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Cardiomyopathies: When the Goliaths of Heart Muscle Hurt

Maegen A. Ackermann and Aikaterini Kontrogianni-Konstantopoulos

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http://dx.doi.org/10.5772/55609

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, nebulette, 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.



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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 cardiomypathy.

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 fourand-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 cardiomyopathylinked 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 non-sense 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 spice 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 in 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 are shown with a magenta background.

2.4. Cardiomyopathy linked mutations within the region of titin that extends into the Mband

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 808 amino acids, respectively. Genetic analysis showed the defects in the *TTN* gene to be homozygous, leaving the heterozygote parents clinically unaffected. These mutations (1bp deletion in exon 360 and an 8bp deletion in exon 358) in the *TTN* gene are the first to be identified that produce both skeletal and cardiac muscle defects.



Figure 4. Schematic representation of the M-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 can be 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.

Although titin has been implicated in cardiomyopathies for over a decade, only recently has its direct role begun to be expounded. Many of the follow-up results on identified cardiomyopathy linked mutations of *TTN* indicate that these mutations can alter titin's binding capacity to its ligands, however, it remains to be proven that this is sufficient to cause DCM and HCM. Further study of the functional consequences of the *TTN* mutations, especially those causing truncated variants, using *in vivo* animal models is still necessary to elucidate titin's role in cardiomyopathies.

3. Nebulin

Nebulin is a giant (~500-800 kDa) sarcomeric protein of striated muscles [26]. Similar to titin, nebulin is oriented longitudinally across the sarcomere, spanning the length of the thin filament [27]. Its NH₂-terminus extends to the pointed ends of thin filaments in the sarcomeric I-band, and its COOH-terminus resides within the Z-disc [28]. The nebulin gene, *NEB*, contains 183 exons and is the product of extensive gene duplication, resulting in a protein of highly repetitive domain structure [29]. Nebulin is mostly composed of tandem nebulin-repeats with the central motifs organized as super-repeats. In addition, nebulin contains a glutamine rich region at its NH₂-terminus, as well as a serine rich region and a Src Homology 3 (SH3) domain at its COOH-terminus. The organization of the nebulin repeats complements the periodicity of actin filaments [28]. Consistent with this, alternative splicing of the *NEB* gene generates proteins of different sizes, which correspond to thin filaments of various lengths [30], [31]. In addition to its role in stabilizing thin filaments, nebulin has also been implicated as a regulator of thin filament length [32], [33].



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, 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 nebulette as it is the only member of the nebulin family that has been linked to cardiomyopathies. Cardiac specific 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 nebulette 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 nebulette 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 nebulette. The mechanism by which this mutation causes disruptions in the heart is unknown, however due to its location this mutation likely disrupts nebulette's incorporation into the Z-disc. Notably, this observation has brought about a new role for nebulette as a genetic marker for patients with non-familial IDC.

Recently, more direct evidence for the involvement of nebulette 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 nebulette 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 nebulette resulting in dramatic structural alterations in I-band and Z-disc proteins. Taken together these studies suggest that nebulette 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 cardiomyopthies.

Although the role of *OBSCN* in cardiomyopthies 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 nebulette, mutations in the *OBSCN* gene lead to the development cardiomyopathies.



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, nebulette, 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*	lg1	Z-disc	Reduced binding to telethonin [1				
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*	lg4	Z-disc	Unknown	[20]			
ARVC	T2896I*	lg16	l-band	Unknown	[9]			
HCM	S3753Y#	N2B	l-band	Increase binding to FLH2	[18]			
DCM	Q4007X#	N2B	l-band	Truncation of titin	[18]			
DCM	Q4249X^	N2B	l-band	Truncation of titin	[6]			
DCM	S4417N#	lg24	l-band	Decrease binding to FHL2	[18]			
DCM	G3470D*	PEVK	l-band	Unknown	[23]			
ARVC	Y8031C*	PEVK	l-band	Unknown	[9]			
НСМ	R8500H*	PEVK	l-band	Increase binding to CARP	[22]			
НСМ	R8604Q*	PEVK	l-band	Increase binding to CARP	[22]			
ARVC	H8848Y*	PEVK	l-band	Unknown	[9]			
DCM	C13771X^	lg94	l-band	Truncation of titin	[6]			
DCM	G16189X^	FNIII17	A-band	Truncation of titin	[6]			
DCM	W16359X^	lg100	A-band	Truncation of titin	[6]			
DCM	R17295X^	FNIII25	A-band	Truncation of titin	[6]			
DCM	R17470X^	lg103	A-band	Truncation of titin [6				
DCM	E17783X^	lg104	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	e Mutation Region on Sarcomeric Effect Effect		Effect	Reference		
DCM	R18858X^	lg107	A-band	Truncation of titin	[6]	
DCM	R18985X^	FNIII37	A-band	Truncation of titin	[6]	
ARVC	A18579T*	FNIII41	A-band	Unknown	[9]	
DCM 🦷	R19560X^	lg109	A-band	Truncation of titin	[6]	
ARVC	A19309S*	lg111	A-band	Unknown	[9]	
DCM	R20858X^	FNIII51	A-band	Truncation of titin	[6]	
DCM	Q25689X^	lg125	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^	lg129	A-band	Truncation of titin	[6]	
DCM	Y27567X^	FNIII100	A-band	Truncation of titin	[6]	
DCM	W29318X^	lg134	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*	lg146	A-band	Unknown	[21]	
ARVC	M33291T*	lg152	A-band	Unknown	[9]	
			Mutation	s 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	lg58		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]				
НСМ	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^	lg85	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^	lg106	A-band	Unknown	[6]				
DCM	IVS254-g>a^	FNIII41	A-band	Unknown	[6]				
DCM	IVS255+g>a^	lg109	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	
		Deletio	on Mutations				
DCM	6247 del g^	R2083fs	lg11	I-band	Unknown	[6]	
DCM	19183 del g^	S6395fs	lg45	I-band	Unknown	[6]	
HCM	23798-23810 del gtcaagatatctg^	G7933fs	lg61	I-band	Unknown	[6]	
DCM	44336 del a^	E14779fs	FNIII6	A-band	Unknown	[6]	
DCM	44725 del t^	D14909fs	FNII18	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	lg129	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	lg139	A-band	Unknown	[6]	
DCM	97824-97831 del agtgacca*	A32606fs	lg147	M-band	Unknown	[6]	
DCM	98964 del a*	K32987fs	lg150	M-band	Unknown	[6]	
		Insertio	on Mutations				
DCM	38621 ins a^	A12873fs	lg85	I-band	Unknown	[6]	
DCM	28kb duplication of ex 72-124^		lg50-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	lg115	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) Mut	tations			
	276 del g ins gt^	T92fs	lg1	Z-disc	Unknown	[5]	
	594-598 del actt ins a^	E198del	lg2	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 lg9	I-band	Unknown	[5]	
	28509-28513 del ctct ins c^	E9503del	lg77	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 tttcctcttcaggagcaa 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	lg80	I-band	Unknown	[5]	
	39252-39254 del ttc ins t^	E13084fs	lg86	I-band	Unknown	[5]	
	65988 del gc ins g^	G21996fs	lg115	A-band	Unknown	[5]	
DCM	67057-67063 del gcatatg ins ta^	A22353fs	lg116	A-band	Unknown	[6]	
DCM	72723-72739 del a ins aga^	S24241fs	lg121	A-band	Unknown	[6]	
	77025 del a ins ag^	L25675fs	lg125	A-band	Unknown	[5]	
	86694-86697 del ttaa ins t^	K28898del	lg133	A-band	Unknown	[5]	
	97995-97998 del actt ins t^	K32665del	lg144	M-band	Unknown	[5]	
101133 del ga ins g^		S33711fs	lg148	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

Acknowledgements

Our research has been supported by grants to A.K.K. through the National Institutes of Health (R21 HL106197) and the American Heart Association (GRNT 3780035) and to M.A.A from the National Institutes of Health (F32 AR058079).

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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