [[CrossRef](#)] [[PubMed](#)]
129. Berciano, J.; Gallardo, E.; Domínguez-Perles, R.; García, A.; García-Barredo, R.; Combarros, O.; Infante, J.; Illa, I. Autosomal-dominant distal myopathy with a myotilin S55F mutation: Sorting out the phenotype. *J. Neurol. Neurosurg. Psychiatry* **2008**, *79*, 205–208. [[CrossRef](#)]
130. Shalaby, S.; Mitsushashi, H.; Matsuda, C.; Minami, N.; Noguchi, S.; Nonaka, I.; Nishino, I.; Hayashi, Y.K. Defective myotilin homodimerization caused by a novel mutation in MYOT exon 9 in the first Japanese limb girdle muscular dystrophy 1A patient. *J. Neuropathol. Exp. Neurol.* **2009**, *68*, 701–707. [[CrossRef](#)] [[PubMed](#)]
131. Ruparelia, A.; Vaz, R.; Bryson-Richardso, R. Myofibrillar Myopathies and the Z-Disk Associated Proteins. *Skelet. Muscle Myogenesis Clin. Relat.* **2012**. [[CrossRef](#)]
132. Olivé, M.; Odgerel, Z.; Martínez, A.; Poza, J.J.; Bragado, F.G.; Zabalza, R.J.; Jericó, I.; Gonzalez-Mera, L.; Shatunov, A.; Lee, H.S.; et al. Clinical and myopathological evaluation of early- and late-onset subtypes of myofibrillar myopathy. *Neuromuscul. Disord.* **2011**, *21*, 533–542. [[CrossRef](#)]
133. Foroud, T.; Pankratz, N.; Batchman, A.P.; Pauciuolo, M.W.; Vidal, R.; Miravalle, L.; Goebel, H.H.; Cushman, L.J.; Azzarelli, B.; Horak, H.; et al. A mutation in myotilin causes spheroid body myopathy. *Neurology* **2005**, *65*, 1936–1940. [[CrossRef](#)]
134. Otey, C.A.; Rachlin, A.; Moza, M.; Arneman, D.; Carpen, O. The palladin/myotilin/myopalladin family of actin-associated scaffolds. *Int. Rev. Cytol.* **2005**, *246*, 31–58. [[CrossRef](#)]
135. Puž, V.; Pavšič, M.; Lenarčič, B.; Djinović-Carugo, K. Conformational plasticity and evolutionary analysis of the myotilin tandem Ig domains. *Sci. Rep.* **2017**, *7*. [[CrossRef](#)]
136. Reilich, P.; Krause, S.; Schramm, N.; Klutzny, U.; Bulst, S.; Zehetmayer, B.; Schneiderat, P.; Walter, M.C.; Schoser, B.; Lochmüller, H. A novel mutation in the myotilin gene (MYOT) causes a severe form of limb girdle muscular dystrophy 1A (LGMD1A). *J. Neurol.* **2011**, *258*, 1437–1444. [[CrossRef](#)] [[PubMed](#)]
137. Schessl, J.; Bach, E.; Rost, S.; Feldkirchner, S.; Kubny, C.; Müller, S.; Hanisch, F.G.; Kress, W.; Schoser, B. Novel recessive myotilin mutation causes severe myofibrillar myopathy. *Neurogenetics* **2014**, *15*, 151–156. [[CrossRef](#)]
138. Rudolf, G.; Suominen, T.; Penttilä, S.; Hackman, P.; Evilä, A.; Lannes, B.; Echaniz-Laguna, A.; Bierry, G.; Tranchant, C.; Udd, B. Homozygosity of the Dominant Myotilin c.179C>T (p.Ser60Phe) Mutation Causes a More Severe and Proximal Muscular Dystrophy. *J. Neuromuscul. Dis.* **2016**, *3*, 275–281. [[CrossRef](#)] [[PubMed](#)]
139. Von Nandelstadh, P.; Souymani, R.; Baumann, M.; Carpen, O. Analysis of myotilin turnover provides mechanistic insight into the role of myotilinopathy-causing mutations. *Biochem. J.* **2011**, *436*, 113–121. [[CrossRef](#)]
140. Djinovic-Carugo, K.; Kostan, J. Structural Insights into F-actin Regulation and Sarcomere Assembly via Myotilin. *Biophys. J.* **2020**. [[CrossRef](#)]
141. Maerkens, A.; Olivé, M.; Schreiner, A.; Feldkirchner, S.; Schessl, J.; Uszkoreit, J.; Barkovits, K.; Güttches, A.K.; Theis, V.; Eisenacher, M.; et al. New insights into the protein aggregation pathology in myotilinopathy by combined proteomic and immunolocalization analyses. *Acta Neuropathol. Commun.* **2016**, *4*. [[CrossRef](#)]
142. Garvey, S.M.; Liu, Y.; Miller, S.E.; Hauser, M.A. Myotilin overexpression enhances myopathy in the LGMD1A mouse model. *Muscle Nerve* **2008**, *37*, 663–667. [[CrossRef](#)]
143. Keduka, E.; Hayashi, Y.K.; Shalaby, S.; Mitsushashi, H.; Noguchi, S.; Nonaka, I.; Nishino, I. In vivo characterization of mutant myotilins. *Am. J. Pathol.* **2012**, *180*, 1570–1580. [[CrossRef](#)]
144. Bührdel, J.B.; Hirth, S.; Keßler, M.; Westphal, S.; Forster, M.; Manta, L.; Wiche, G.; Schoser, B.; Schessl, J.; Schröder, R.; et al. In vivo characterization of human myofibrillar myopathy genes in zebrafish. *Biochem. Biophys. Res. Commun.* **2015**, *461*, 217–223. [[CrossRef](#)]



145. Olivé, M.; van Leeuwen, F.W.; Janué, A.; Moreno, D.; Torrejón-Escribano, B.; Ferrer, I. Expression of mutant ubiquitin (UBB+1) and p62 in myotilinopathies and desminopathies. *Neuropathol. Appl. Neurobiol.* **2008**, *34*, 76–87. [[CrossRef](#)]
146. Valle, G.; Faulkner, G.; De Antoni, A.; Pacchioni, B.; Pallavicini, A.; Pandolfo, D.; Tiso, N.; Toppo, S.; Trevisan, S.; Lanfranchi, G. Telethonin, a novel sarcomeric protein of heart and skeletal muscle. *FEBS Lett.* **1997**, *415*, 163–168. [[CrossRef](#)]
147. Sadikot, T.; Hammond, C.R.; Ferrari, M.B. Distinct roles for telethonin N-versus C-terminus in sarcomere assembly and maintenance. *Dev. Dyn.* **2010**, *239*, 1124–1135. [[CrossRef](#)]
148. Faulkner, G.; Lanfranchi, G.; Valle, G. Telethonin and other new proteins of the Z-disc of skeletal muscle. *IUBMB Life* **2001**, *51*, 275–282.
149. Knöll, R.; Linke, W.A.; Zou, P.; Miodic, S.; Kostin, S.; Buyandelger, B.; Ku, C.H.; Neef, S.; Bug, M.; Schäfer, K.; et al. Telethonin deficiency is associated with maladaptation to biomechanical stress in the mammalian heart. *Circ. Res.* **2011**, *109*, 758–769. [[CrossRef](#)]
150. Hayashi, T.; Arimura, T.; Itoh-Satoh, M.; Ueda, K.; Hohda, S.; Inagaki, N.; Takahashi, M.; Hori, H.; Yasunami, M.; Nishi, H.; et al. Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy. *J. Am. Coll. Cardiol.* **2004**, *44*, 2192–2201. [[CrossRef](#)]
151. Bos, J.M.; Poley, R.N.; Ny, M.; Tester, D.J.; Xu, X.; Vatta, M.; Towbin, J.A.; Gersh, B.J.; Ommen, S.R.; Ackerman, M.J. Genotype-phenotype relationships involving hypertrophic cardiomyopathy-associated mutations in titin, muscle LIM protein, and telethonin. *Mol. Genet. Metab.* **2006**, *88*, 78–85. [[CrossRef](#)]
152. Perrot, A.; Sigusch, H.H.; Nägele, H.; Genschel, J.; Lehmkühl, H.; Hetzer, R.; Geier, C.; Leon Perez, V.; Reinhard, D.; Dietz, R.; et al. Genetic and phenotypic analysis of dilated cardiomyopathy with conduction system disease: Demand for strategies in the management of presymptomatic lamin A/C mutant carriers. *Eur. J. Heart Fail.* **2006**, *8*, 484–493. [[CrossRef](#)]
153. Marziliano, N.; Pilotto, A.; Grasso, M.; Pasotti, M.; Arbustini, E. Deletion of Glu at codon 13 of the TCAP gene encoding the titin-cap-telethonin is a rare polymorphism in a large Italian population. *Mol. Genet. Metab.* **2006**, *89*, 286–287. [[CrossRef](#)]
154. Toste, A.; Perrot, A.; Özcelik, C.; Cardim, N. Identification of a novel titin-cap/telethonin mutation in a Portuguese family with hypertrophic cardiomyopathy. *Rev. Port. Cardiol.* **2020**, *39*, 317–327. [[CrossRef](#)]
155. Hershberger, R.E.; Parks, S.B.; Kushner, J.D.; Li, D.; Ludwigsen, S.; Jakobs, P.; Nauman, D.; Burgess, D.; Partain, J.; Litt, M. Coding sequence mutations identified in MYH7, TNNT2, SCN5A, CSRP3, LBD3, and TCAP from 313 patients with familial or idiopathic dilated cardiomyopathy. *Clin. Transl. Sci.* **2008**, *1*, 21–26. [[CrossRef](#)]
156. Hirtle-Lewis, M.; Desbiens, K.; Ruel, I.; Rudzicz, N.; Genest, J.; Engert, J.C.; Giannetti, N. The genetics of dilated cardiomyopathy: A prioritized candidate gene study of LMNA, TNNT2, TCAP, and PLN. *Clin. Cardiol.* **2013**, *36*, 628–633. [[PubMed](#)]
157. Martins, E.; Sousa, A.; Canedo, P.; Leite, S.; Pinto, R.; Campelo, M.; Amorim, S.; Moura, B.; Lopes, J.M.; Machado, J.C.; et al. Genetic variants identified by target next-generation sequencing in heart transplant patients with dilated cardiomyopathy. *Rev. Port. Cardiol.* **2019**, *38*, 441–447. [[CrossRef](#)] [[PubMed](#)]
158. Moreira, E.S.; Wiltshire, T.J.; Faulkner, G.; Nilforoushan, A.; Vainzof, M.; Suzuki, O.T.; Valle, G.; Reeves, R.; Zatz, M.; Passos-Bueno, M.R.; et al. Limb-girdle muscular dystrophy type 2G is caused by mutations in the gene encoding the sarcomeric protein telethonin. *Nat. Genet.* **2000**. [[CrossRef](#)]
159. Olivé, M.; Shatunov, A.; Gonzalez, L.; Carmona, O.; Moreno, D.; Quereda, L.G.; Martinez-Matos, J.A.; Goldfarb, L.G.; Ferrer, I. Transcription-terminating mutation in telethonin causing autosomal recessive muscular dystrophy type 2G in a European patient. *Neuromuscul. Disord.* **2008**, *18*, 929–933. [[CrossRef](#)] [[PubMed](#)]
160. Francis, A.; Sunitha, B.; Vinodh, K.; Polavarapu, K.; Katkam, S.K.; Modi, S.; Bharath, M.M.; Gayathri, N.; Nalini, A.; Thangaraj, K. Novel TCAP mutation c.32C>A causing limb girdle muscular dystrophy 2G. *PLoS ONE* **2014**, *9*, e102763. [[CrossRef](#)] [[PubMed](#)]
161. Waddell, L.B.; Lek, M.; Bahlo, M.; Bromhead, C.; Jones, K.; North, K.N.; Clarke, N.F. GP 41 The identification of LGMD2G (TCAP) in Australia. *Neuromuscul. Disord.* **2012**, *22*, 831–832. [[CrossRef](#)]
162. Barresi, R.; Morris, C.; Hudson, J.; Curtis, E.; Pickthall, C.; Bushby, K.; Davies, N.P.; Straub, V. Conserved expression of truncated telethonin in a patient with limb-girdle muscular dystrophy 2G. *Neuromuscul. Disord.* **2015**, *25*, 349–352. [[CrossRef](#)]
163. Ikenberg, E.; Karin, I.; Ertl-Wagner, B.; Abicht, A.; Bulst, S.; Krause, S.; Schoser, B.; Reilich, P.; Walter, M.C. Rare diagnosis of telethoninopathy (LGMD2G) in a Turkish patient. *Neuromuscul. Disord.* **2017**, *27*, 856–860. [[CrossRef](#)]
164. Brusa, R.; Magri, F.; Papadimitriou, D.; Govoni, A.; Del Bo, R.; Ciscato, P.; Savarese, M.; Cinnante, C.; Walter, M.C.; Abicht, A.; et al. A new case of limb girdle muscular dystrophy 2G in a Greek patient, founder effect and review of the literature. *Neuromuscul. Disord.* **2018**, *28*, 532–537. [[CrossRef](#)]
165. Chamova, T.; Bichev, S.; Todorov, T.; Gospodinova, M.; Taneva, A.; Kastreva, K.; Zlatareva, D.; Krupev, M.; Hadjiivanov, R.; Guerguelcheva, V.; et al. Limb girdle muscular dystrophy 2G in a religious minority of Bulgarian Muslims homozygous for the c.75G>A, p.Trp25X mutation. *Neuromuscul. Disord.* **2018**, *28*, 625–632. [[CrossRef](#)]
166. Yee, W.; Pramono, Z.; Tan, C.; Kathioravelu, P.; Lai, P.G.P. 8.15 Limb girdle muscular dystrophy 2G and novel TCAP mutations in ethnic Chinese. *Neuromuscul. Disord.* **2007**, *17*, 814. [[CrossRef](#)]
167. Chen, H.; Xu, G.; Lin, F.; Jin, M.; Cai, N.; Qiu, L.; Ye, Z.; Wang, L.; Lin, M.; Wang, N. Clinical and genetic characterization of limb girdle muscular dystrophy R7 telethonin-related patients from three unrelated Chinese families. *Neuromuscul. Disord.* **2020**, *30*, 137–143. [[CrossRef](#)]
168. Markert, C.D.; Meaney, M.P.; Voelker, K.A.; Grange, R.W.; Dalley, H.W.; Cann, J.K.; Ahmed, M.; Bishwokarma, B.; Walker, S.J.; Yu, S.X.; et al. Functional muscle analysis of the Tcap knockout mouse. *Hum. Mol. Genet.* **2010**, *19*, 2268–2283. [[CrossRef](#)] [[PubMed](#)]

169. Zhou, Q.; Chu, P.H.; Huang, C.; Cheng, C.F.; Martone, M.E.; Knoll, G.; Shelton, G.D.; Evans, S.; Chen, J. Ablation of Cypher, a PDZ-LIM domain Z-line protein, causes a severe form of congenital myopathy. *J. Cell Biol.* **2001**, *155*, 605–612. [[CrossRef](#)] [[PubMed](#)]
170. Sheikh, F.; Bang, M.L.; Lange, S.; Chen, J. “Z”eroing in on the Role of Cypher in Striated Muscle Function, Signaling, and Human Disease. *Trends Cardiovasc. Med.* **2007**, *17*, 258–262. [[CrossRef](#)] [[PubMed](#)]
171. Martinelli, V.C.; Kyle, W.B.; Kojic, S.; Vitulo, N.; Li, Z.; Belgrano, A.; Maiuri, P.; Banks, L.; Vatta, M.; Valle, G.; et al. ZASP interacts with the mechanosensing protein Ankrd2 and p53 in the signalling network of striated muscle. *PLoS ONE* **2014**, *9*, e92259. [[CrossRef](#)] [[PubMed](#)]
172. Faulkner, G.; Pallavicini, A.; Formentin, E.; Comelli, A.; Ievolella, C.; Trevisan, S.; Bortoletto, G.; Scannapieco, P.; Salamon, M.; Mouly, V.; et al. ZASP: A new Z-band alternatively spliced PDZ-motif protein. *J. Cell Biol.* **1999**, *146*, 267–272. [[CrossRef](#)] [[PubMed](#)]
173. Au, Y.; Atkinson, R.A.; Guerrini, R.; Kelly, G.; Joseph, C.; Martin, S.R.; Muskett, F.W.; Pallavicini, A.; Faulkner, G.; Pastore, A. Solution structure of ZASP PDZ domain: Implications for sarcomere ultrastructure and enigma family redundancy. *Structure* **2004**, *12*, 611–622. [[CrossRef](#)]
174. Knappeis, G.G.; Carlsen, F. The ultrastructure of the Z disc in skeletal muscle. *J. Cell Biol.* **1962**, *13*, 323–335. [[CrossRef](#)]
175. González-Morales, N.; Xiao, Y.S.; Schilling, M.A.; Marescal, O.; Liao, K.A.; Schöck, F. Myofibril diameter is set by a finely tuned mechanism of protein oligomerization in. *Elife* **2019**. [[CrossRef](#)]
176. Lin, X.; Ruiz, J.; Bajraktari, I.; Ohman, R.; Banerjee, S.; Gribble, K.; Kaufman, J.D.; Wingfield, P.T.; Griggs, R.C.; Fischbeck, K.H.; et al. Z-disc-Associated, alternatively spliced, PDZ motif-containing protein (ZASP) mutations in the actin-binding domain cause disruption of skeletal muscle actin filaments in myofibrillar myopathy. *J. Biol. Chem.* **2014**, *289*, 13615–13626. [[CrossRef](#)]
177. Van der Meer, D.L.M.; Marques, I.J.; Leito, J.T.D.; Besser, J.; Bakkers, J.; Schoonheere, E.; Bagowski, C.P. Zebrafish cypher is important for somite formation and heart development. *Dev. Biol.* **2006**, *299*, 356–372. [[CrossRef](#)]
178. Vatta, M.; Mohapatra, B.; Jimenez, S.; Sanchez, X.; Faulkner, G.; Perles, Z.; Sinagra, G.; Lin, J.H.; Vu, T.M.; Zhou, Q.; et al. Mutations in Cypher/ZASP in Patients with Dilated Cardiomyopathy and Left Ventricular Non-Compaction. *J. Am. Coll. Cardiol.* **2003**, *42*, 2014–2027. [[CrossRef](#)]
179. Arimura, T.; Hayashi, T.; Terada, H.; Lee, S.Y.; Zhou, Q.; Takahashi, M.; Ueda, K.; Nouchi, T.; Hohda, S.; Shibutani, M.; et al. A Cypher/ZASP Mutation Associated with Dilated Cardiomyopathy Alters the Binding Affinity to Protein Kinase C. *J. Biol. Chem.* **2004**, *279*, 6746–6752. [[CrossRef](#)] [[PubMed](#)]
180. Lopez-Ayala, J.M.; Ortiz-Genga, M.; Gomez-Milanes, I.; Lopez-Cuenca, D.; Ruiz-Espejo, F.; Sanchez-Munoz, J.J.; Oliva-Sandoval, M.J.; Monserrat, L.; Gimeno, J.R. A mutation in the Z-line Cypher/ZASP protein is associated with arrhythmogenic right ventricular cardiomyopathy. *Clin. Genet.* **2015**, *88*, 172–176. [[CrossRef](#)]
181. Xing, Y.; Ichida, F.; Matsuoka, T.; Isobe, T.; Ikemoto, Y.; Higaki, T.; Tsuji, T.; Haneda, N.; Kuwabara, A.; Chen, R.; et al. Genetic analysis in patients with left ventricular noncompaction and evidence for genetic heterogeneity. *Mol. Genet. Metab.* **2006**, *88*, 71–77. [[CrossRef](#)]
182. Watts, N.R.; Zhuang, X.; Kaufman, J.D.; Palmer, I.W.; Dearborn, A.D.; Coscia, S.; Blech-Hermoni, Y.; Alfano, C.; Pastore, A.; Mankodi, A.; et al. Expression and Purification of ZASP Subdomains and Clinically Important Isoforms: High-Affinity Binding to G-Actin. *Biochemistry* **2017**, *56*, 2061–2070. [[CrossRef](#)] [[PubMed](#)]
183. Arimura, T.; Inagaki, N.; Hayashi, T.; Shichi, D.; Sato, A.; Hinohara, K.; Vatta, M.; Towbin, J.A.; Chikamori, T.; Yamashina, A.; et al. Impaired binding of ZASP/Cypher with phosphoglucosylase 1 is associated with dilated cardiomyopathy. *Cardiovasc. Res.* **2009**, *83*, 80–88. [[CrossRef](#)]
184. Cai, H.; Yabe, I.; Sato, K.; Kano, T.; Nakamura, M.; Hozen, H.; Sasaki, H. Clinical, pathological, and genetic mutation analysis of sporadic inclusion body myositis in Japanese people. *J. Neurol.* **2012**, *259*, 1913–1922. [[CrossRef](#)] [[PubMed](#)]
185. Selcen, D. Myofibrillar myopathies. *Handb. Clin. Neurol.* **2011**, *101*, 143–154. [[CrossRef](#)]
186. Selcen, D.; Engel, A.G. Mutations in ZASP define a novel form of muscular dystrophy in humans. *Ann. Neurol.* **2005**, *57*, 269–276. [[CrossRef](#)]
187. Griggs, R.; Vihola, A.; Hackman, P.; Talvinen, K.; Haravuori, H.; Faulkner, G.; Eymard, B.; Richard, I.; Selcen, D.; Engel, A.; et al. Zaspopathy in a large classic late-onset distal myopathy family. *Brain* **2007**, *130*, 1477–1484. [[CrossRef](#)]
188. Laneuville, M.; Woulfe, J.; Bourque, P.; McMillan, H.; Dymont, D.; Warman Chardon, J. LDB3/ZASP-related myofibrillar myopathy associated with marked phenotypic variability. *Neuromuscular Disord.* **2016**, *26*, S192. [[CrossRef](#)]
189. Zheng, J.; Chen, S.; Chen, Y.; Zhu, M.; Hong, D. A novel mutation in the PDZ-like motif of ZASP causes distal ZASP-related myofibrillar myopathy. *Neuropathology* **2017**, *37*, 45–51. [[CrossRef](#)]
190. Cassandrini, D.; Merlini, L.; Pilla, F.; Cenni, V.; Santi, S.; Faldini, C.; Santorelli, F.M.; Sabatelli, P. Protein aggregates and autophagy involvement in a family with a mutation in Z-band alternatively spliced PDZ-motif protein. *Neuromuscul. Disord.* **2020**, *31*, 44–51. [[CrossRef](#)]
191. Ohsawa, N.; Koebis, M.; Suo, S.; Nishino, I.; Ishiura, S. Alternative splicing of PDLIM3/ALP, for  $\alpha$ -actinin-associated LIM protein 3, is aberrant in persons with myotonic dystrophy. *Biochem. Biophys. Res. Commun.* **2011**, *409*, 64–69. [[CrossRef](#)]
192. Machuca-Tzili, L.; Thorpe, H.; Robinson, T.E.; Sewry, C.; Brook, J.D. Flies deficient in Muscleblind protein model features of myotonic dystrophy with altered splice forms of Z-band associated transcripts. *Hum. Genet.* **2006**, *120*, 487–499. [[CrossRef](#)] [[PubMed](#)]

193. Claeys, K.G.; van der Ven, P.F.; Behin, A.; Stojkovic, T.; Eymard, B.; Dubourg, O.; Laforêt, P.; Faulkner, G.; Richard, P.; Vicart, P.; et al. Differential involvement of sarcomeric proteins in myofibrillar myopathies: A morphological and immunohistochemical study. *Acta Neuropathol.* **2009**, *117*, 293–307. [[CrossRef](#)]
194. Karczewski, K.J.; Francioli, L.C.; Tiao, G.; Cummings, B.B.; Alföldi, J.; Wang, Q.; Collins, R.L.; Laricchia, K.M.; Ganna, A.; Birnbaum, D.P.; et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* **2020**, *581*, 434–443. [[CrossRef](#)] [[PubMed](#)]
195. Danisovic, L.; Culenova, M.; Csobonyeiova, M. Induced Pluripotent Stem Cells for Duchenne Muscular Dystrophy Modeling and Therapy. *Cells* **2018**, *7*, 253. [[CrossRef](#)] [[PubMed](#)]
196. Doudna, J.A.; Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. *Science* **2014**, *346*, 1258096. [[CrossRef](#)]