Review Article

The Role of *TTN* Gene Variants in Dilated Cardiomyopathy

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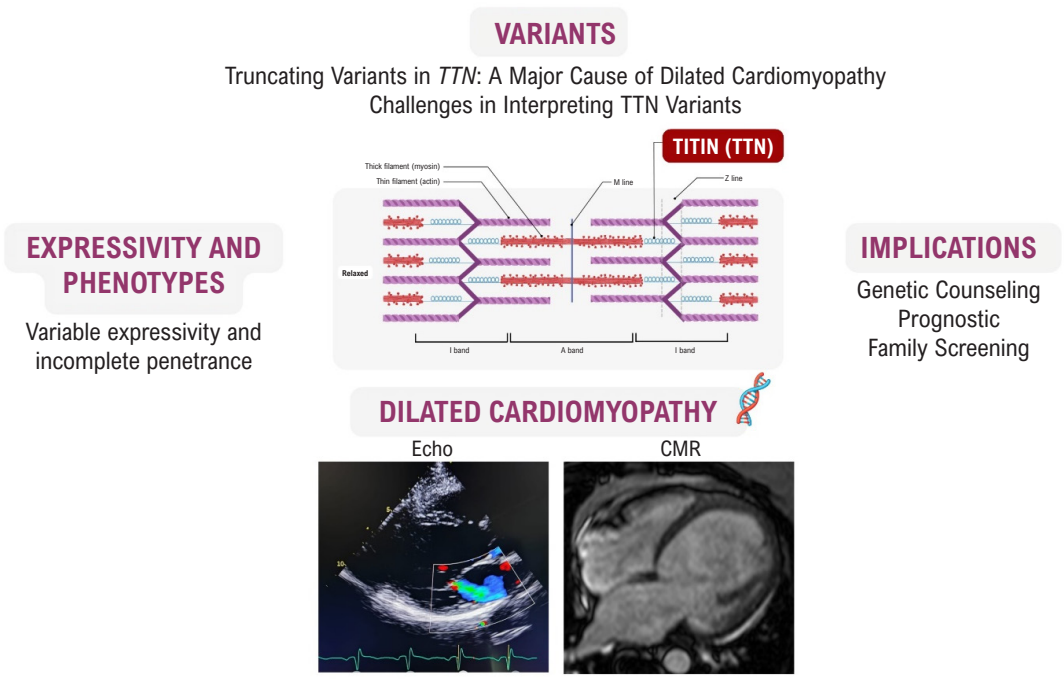
Central Illustration: The Role of TTN Gene Variants in Dilated Cardiomyopathy



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Echo: echocardiogram; CMR: cardiac magnetic resonance.

Keywords

Dilated Cardiomyopathy; Genes; Titin.

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Abstract

Titin, encoded by the *TTN* gene, is the largest human protein, essential for maintaining muscle cell stability and regulating myocardial contraction. *TTN* truncating variants (*TTN*tv) have emerged as the most common genetic cause of dilated cardiomyopathy (DCM), accounting for up to 25% of familial cases and 18% of sporadic cases. This review examines the complex relationship between *TTN* variants and DCM, highlighting the challenges posed by incomplete penetrance and variable expressivity. While truncating variants primarily in the A-band region are linked to DCM, not all carriers

exhibit clinical symptoms, indicating the influence of additional genetic and environmental factors. We also discuss the prognostic implications of *TTN* variants, which, despite similar clinical outcomes to other DCM cases, demonstrate a higher rate of reverse remodeling in response to heart failure treatment. Emerging therapeutic strategies targeting the underlying molecular mechanisms of *TTN* variants, including mTOR inhibitors and CRISPR-based gene editing, offer promising avenues for personalized treatment. Understanding the genetic underpinnings of *TTN*-related DCM is crucial for improving diagnosis, family screening, and therapeutic outcomes, paving the way for more effective management of this complex condition.

Introduction

Titin and the Sarcomere: Structure and Function

The sarcomere, the functional unit of the myocardium, is composed of proteins with both contractile and supportive roles (Figure 1). Titin, a protein encoded by the *TTN* gene, is a critical component of the sarcomere, responsible for assisting in the regulation of myocardial contraction.[1,2] As the largest known human protein, composed of approximately 33,000 amino acids, titin is essential for maintaining the structural stability of muscle cells.[1] Its large size also makes it susceptible to dysregulation, which can lead to various cardiomyopathies.[3] Titin exists in multiple isoforms, with N2B and N2Ba being the most prevalent in the heart.[2] A seminal study highlighted how changes in the *TTN* gene can cause dilated cardiomyopathy (DCM).[3] Understanding the mechanisms and pathophysiology of pathogenic variants in this gene remains a challenge for accurate genotype-phenotype correlation, ultimately improving care for patients and their families.

Isoforms of Titin: Diversity and Functional Implications

Within the sarcomere, titin spans from the Z-disc to the M-band,[2] making it the third most abundant myofilament

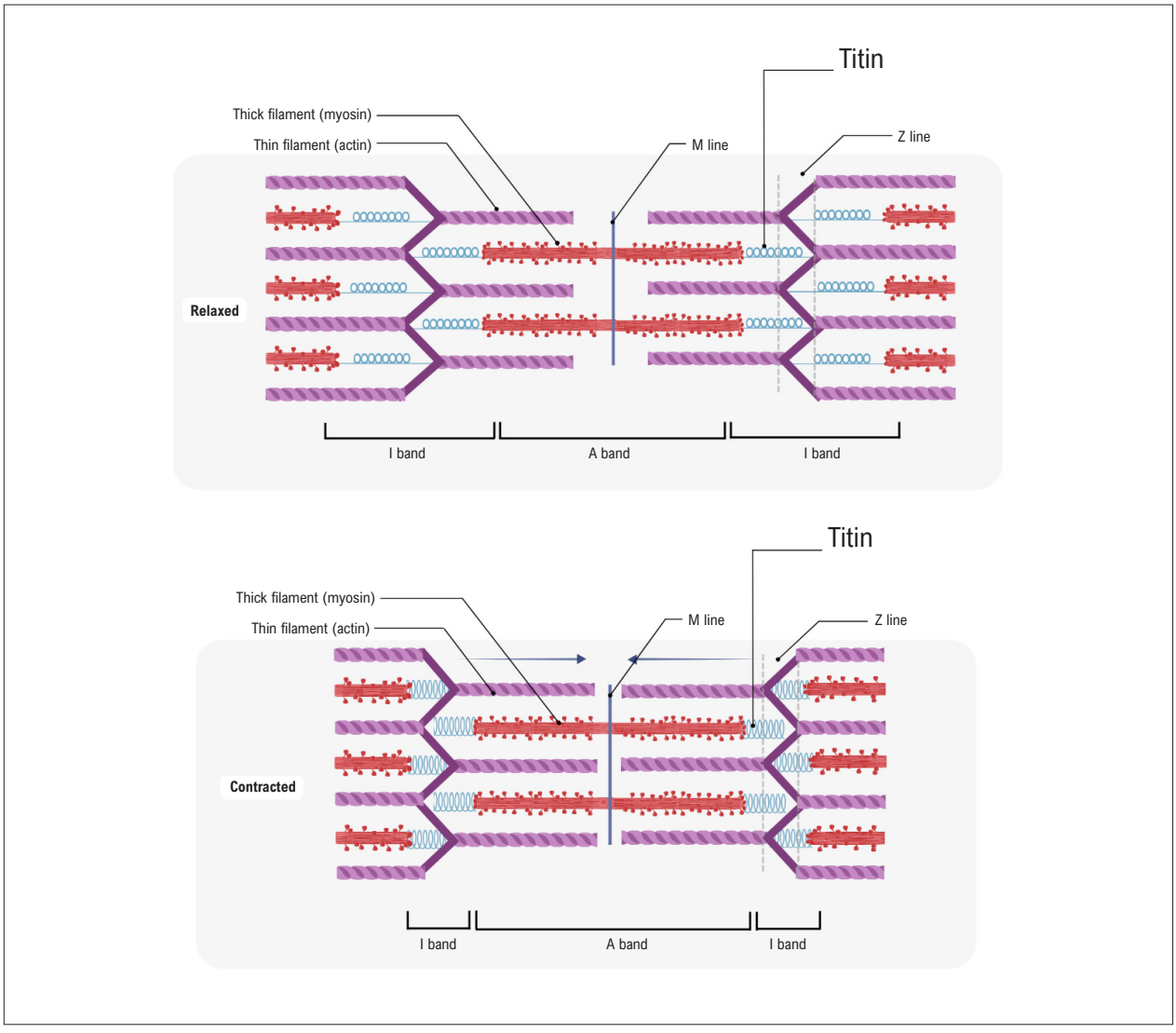

Figure 1 – *Sarcomere representation.*

in striated muscle, with a size of approximately 33,000 amino acids.[3] The properties of titin vary along its length, depending on its location within the sarcomere.[2] Several isoforms of titin exist, with N2B and N2BA being predominant in the heart. The *TTN* gene also encodes a distinct cardiac isoform, novex-3 titin, which is 5,600 amino acids long and lacks the A-band and M-band segments of titin.[3] The functional characteristics of titin are significantly influenced by alternative splicing of the *TTN* gene. More than a third of *TTN*'s 363 exons, particularly those in the I-band, can undergo alternative splicing, generating a variety of isoforms. These isoforms result in titin proteins with different lengths and stiffness, affecting both passive tension and force production within the sarcomere. During fetal development, longer and more flexible isoforms are predominant, while shorter and stiffer isoforms, such as N2BA and N2B, replace them after birth. In adult hearts, these isoforms exist in a typical ratio of 70:30 to 60:40, respectively. Isoform switching, regulated by the master splicing regulator RNA binding motif 20 (*RBM20*), modulates sarcomere tension in response to changes in loading conditions and pathological processes. Variants affecting *RBM20* are also linked to the DCM phenotype. Additionally, post-translational modifications, like phosphorylation, rapidly alter titin's stiffness in response to physiological and pathological stress.[4]

Variants

Truncating Variants in *TTN*: A Major Cause of Dilated Cardiomyopathy

Truncating variants in the *TTN* gene (*TTN*tv), including nonsense variants that result in premature termination codons, insertions and deletions (indels) causing frameshifts, and variants affecting canonical splice sites, are now recognized as a major genetic cause of DCM. These variants are predominantly found in the A-band of titin. Cohort studies have analyzed the distribution of truncating variants in *TTN* among DCM patients and controls. The titin protein is segmented into different bands: Z-disc (red), I-band (blue), A-band (green), and M-band (purple) (Figure 2).[5]

Challenges in Interpreting *TTN* Variants

Interpreting *TTN* variants is challenging due to the presence of multiple transcripts with variable expression across different tissues (table 1). Alternative splicing means not all exons are included in every transcript, which can affect the impact of a variant depending on the exon involved. The recommended approach for variant reporting involves the inferred full meta-transcript, as annotated by the Havana Group. Truncating variants in the A-band region of cardiac isoforms N2B and N2BA, which are more highly expressed in cardiac tissue, are particularly associated with DCM. *TTN* contains various domains, including Ig-like, Z-repeat, PEVK, Fibronectin type III, and Protein kinase domains. For more details on *TTN* exons, transcripts, and domains, resources like https://www.cardiodb.org/ are valuable. Variant interpretation requires careful consideration of phenotype, inheritance pattern, and variant classification, following the American College of Medical Genetics and Genomics (ACMG) guidelines.[6] Variants are classified as benign, likely benign, variants of uncertain significance, likely pathogenic, and pathogenic, based on criteria including variant type (nonsense, missense, frameshift) and frequency in control populations. Although missense variants in *TTN* are frequently found during genomic analysis, they are rarely reported as the primary pathogenic mechanism in *TTN*-related DCM is truncating variants. Variants of uncertain significance should be periodically re-evaluated as new data emerge, and family segregation studies can aid in reclassification. Determining pathogenicity for *TTN* truncating variants requires considering the variant's position in the gene and correlating with clinical or functional data, such as previous descriptions in symptomatic individuals and the associated clinical phenotype (DCM, myopathy). Thus, caution is advised when interpreting *TTN* variants.

Expressivity and Phenotypes

Variable Expressivity and Incomplete Penetrance of *TTN* Variants

The expressivity of *TTN* gene variants refers to the spectrum of phenotypes observed in individuals carrying the same variant. *TTN* variants have a well-established link with DCM, typically

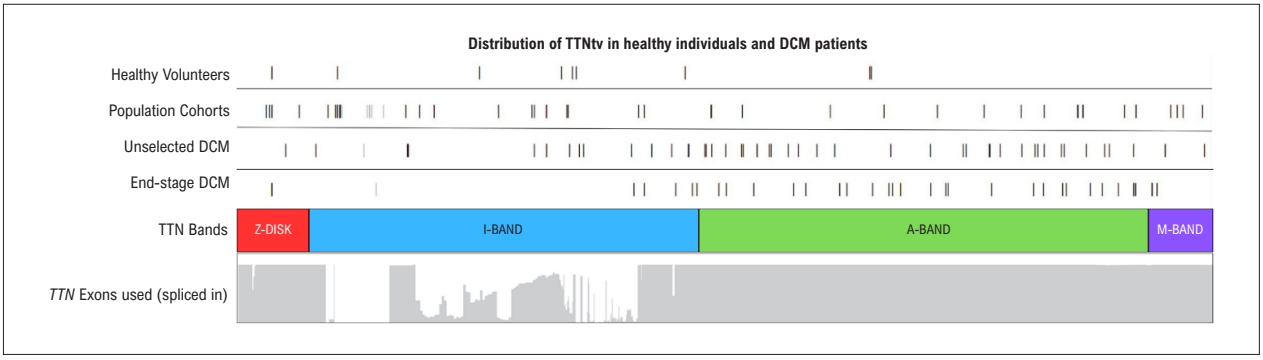

Figure 2 – *Distribution of TTNtv in healthy individuals and DCM patients, subdivided into unselected and end-stage DCM. TTN exon usage based on spliced in data, for the two principal adult cardiac isoforms N2BA and N2B. TTN protein bands are colored as follows: Z-disc (red), I-band (blue), A-band (green), and M-band (purple). Most TTNtv cases are located in the A-band. Adapted from Roberts et al.*[5]

Table 1 – *TTN* Transcripts

Transcript	Description	Length (aa)	No. exons	Transcript Ensembl	Protein Ensembl	Transcript Refseq	Protein Refseq
Meta	Inferred complete meta-transcript	35991	363	ENST00000589042	ENSP00000467141	NM_001267550.1	NP_001254479
N2BA	Principle cardiac long isoform	34350	313	ENST00000591111	ENSP00000465570	NM_001256850.1	NP_001243779
N2B	Principle cardiac short isoform	26926	191	ENST00000460472	ENSP00000434586	NM_003319.4	NP_003310.4
N2A	Soleus / skeletal long isoform	33423	312	ENST00000342992	ENSP00000343764	NM_133378.4	NP_596869.4
Novex-1	Minor cardiac short isoform	27051	192	ENST00000359218	ENSP00000352154	NM_133432.3	NP_597676.3
Novex-2	Minor cardiac short isoform	27118	192	ENST00000342175	ENSP00000340554	NM_133437.3	NP_597681.3
Novex-3	Minor small cardiac isoform	5604	46	ENST00000360870	ENSP00000354117	NM_133379.3	NP_596870.2

showing an autosomal dominant inheritance pattern, and *TTN*-related myopathy, which generally follows an autosomal recessive pattern (https://clinicalgenome.org/). The association of *TTN* variants with hypertrophic cardiomyopathy is limited.[3]

Phenotypic Spectrum of *TTN*-Related Dilated Cardiomyopathy

DCM can be caused by variants in several genes, but *TTN* truncating variants are responsible for most genetic cases. *TTN*tv variants in the A-band are present in up to 25% of familial DCM cases and 18% of sporadic cases.[7] Family studies suggest that the penetrance of truncating *TTN* variants in individuals over 40 years old is close to 95%.[3] However, *TTN* truncating variants are also found in about 1% of healthy individuals, indicating incomplete penetrance and variable expressivity, which may involve other yet-to-be-understood mechanisms. When evaluating *TTN* variants, it is important to consider the phenomena of incomplete penetrance and variable expressivity. Not everyone with a pathogenic *TTN* variant will exhibit related signs or symptoms, and clinical presentations may vary even within the same family. This variability complicates the prediction of cardiomyopathy onset. Additional genetic and environmental factors, such as hemodynamic stress,[8] alcohol consumption,[9] chemotherapy,[10] and pregnancy,[11] may contribute to this phenotypic variability, although the precise mechanisms remain unclear.

Implications

Prognostic Implications of *TTN* Variants in DCM

Identifying a causal *TTN* variant has important prognostic implications. Compared to DCM patients without identified variants, those with *TTN* variants exhibit similar clinical manifestations, morbidity, and mortality.[3] However, DCM patients with *TTN* variants show a higher rate of reverse remodeling in response to heart failure treatment, with 53.2% responding favorably compared to only 11.1% of patients with desmosomal gene variants.[12]

Family Screening and Genetic Counseling in *TTN*-Related DCM

Genetic evaluation offers benefits beyond the index patient, extending to family members, even during subclinical phases. Family screening enables early diagnosis, allowing for early treatment and potentially better outcomes.[7] Genetic counseling and evaluation of first-degree relatives of DCM patients are recommended with a Class I recommendation and Level of Evidence B.[7]

Therapeutic Perspectives

Emerging Therapeutic Strategies Targeting *TTN* Variants

Although established heart failure treatments, such as renin-angiotensin-aldosterone system blockade and beta-adrenergic antagonism, effectively reduce mortality and improve quality of life, they do not directly address the underlying mechanisms caused by *TTN* variants. Understanding the pathophysiology and molecular pathways involved in titin modification opens new avenues for promising therapeutic strategies and targets.[13] Some truncated *TTN* variants lead to overexpression of the mTOR (mammalian target of rapamycin) complex, suggesting that mTOR inhibitors like rapamycin could be evaluated in randomized clinical trials.[14] In animal models, antisense oligonucleotides have been shown to mediate and alter splicing processes, excluding the reading of the mutated exon and preventing premature termination of titin translation. This strategy has proven effective in other conditions, such as Duchenne muscular dystrophy, and is already FDA-approved for that indication.[15] A recent study demonstrated that using CRISPR technology could increase *TTN* gene expression and titin production in a cell model, improving cellular contractile capacity in patients with haploinsufficiency.[16]

Take-home Messages

- Titin, encoded by the *TTN* gene, is one of the largest human proteins and plays a crucial role in maintaining muscle cell stability and regulating myocardial contraction.
- Truncating variants in the *TTN* gene are the most frequent genetic cause of dilated cardiomyopathy.
- The expressivity of *TTN* gene zvariants refers to the range of phenotypes observed in individuals carrying the same variant, which can be influenced by incomplete penetrance and variable expressivity. Environmental factors like alcohol consumption, chemotherapy, pregnancy, and hemodynamic stress may contribute to this variability.
- Compared to DCM patients without identified variants, those with *TTN* variants exhibit similar clinical manifestations, morbidity, and mortality, but show a higher rate of reverse remodeling in response to heart failure treatment.
- Conventional heart failure treatments have been effective in reducing mortality and improving quality of life, but they do not directly address the underlying mechanisms caused by *TTN* variants.
- The mTOR pathway and CRISPR technology represent emerging therapeutic alternatives that hold promise for treating *TTN*-related DCM.

Conclusion

Titin, encoded by the *TTN* gene, is a protein with variable stiffness and size depending on its isoforms and is essential for myocardial contraction. Truncating variants in the *TTN* gene are the most frequent genetic cause of dilated cardiomyopathy. Understanding the genetic aspects is fundamental for better genotype-phenotype correlation, with important implications for prognosis, diagnosis, family screening, and therapeutic strategies.

Author Contributions

Conception and design of the research: Furquim SR, Senra PM, Linnenkamp BDW, Vilalva KH, Mizuta MH; Acquisition of data: Silva EA, Andrade FA; Writing of the manuscript: Furquim SR, Senra PM, Linnenkamp BDW, Vilalva KH, Mizuta MH; Critical revision of the manuscript for content: Vilalva KH, Mizuta MH, Santos BM, Stephan BO, Buriti NA, Val VP, Krieger JE.

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Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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