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Cardiomyopathies: When the Goliaths of Heart Muscle Hurt

Maegen A. Ackermann and
Aikaterini Kontrogianni-Konstantopoulos

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1. Introduction

Cardiomyopathy, a primary cause of human death, is defined as a disease of the myocardium, which results in insufficient pumping of the heart. It is classified into four major forms; hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RMC), and arrhythmogenic right ventricular cardiomyopathy (ARVC) [1]. These are characterized by extensive remodeling of the myocardium initially manifested as hypertrophy, evidenced by an increase in the thickness of the left ventricular wall and interventricular septum due to interstitial fibrosis and enlarged myocyte size. Following hypertrophy the heart muscle reverts to a dilated state, characterized by a profound expansion of the intraventricular volume and a modest increase in ventricular wall thickness [2]. These changes, initially compensatory, eventually become maladaptive.

During the past ~20 years, several mutations in genes encoding sarcomeric proteins have been causally linked to cardiomyopathies [1]. Among the long list of affected proteins are three members of the family of giant sarcomeric proteins of striated muscles: titin, nebulin, a member of the nebulin subfamily, and obscurin, each encoded by single genes namely *TTN*, *NEBL*, and *OBSCN*, respectively [3]-[10]. This chapter will briefly describe the molecular structure of these genes, assisting the reader to excellent detailed reviews when appropriate, and further provide a comprehensive and up-to-date listing of the mutations that have been identified and directly linked to the development of cardiomyopathy.

2. Titin

Titin, the largest member of the superfamily of giant sarcomeric proteins, is a 3-4 MDa protein encoded by the single *TTN* gene. The 363 exons that make up *TTN* undergo extensive alternative splicing resulting in the expression of several large variants of the protein [11]. A single titin molecule spans a half sarcomere, with its NH₂-terminus anchored to the Z-disc and its COOH-terminus extending into the M-band [11]-[14]. Titin possesses a modular structure, composed mainly of immunoglobulin (Ig) and fibronectin type III (FN-III) domains. Specifically, its Z-disc portion is composed of Ig domains along with ~45-residue long repeats unique to that region (Z-repeats) and possesses binding sites for several sarcomeric proteins (Figure 1) [7]. The portion of titin that spans the I-band is composed mainly of Ig domains intersected by titin specific N2A and N2B regions along with several ~30-residue long PEVK repeats (Figure 2). Titin's I-band region holds binding sites for actin and thin filament proteins, as well as docking sites for many signaling molecules [7]. Within its A-band portion, titin is organized in repeats containing numerous FN-III domains interspersed by Ig domains, which provide repetitive binding sites for myosin and thick filament associated proteins (Figure 3) [7]. The portion of titin that extends into the M-band begins with a Ser/Thr kinase domain followed by additional Ig domains and M-band specific insertions (Figure 4) [7]. Through this region, titin interacts with many other structural proteins to form a scaffold at the M-band.

Within the sarcomere, titin, through its PEVK domain, functions as a “molecular spring,” contributing to the biomechanical properties and structural integrity of striated muscle cells during the contractile cycle [15], [16]. In addition, it acts as a “molecular blueprint” coordinating the assembly of structural, regulatory, and contractile proteins [17]. Given the elastic nature and scaffolding role of titin, it is not surprising that mutations along the length of *TTN* are intimately associated with the development of cardiomyopathy.

To date, at least 107 mutations in *TTN* have been causally linked to HCM, DCM, and ARVC. Many of these mutations occur within essential binding sites along its length, with a high incidence of mutations occurring within the region that spans the A-band, disrupting the ability of titin to bind to myosin thick filaments. The remaining mutations are present within the extensible region of titin affecting its ability to respond to the constant stretching of the sarcomere during repeating cycles of contraction and relaxation. Notably, 84 of these mutations, found exclusively in the I- and A-band regions of titin, alter the length of the protein. Thus, mutations within *TTN* compromise the structural integrity of sarcomeres and lead to impaired contractile activity of cardiac muscle cells.

2.1. Cardiomyopathy linked mutations within titin's Z-disc region

The extreme NH₂-terminus of titin is only mildly affected by cardiomyopathy-causing mutations; only 4 missense mutations and 3 insertion/deletion (indel) polymorphisms have been identified within the Z-disc portion of titin. A missense mutation identified in codon 54 leads to conversion of a valine residue to methionine (V54M) in the region encoding the first Ig domain of titin [18]. The V54M mutation is located in the telethonin-binding domain of titin and functional analysis revealed a decrease of titin's ability to interact with telethonin in the

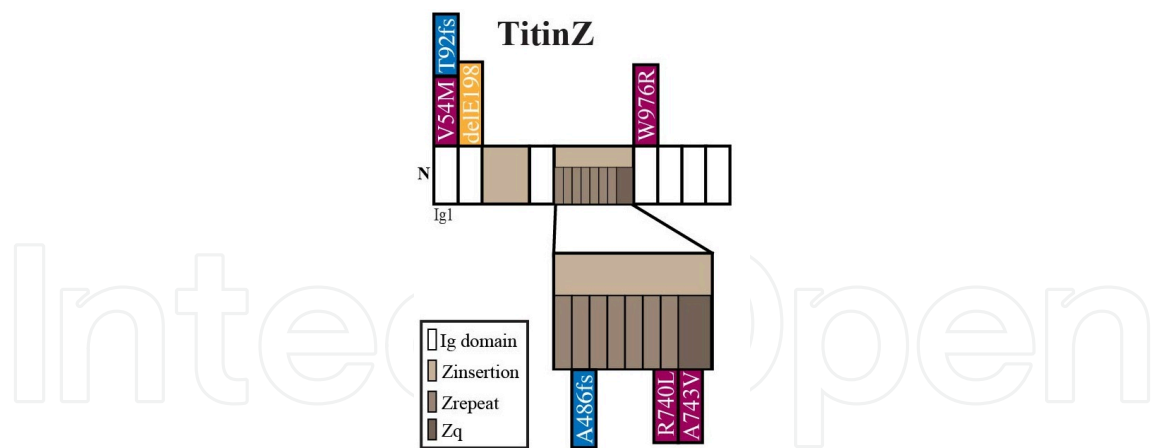


Figure 1. Schematic representation of the Z-disc region of titin, illustrating its motifs and cardiomyopathy associated mutations. Insertions or deletions predicted to cause frame shifts (fs) and single amino acid deletions (del) are noted in blue and yellow, respectively. Missense mutations are shown with a magenta background. Mutations in the *TTN* gene within this region are shown relative to the domains in which they are found.

presence of this mutation [18]. Sequencing of the DNA encoding the Z-disc region of titin in patients with HCM revealed a G to T transversion in codon 740 that is located within the 7th Z-repeat of titin and results in the replacement of an arginine residue with leucine (R740L) [19]. The mutation was not found in DNA from corresponding controls, suggesting it is not a polymorphism. Yeast two-hybrid assays showed that the mutation increased binding to α -actinin by ~40% [19]. Interestingly the opposite biochemical effect was observed in a father and daughter with DCM where a point mutation in codon 743 resulting in an alanine to valine (A743V) conversion in the Zq region of titin was identified [18]. The A743V mutation, which is also localized within the α -actinin binding site on titin, significantly decreases the binding capacity of titin for α -actinin [18]. Additionally, a kindred with autosomal dominant DCM was analyzed and shown to have a point mutation in exon 18 encoding Ig4 that results in the conversion of a tryptophan residue to arginine (W976R), but the functional consequence of this mutation is currently unknown [20]. Recently, Golbus et al identified 3 indels within the Z-disc portion of titin in a large population of individuals exhibiting cardiac disease [5]. Due to the nature of this extensive study, phenotypic data regarding the subjects is unavailable, however they have been linked to either DCM or HCM.

2.2. Cardiomyopathy linked mutations within the region of titin that spans the I-band region

To date, several mutations within the region of titin that spans the I-band have been linked to DCM and HCM. In particular, three mutations within titin's N2B region at the beginning of its I-band portion have been identified. A missense mutation in the N2B region of titin (S3753Y) was identified in two siblings with familial HCM [18], and shown to increase binding to four-and-a-half-LIM domain protein 2 (FLH2) by ~26% in a yeast two-hybrid assay [21]. In addition, in patients exhibiting a DCM phenotype a transversion of C to T in codon 4007 was found to result in the conversion of a glutamine residue to an early termination codon (Q4007X), and another missense mutation in codon 4417 was identified to replace a serine residue with an asparagine (S4417N) [18]. The premature stop codon (Q4007X) occurs just prior to the binding

site for FHL2 while the S4417N mutation decreases the binding capacity of titin for FHL2, as determined by yeast two-hybrid studies [21]. Within titin's PEVK region 3 missense mutations have been identified in DCM and HCM patients (G3470D, R8500H, and R8604Q) [22], [23]. Mutations R8500H and R8604Q were shown to increase the binding capacity of titin for cardiac ankyrin repeat protein (CARP) as determined by coimmunoprecipitation assays [22]. The pathogenicity of the G3470D is still unknown, however. Moreover, using population based studies of DCM and HCM patients, Golbus et al recently identified 9 indels within the portion of titin that spans the I-band [5].

In a large study, using next generation sequencing, Herman et al analyzed 203 and 231 patients with DCM and HCM, respectively and the corresponding control subjects for mutations in the *TTN* gene [6]. The frequency of *TTN* polymorphisms was significantly higher in DCM than HCM patients or normal subjects. Interestingly, of the mutations identified, all were shown to cause alterations in full-length titin, many of which caused early termination. Two of these truncations occurred as a result of missense mutations within the N2B region and also within Ig94 following the PEVK region. In addition, several splice site donor/acceptor mutations were found to cause truncations within the PEVK region and Ig85. The study also revealed 3 deletion and 2 insertion mutations affecting the I-band region of titin. Specifically, frameshift mutations within Ig11, 45, 61, and 85 alter the length of the protein, while a large duplication of exons 72-124 corresponding to Ig50 through the PEVK region increased *TTN*'s already large size by ~28kb. The functional significance of these mutations, which alter the length of full-length titin, is currently unknown.

Studies linking *TTN* to the development of DCM and HCM date back about a decade, however, it is only recently that *TTN* has been linked to ARVC. A recent study using DNA screening of patients diagnosed with ARVC revealed 3 missense mutations along the I-band region of titin [9]. The study identified a threonine to isoleucine transversion within codon 2896 (T2896I) located in Ig16 as well as two mutations within the PEVK region (Y8031C and H8848Y). Proteomic techniques revealed that the T2896I mutation reduces the structural stability of Ig16 and increases its propensity for degradation [9]. The pathogenicity of the other two mutations has not yet been determined.

2.3. Cardiomyopathy linked mutations within titin's A-band region

The region of titin spanning the A-band can be considered a "hot spot" for cardiomyopathy-linked changes with an overwhelming 63 identified mutations. In the early 2000's, Gerull et al analyzed two siblings with autosomal dominant DCM and identified a unique mutation in titin [20]. A 2-bp insertion mutation in exon 326, caused a frameshift at K20995 within Ig115 resulting in a premature stop codon leading to proteolytic degradation of titin, probably near or within the PEVK domain, as determined by antibody labeling [20]. A few years later, the same group discovered a frameshift mutation at A27460 within FN-III107, also resulting in a premature stop codon, and proteolytic degradation of titin [24].

Recently, two additional insertions within FN-III domains present at the A-band have been identified in patients with DCM. The first causes a frame shift at S19628 and the second a frameshift at G26124 resulting in early termination within domains FN-III 42 and 97, respec-

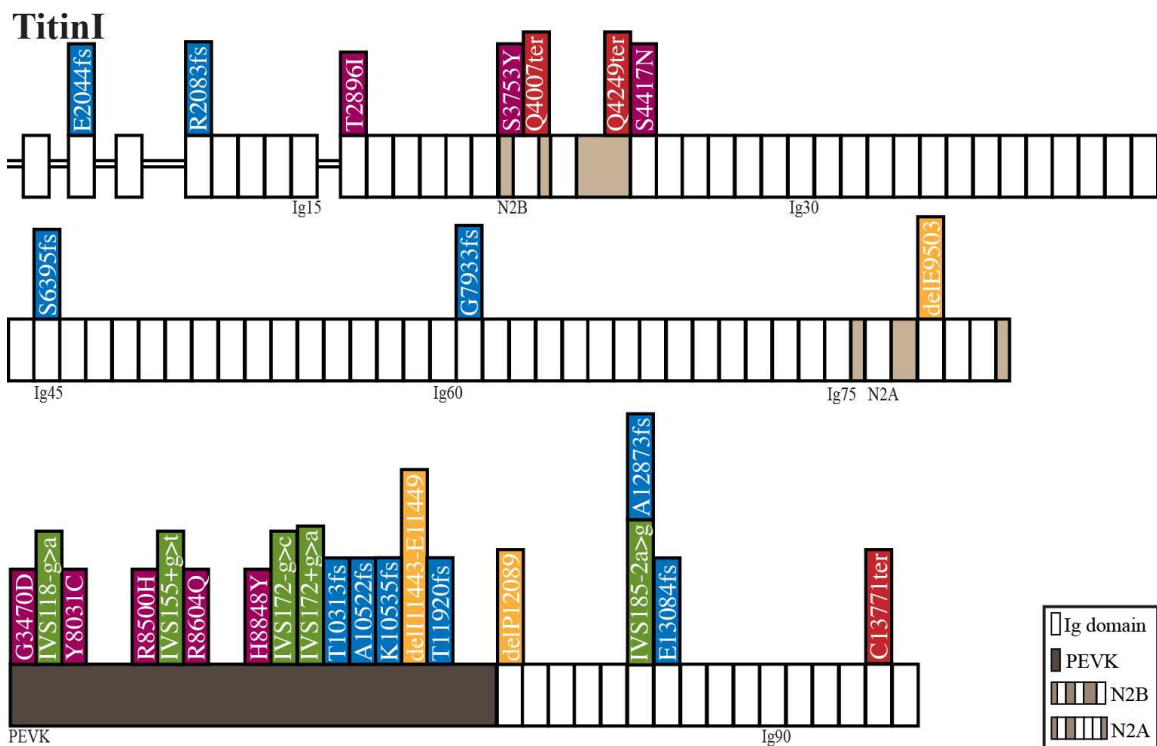


Figure 2. Schematic representation of the I-band region of titin, illustrating its motifs and cardiomyopathy associated mutations. Mutations in the *TTN* gene within this region are shown relative to the domains in which they are found. Insertions or deletions predicted to cause frame shifts (fs) and single amino acid deletions (del) are noted in blue and yellow, respectively. Missense mutations are shown with a magenta background. A red background indicates nonsense mutations resulting in premature stop codons (ter). Splice site donor/acceptor mutations are shown in green.

tively [10]. In addition to the several length altering mutations noted within the I-band region of titin by Herman et al, many more have been found within the region spanning the A-band [6]. An astonishing 23 missense mutations have been identified to cause early termination within several of the Ig and FN-III domains throughout the A-band. Another 11 splice site donor/acceptor mutations were found to cause truncations throughout the A-band. In addition, 13 deletion, 4 insertion, and 2 insertion/deletion mutations have been shown to cause frameshifts in the coding region of titin, resulting in altered full-length titin protein. The functional significance of these mutations is not yet known, however. Moreover, Golbus et al recently identified 3 indels within the portion of titin that spans the A-band in a large population of individuals exhibiting cardiac disease [5]. Due to the nature of this extensive study phenotypic data regarding the subjects is unavailable, however they have been linked to either DCM or HCM.

In addition, 4 missense mutations affecting patients with ARVC have been identified within the A-band region of titin. DNA screening of patients exhibiting signs of ARVC revealed 4 missense mutations affecting both FN-III and Ig domains along the A-band region of titin (I16949T, A18579T, A19309S, P30847L) [9]. The molecular effects of these mutations have not yet been determined.

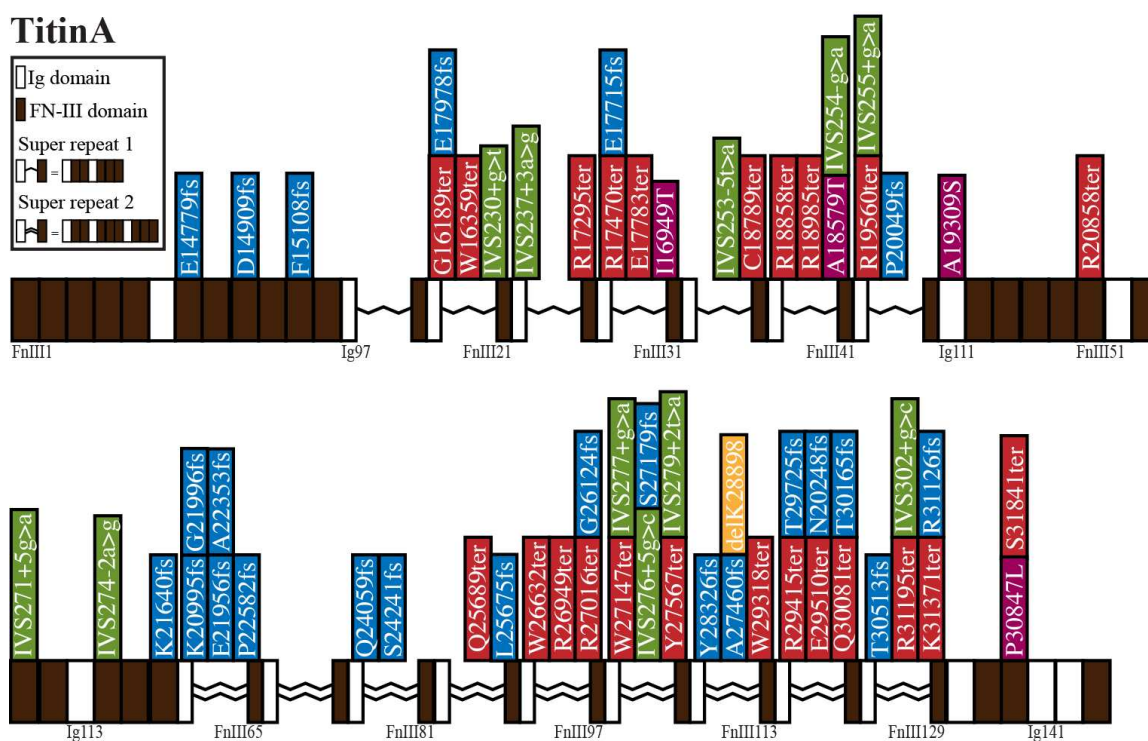


Figure 3. Schematic representation of the A-band region of titin, illustrating its motifs and cardiomyopathy-associated mutations. Mutations in the *TTN* gene within this region are shown relative to the domains in which they are found. Insertions or deletions predicted to cause frame shifts (fs) and single amino acid deletions (del) are noted in blue and yellow, respectively. A red background indicates nonsense mutations resulting in premature stop codons (ter). Splice site donor/acceptor mutations are shown in green. Missense mutations are shown with a magenta background.

2.4. Cardiomyopathy linked mutations within the region of titin that extends into the M-band

Similar to the NH₂-terminus of titin, the COOH-terminus remains relatively unaffected by cardiomyopathy causing mutations. A total of 6 mutations have been described within the portion of titin that extends into the M-band. Sequencing of DNA from patients with DCM and ARVC has identified 2 missense mutations localized to the M-band region of titin. Specifically, in two related individuals exhibiting late-onset DCM, an arginine to glutamine conversion at amino acid 32069 was identified (R32069Q) [21]. In addition, a patient diagnosed with ARVC possessed a methionine to threonine transition at codon 33291 (M33291T) [9]. These mutations localize to Ig146 and Ig152, respectively, however, their pathogenicity has not yet been determined. A recent study using population based analysis of DCM and HCM patients, identified 2 indels within the M-band portion of titin [5]. Phenotypic data regarding the subjects, as well as the mechanistic affects of the mutations are unavailable. Interestingly, 2 deletion mutations within the M-band region of titin were identified in 2 non-related families exhibiting early onset myopathy, affecting skeletal muscle, with fatal cardiomyopathy. Sequence analysis indicated a deletion mutation of 1 bp in exon 360 (Mex3) and an 8 bp deletion in exon 358 (Mex1) [25]. Both deletions left the titin kinase domain intact but resulted in premature stop codons at Ig domains 147 and 150 and a loss of the COOH-terminal 447 and

Nebulin Superfamily

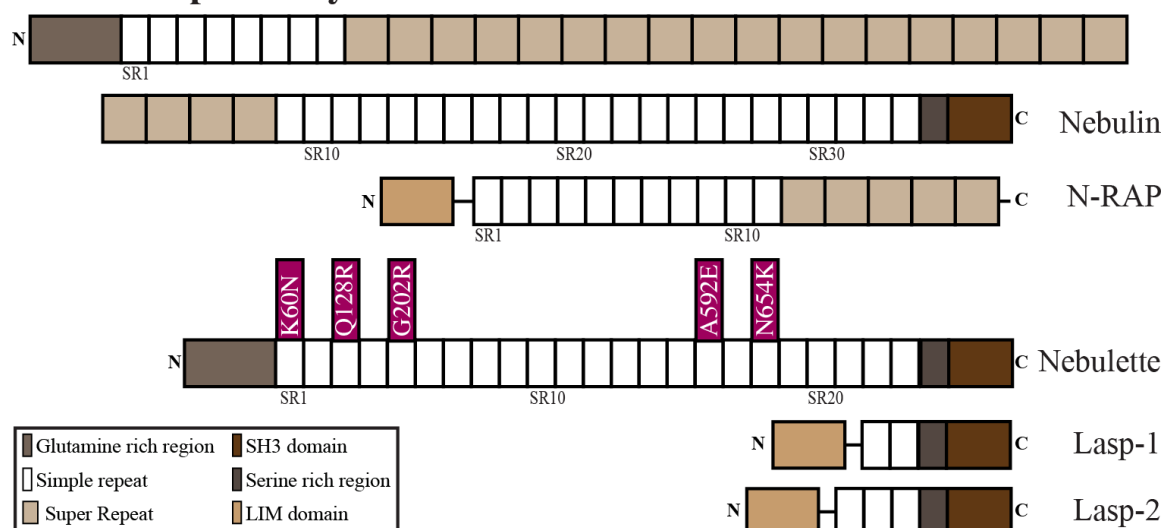


Figure 5. Schematic representation of the nebulin superfamily members, illustrating their motifs and cardiomyopathy associated mutations. Mutations in the *NEBL* gene are shown relative to the domains in which they are found. Missense mutations are shown with a magenta background.

In addition to being a member of the family of large sarcomeric proteins of striated muscle, nebulin is also a member of a family of actin-binding cytoskeletal proteins, which includes N-RAP, nebulin, nebulin-like protein 1 (Nebulette), LASP-1, and LASP-2 (Figure 5). The unifying domain of nebulin family members is the actin binding nebulin-repeat, of ~35 amino acids in length, each containing an SDxxYK motif [34]. For the remainder of the chapter, we will focus on nebulin-like protein 1 (Nebulette) as it is the only member of the nebulin family that has been linked to cardiomyopathies. Cardiac specific nebulin-like protein 1 (Nebulette) is functionally similar to nebulin whereby it aids in the stabilization of actin filaments [26]. Nebulette localizes to the Z-disc [35], where it interacts with the thin filament proteins troponin and tropomyosin [36]. Mutations in the nebulin-like protein 1 gene, *NEBL*, which cause disruption of the stabilization of the Z-disc, have been linked to the development of cardiomyopathy [4], [8].

Nebulette's involvement in cardiomyopathies was first identified in the early 2000's when Arimura et al screened *NEBL* paired normal subjects and patients with idiopathic dilated cardiomyopathy (IDC) for mutations in the nebulin-like protein 1 gene [4]. The study identified several polymorphisms in *NEBL* with one variant showing a high frequency in patients with non-familial IDC. Patients carrying this variant possess a missense mutation, N654K, in the 18th nebulin repeat of nebulin-like protein 1. The mechanism by which this mutation causes disruptions in the heart is unknown, however due to its location this mutation likely disrupts nebulin-like protein 1's incorporation into the Z-disc. Notably, this observation has brought about a new role for nebulin-like protein 1 as a genetic marker for patients with non-familial IDC.

Recently, more direct evidence for the involvement of nebulin-like protein 1 in the development of heart disease was demonstrated in several patients diagnosed with DCM [8]. Linkage analysis revealed four sequence variations in the *NEBL* gene in regions encoding nebulin-repeats along the length of the molecule. Specifically, variants K60N, Q128R, and G202R are located in

nebulin-repeats that bind to F-actin and the tropomyosin-troponin complex, while A592E is located in the region that is incorporated into the Z-disc. Variants K60N and G202R found in nebulin-repeats 1 and 5, respectively, were identified in adult patients developing clinical manifestations of DCM. On the contrary variant Q128R, located in nebulin-repeat 3, was identified in a newborn patient diagnosed with DCM and endocardial fibroelastosis. The remaining variant, A592E, located in nebulin-repeat 16 was also found in a newborn displaying clinical features of DCM. *In vivo* studies using cardiac specific nebulin mutant mice were able to recapitulate human cardiac disease phenotypes and begun to unravel the mechanisms by which these mutations affect cardiac function [8]. Specifically, variants K60N and Q128R were embryonic lethal with hearts exhibiting structural abnormalities. Additionally, mutant mice carrying G202R or A592E variants resulted in left ventricular dilation and impaired cardiac function. These functional defects were coupled with improper localization of mutant nebulin resulting in dramatic structural alterations in I-band and Z-disc proteins. Taken together these studies suggest that nebulin is required for normal maintenance of the sarcomere and stability of the Z-disc and identifies *NEBL* as a contributor to the development of ICD and DCM.

4. Obscurin

Obscurin is the third giant protein of the contractile apparatus of striated muscles. Similar to titin and nebulin the obscurin gene, *OBSCN*, gives rise to a large (~720 kDa; obscurin A) multidomain protein composed mainly of Ig and FN-III domains [7]. In addition, obscurin possesses several signaling motifs within its COOH-terminal half, including an IQ motif, an SH3 domain, as well as tandem Rho Guanine Nucleotide Exchange Factor (RhoGEF) and Pleckstrin Homology (PH) motifs. Similar to *TTN* and *NEB*, *OBSCN* is also subjected to alternative splicing giving rise to several isoforms of differing sizes (Figure 6) [37]. Specifically, the *OBSCN* gene gives rise to another large isoform, referred to as obscurin-B, which has a molecular mass of ~870 kDa. Obscurin B contains two serine/threonine kinase domains, which replace the non-modular COOH-terminus of obscurin A. The kinase domains may be expressed independently as smaller isoforms, containing one or both kinase domains (sMLCK or tMLCK, respectively). Unlike its counterparts, obscurins surround the sarcomere at the level of the Z-disc and M-band, where they are appropriately positioned to interact with several ligands and participate in their assembly and integration into the sarcomere and internal membrane systems [7]. Despite its large size, it is only recently that we learn of *OBSCN*'s linkage to cardiomyopathies.

Although the role of *OBSCN* in cardiomyopathies is still unclear, several studies have documented an upregulation of obscurins during cardiac hypertrophic responses to pressure overload and myopathic responses to mutations in titin [38]-[40]. In addition, up-regulation of different *OBSCN* gene products, including full length obscurin and several of the smaller MLCK variants was reported to occur in mice with myocardial hypertrophy induced by aortic constriction [38]. This increase in expression was mainly observed in obscurin isoforms that contained the RhoGEF and kinase signaling motifs, and occurred early in the hypertrophic

response and also during hypertrophic growth. Concurrent with this, targeted loss of the obscurin RhoGEF domain resulted in myocytes lacking intercalated discs and in more severe cases in failure of the contractile filaments to organize into mature sarcomeres [41]. It is likely that upregulation of obscurins, is associated with the increase in contractile structures observed during hypertrophy, however, the mechanism by which this occurs remains unresolved.

More direct evidence for the involvement of *OBSCN* in the development of heart disease was demonstrated in a single patient with HCM [3]. Linkage analysis revealed a sequence variation in the *OBSCN* gene in the region encoding the site of interaction for the Z-disc region of titin (Ig58/59), specifically an R4344Q variant in the Ig58 domain of obscurin. *In vitro* studies showed that this variant resulted in decreased binding of obscurin to titin as well as mis-localization of obscurin to the Z-disc. Despite this single case, it suggests that, like titin and nebulin, mutations in the *OBSCN* gene lead to the development cardiomyopathies.

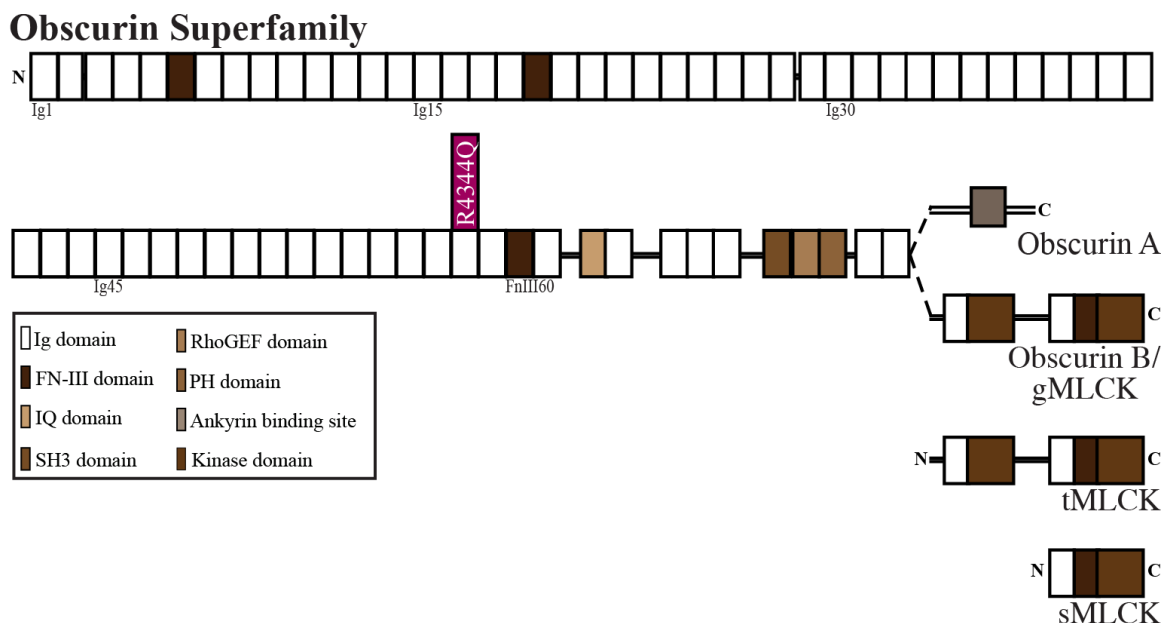


Figure 6. Schematic representation of the obscurin isoforms, illustrating their motifs and cardiomyopathy associated mutation. The missense mutation in the *OBSCN* gene is shown relative to the domain in which it is localized with a magenta background.

5. Concluding remarks

Over two decades ago the first HCM-causing mutation in a sarcomeric gene was identified in β -myosin heavy chain. Since then both HCM and DCM have come to be known as diseases of the sarcomere. In fact, sarcomeric dysfunction is the underlying cause of many genetically mediated HCM and DCM disorders and accounts for ~60% of HCM and ~10-20% of DCM reported cases [42]-[45]. To date, more than 1500 distinct mutations of sarcomeric proteins have been linked to cardiomyopathies [46], [47]. Given the many roles that have been described for

titin, nebulin, and obscurin in cardiac muscle, and the effects of the identified mutations in their localization, activity, and regulation, it is not surprising that many human diseases of heart muscle have been linked to these proteins. Notably, a striking 50 missense mutations within *TTN*, *NEBL*, and *OBSCN* (Table 1) with an additional 16 splice site donor/acceptor mutations (Table 2) and 47 deletion or insertion mutations within *TTN* (Table 3) have been associated with the development of different forms of cardiomyopathy. The severity of these diseases can vary from moderate to severe, depending on the nature of the mutation. The characterization of these mutations and their effects on cardiac pathophysiology is just beginning to be elucidated, however it is clear that this is just the tip of the iceberg. Understanding how these mutations alter sarcomeric structure and contractile activity could aid in improving clinical diagnosis and developing individualized therapies for cardiomyopathic patients.

| Missense Mutations of Titin, Nebulette, and Obscurin | | | | | |
|--|----------|-------------------|-------------------|---------------------------------------|-----------|
| Disease | Mutation | Region on Protein | Sarcomeric Region | Effect | Reference |
| Mutations of TTN | | | | | |
| DCM | V54M* | Ig1 | Z-disc | Reduced binding to telethonin | [18] |
| HCM | R740L* | Z-repeat 7 | Z-disc | Increase binding to α -actinin | [19] |
| DCM | A743V* | Zq region | Z-disc | Reduce binding to α -actinin | [18] |
| DCM | W976R* | Ig4 | Z-disc | Unknown | [20] |
| ARVC | T2896I* | Ig16 | I-band | Unknown | [9] |
| HCM | S3753Y# | N2B | I-band | Increase binding to FLH2 | [18] |
| DCM | Q4007X# | N2B | I-band | Truncation of titin | [18] |
| DCM | Q4249X^ | N2B | I-band | Truncation of titin | [6] |
| DCM | S4417N# | Ig24 | I-band | Decrease binding to FHL2 | [18] |
| DCM | G3470D* | PEVK | I-band | Unknown | [23] |
| ARVC | Y8031C* | PEVK | I-band | Unknown | [9] |
| HCM | R8500H* | PEVK | I-band | Increase binding to CARP | [22] |
| HCM | R8604Q* | PEVK | I-band | Increase binding to CARP | [22] |
| ARVC | H8848Y* | PEVK | I-band | Unknown | [9] |
| DCM | C13771X^ | Ig94 | I-band | Truncation of titin | [6] |
| DCM | G16189X^ | FNIII17 | A-band | Truncation of titin | [6] |
| DCM | W16359X^ | Ig100 | A-band | Truncation of titin | [6] |
| DCM | R17295X^ | FNIII25 | A-band | Truncation of titin | [6] |
| DCM | R17470X^ | Ig103 | A-band | Truncation of titin | [6] |
| DCM | E17783X^ | Ig104 | A-band | Truncation of titin | [6] |
| ARVC | I16949T* | FNIII29 | A-band | Unknown | [9] |
| DCM | C18789X^ | FNIII36 | A-band | Truncation of titin | [6] |

| Missense Mutations of Titin, Nebulette, and Obscurin | | | | | |
|--|----------------------|-------------------|-------------------|--|-----------|
| Disease | Mutation | Region on Protein | Sarcomeric Region | Effect | Reference |
| DCM | R18858X [^] | Ig107 | A-band | Truncation of titin | [6] |
| DCM | R18985X [^] | FNIII37 | A-band | Truncation of titin | [6] |
| ARVC | A18579T [*] | FNIII41 | A-band | Unknown | [9] |
| DCM | R19560X [^] | Ig109 | A-band | Truncation of titin | [6] |
| ARVC | A19309S [*] | Ig111 | A-band | Unknown | [9] |
| DCM | R20858X [^] | FNIII51 | A-band | Truncation of titin | [6] |
| DCM | Q25689X [^] | Ig125 | A-band | Truncation of titin | [6] |
| DCM | W26632X [^] | FNIII94 | A-band | Truncation of titin | [6] |
| DCM | R26949X [^] | FNIII96 | A-band | Truncation of titin | [6] |
| DCM | K27016X [^] | FNIII97 | A-band | Truncation of titin | [6] |
| DCM | W27147X [^] | Ig129 | A-band | Truncation of titin | [6] |
| DCM | Y27567X [^] | FNIII100 | A-band | Truncation of titin | [6] |
| DCM | W29318X [^] | Ig134 | A-band | Truncation of titin | [6] |
| DCM | R29415X [^] | FNIII114 | A-band | Truncation of titin | [6] |
| DCM | E29510X [^] | FNIII115 | A-band | Truncation of titin | [6] |
| DCM | Q30081X [^] | FNIII119 | A-band | Truncation of titin | [6] |
| DCM | R31195X [^] | FNIII127 | A-band | Truncation of titin | [6] |
| DCM | K31371X [^] | FNIII129 | A-band | Truncation of titin | [6] |
| ARVC | P30847L [*] | FNIII131 | A-band | Unknown | [9] |
| DCM | S31841X [^] | FNIII131 | A-band | Truncation of titin | [6] |
| DCM | R32069Q [*] | Ig146 | A-band | Unknown | [21] |
| ARVC | M33291T [*] | Ig152 | A-band | Unknown | [9] |
| Mutations of NEBL | | | | | |
| DCM | K60N | Repeat 1 | | Sarcomeric structural abnormalities | [8] |
| DCM | Q128R | Repeat 3 | | Sarcomeric structural abnormalities | [8] |
| DCM | G202R | Repeat 5 | | Disruption of I-band and Z-disc proteins | [8] |
| DCM | A592E | Repeat 16 | | Disruption of I-band and Z-disc proteins | [8] |
| IDC | N654K | Repeat 18 | | Unknown | [4] |
| Mutations of OBSCN | | | | | |
| HCM | R4344Q | Ig58 | | Loss of titin Binding | [3] |

Table 1. Listing of missense mutations found in *TTN*, *NEBL*, and *OBSCN* that have been causally linked to the development of cardiomyopathies. Sequences correspond to the following accession numbers: NM_133378.4^{*}, NM_003319.4[#], and NM_001256850.1[^] for titin; NM_006393.2 for nebulette; and NM_052843.2 for obscurin. DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy, ARVC: arrhythmogenic right ventricular cardiomyopathy, IDC: idiopathic dilated cardiomyopathy

| Splice Site Donor/Acceptor Mutations of Titin | | | | | |
|---|--------------------------|-------------------|-------------------|---------|-----------|
| Disease | Mutation | Region on Protein | Sarcomeric Region | Effect | Reference |
| Mutations of TTN | | | | | |
| DCM | IVS118-g>a [^] | PEVK | I-band | Unknown | [6] |
| HCM | IVS155+g>t [^] | PEVK | I-band | Unknown | [6] |
| DCM | IVS172-g>c [^] | PEVK | I-band | Unknown | [6] |
| DCM | IVS172+g>a [^] | PEVK | I-band | Unknown | [6] |
| DCM | IVS185-2a>g [^] | Ig85 | I-band | Unknown | [6] |
| DCM | IVS230+g>t [^] | FNIII19 | A-band | Unknown | [6] |
| DCM | IVS237+3a>g [^] | FNIII22 | A-band | Unknown | [6] |
| DCM | IVS253-5t>a [^] | Ig106 | A-band | Unknown | [6] |
| DCM | IVS254-g>a [^] | FNIII41 | A-band | Unknown | [6] |
| DCM | IVS255+g>a [^] | Ig109 | A-band | Unknown | [6] |
| DCM | IVS271+5g>a [^] | FNIII53 | A-band | Unknown | [6] |
| DCM | IVS274-2a>g [^] | FNIII55 | A-band | Unknown | [6] |
| DCM | IVS276+5g>c [^] | FNIII99 | A-band | Unknown | [6] |
| DCM | IVS277+g>a [^] | FNIII100 | A-band | Unknown | [6] |
| DCM | IVS279+2t>a [^] | FNIII102 | A-band | Unknown | [6] |
| DCM | IVS302+g>c [^] | FNIII128 | A-band | Unknown | [6] |

Table 2. Listing of splice site donor/acceptor mutations found in *TTN* that have been causally linked to the development of cardiomyopathies. Sequence correspond to accession number NM_001256850.1[^] for titin. DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy, IVS: intron

| Deletion and Insertion Mutations of Titin | | | | | | |
|---|--|------------|-------------------|-------------------|---------------------|-----------|
| Disease | Mutation | Amino Acid | Region on Protein | Sarcomeric Region | Effect | Reference |
| Deletion Mutations | | | | | | |
| DCM | 6247 del g [^] | R2083fs | Ig11 | I-band | Unknown | [6] |
| DCM | 19183 del g [^] | S6395fs | Ig45 | I-band | Unknown | [6] |
| HCM | 23798-23810 del gtcaagatatctg [^] | G7933fs | Ig61 | I-band | Unknown | [6] |
| DCM | 44336 del a [^] | E14779fs | FNIII6 | A-band | Unknown | [6] |
| DCM | 44725 del t [^] | D14909fs | FNIII8 | A-band | Unknown | [6] |
| DCM | 45322 del t [^] | F15108fs | FNIII10 | A-band | Unknown | [6] |
| DCM | 53935 del c [^] | E17978fs | FNIII20 | A-band | Unknown | [6] |
| HCM | 60147 del c [^] | P20049fs | FNIII45 | A-band | Unknown | [6] |
| DCM | 64925 del t [^] | K21640fs | FNIII57 | A-band | Unknown | [6] |
| DCM | 65867 del a [^] | E21956fs | FNIII59 | A-band | Unknown | [6] |
| DCM | 67745 del t [^] | P22582fs | FNIII64 | A-band | Unknown | [6] |
| DCM | 81536-81537 del ct [^] | S27179fs | Ig129 | A-band | Unknown | [6] |
| DCM | 84977-84980 del atta [^] | Y28326fs | FNIII106 | A-band | Unknown | [6] |
| DCM | 82381 del g [*] | A27460 | FNIII107 | A-band | Truncation of titin | [24] |
| DCM | 89180-89184 del ttaaa [^] | T29725fs | FNIII116 | A-band | Unknown | [6] |

| Deletion and Insertion Mutations of Titin | | | | | | |
|---|--|------------------|-------------------|-------------------|---------------------|-----------|
| Disease | Mutation | Amino Acid | Region on Protein | Sarcomeric Region | Effect | Reference |
| DCM | 91043 del a [^] | N30348fs | FNIII121 | A-band | Unknown | [6] |
| DCM | 93376-93377 del ag [^] | R31126fs | Ig139 | A-band | Unknown | [6] |
| DCM | 97824-97831 del agtgacca* | A32606fs | Ig147 | M-band | Unknown | [6] |
| DCM | 98964 del a* | K32987fs | Ig150 | M-band | Unknown | [6] |
| Insertion Mutations | | | | | | |
| DCM | 38621 ins a [^] | A12873fs | Ig85 | I-band | Unknown | [6] |
| DCM | 28kb duplication of ex 72-124 [^] | | Ig50-PEVK | I-band | Unknown | [6] |
| DCM | 53145 ins g [^] | E17715fs | FNIII28 | A-band | Unknown | [6] |
| DCM | 58880 insert a* | S19628fs | FNIII42 | A-band | Truncation of titin | [10] |
| DCM | 62986-62987 ins at ex. 326* | K20995fs | Ig115 | A-band | Truncation of titin | [20] |
| DCM | 72178 ins t [^] | Q24059fs | FNIII75 | A-band | Unknown | [6] |
| DCM | 78372 ins a* | G26124fs | FNIII97 | A-band | Unknown | [10] |
| DCM | 90493 ins cct [^] | T30165fs | FNIII120 | A-band | Unknown | [6] |
| DCM | 91537 ins a [^] | T30513fs | FNIII122 | A-band | Unknown | [6] |
| Indel (Insertion/Deletion) Mutations | | | | | | |
| | 276 del g ins gt [^] | T92fs | Ig1 | Z-disc | Unknown | [5] |
| | 594-598 del actt ins a [^] | E198del | Ig2 | Z-disc | Unknown | [5] |
| | 1458 del c ins ct [^] | A486fs | Z-repeat 2 | Z-disc | Unknown | [5] |
| | 6131 del c ins ct [^] | E2044fs | 3' to Ig9 | I-band | Unknown | [5] |
| | 28509-28513 del ctct ins c [^] | E9503del | Ig77 | I-band | Unknown | [5] |
| | 30939 del gt ins g [^] | T10313fs | PEVK | I-band | Unknown | [5] |
| | 31566 del a ins ag [^] | A10522fs | PEVK | I-band | Unknown | [5] |
| | 31605 del tc ins t [^] | K10535fs | PEVK | I-band | Unknown | [5] |
| | 34329-34347 del ttcctcttcaggagcaa ins t [^] | I11443-E11449del | PEVK | I-band | Unknown | [5] |
| | 35760 del a ins ag [^] | T11920fs | PEVK | I-band | Unknown | [5] |
| | 36267-36271 del cagg ins c [^] | P12089del | Ig80 | I-band | Unknown | [5] |
| | 39252-39254 del ttc ins t [^] | E13084fs | Ig86 | I-band | Unknown | [5] |
| | 65988 del gc ins g [^] | G21996fs | Ig115 | A-band | Unknown | [5] |
| DCM | 67057-67063 del gcatatg ins ta [^] | A22353fs | Ig116 | A-band | Unknown | [6] |
| DCM | 72723-72739 del a ins aga [^] | S24241fs | Ig121 | A-band | Unknown | [6] |
| | 77025 del a ins ag [^] | L25675fs | Ig125 | A-band | Unknown | [5] |
| | 86694-86697 del ttaa ins t [^] | K28898del | Ig133 | A-band | Unknown | [5] |
| | 97995-97998 del actt ins t [^] | K32665del | Ig144 | M-band | Unknown | [5] |
| | 101133 del ga ins g [^] | S33711fs | Ig148 | M-band | Unknown | [5] |

Table 3. Listing of deletion and insertion mutations found in TTN that have been causally linked to the development of cardiomyopathies. Sequences correspond to the following accession numbers: NM_133378.4* and NM_001256850.1[^] for titin DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy, del: deletion, ins: insertion, fs: frameshift

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Author details

Maegen A. Ackermann and Aikaterini Kontrogianni-Konstantopoulos

Department of Biochemistry and Molecular Biology, University of Maryland, School of Medicine, Baltimore, MD, USA

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