Biophysical Reviews
https://doi.org/10.1007/s12551-026-01411-4

REVIEW

Mending the Achilles heels of titin in cardiac and musculoskeletal disease

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Received: 16 December 2025 / Accepted: 27 January 2026

Abstract

Titin, the largest known human protein, spans the sarcomere from Z-disk to M-line and is central to muscle elasticity, force transmission, and structural integrity. Maybe not surprisingly, accumulated evidence over the last years shows that titin, despite its titanic size, is not devoid of molecular *Achilles heels* that can lead to dysfunction and disease. In this review, we summarize the fundamental roles of titin in muscle mechanics, mechanosignaling, and physiology as well as in genetic and acquired disorders of cardiac and skeletal muscle. We discuss the current understanding of how mutations and posttranslational processing (dys)regulate titin, while highlighting gaps of knowledge regarding underlying molecular mechanisms. Finally, we analyze emerging experimental titin-cleavage models that are uncovering novel pathways of titin-based pathogenesis, positioning the protein not only as a central player in myocyte biomechanics but also as a determinant of pathological tissue remodeling. A main driving force in the field is to exploit the accumulated knowledge on titin to find new avenues for therapeutic intervention in cardiac and musculoskeletal disease.

Keywords Muscle · Heart · Sarcomere · Mechanics · Cardiomyopathy · Myopathy

Introduction

Muscle function is fundamental to life — driving movement, maintaining posture, and powering the rhythmic beating of the heart. The proper function of muscle depends not only on the muscle's ability to actively generate force at sarcomeres (Gautel and Djinović-Carugo 2016) but also on its finely tuned mechanical properties, including viscoelasticity and tensile strength. Therefore, it is not surprising that disruption of these mechanical properties is being increasingly recognized as a contributing factor to disease (Münch and Abdelilah-Seyfried 2021).

Muscle organization allows for a good understanding of macroscopic tissue mechanics from the mechanical properties of cellular and subcellular components, including force-generating myofibrils, the linear association of sarcomeres (Fig. 1A). While the fundamental contribution of actomyosin filaments to active contraction is well established (Szent-Györgyi 2004), elegant recent work by Loescher et al. (2023) has confirmed that the primary source of passive forces in cardiac muscles at all levels of deformation is the cellular components of cardiomyocytes. Among these, the giant protein titin stands out as one of the most important contributors to stiffness, both in skeletal and in cardiac muscle (Y. Li et al. 2020) (Fig. 1A). In addition, titin significantly accounts for the viscous component of these forces together with other cytoskeletal components, mainly the microtubule network (Uchida et al. 2022), but also actin and intermediate filaments (Grimes et al. 2019; Loescher et al. 2023).

The mechanical properties of titin can be extensively modulated both transcriptionally and posttranslationally, providing a fine regulatory mechanism for muscle mechanics (Linke and Hamdani 2014; Loescher et al. 2022; H. L. Granzier and Labeit 2025). Likewise, pathological alteration of titin's physical properties (e.g., mechanics and protein stability) can affect its function and lead to the

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Published online: 14 February 2026

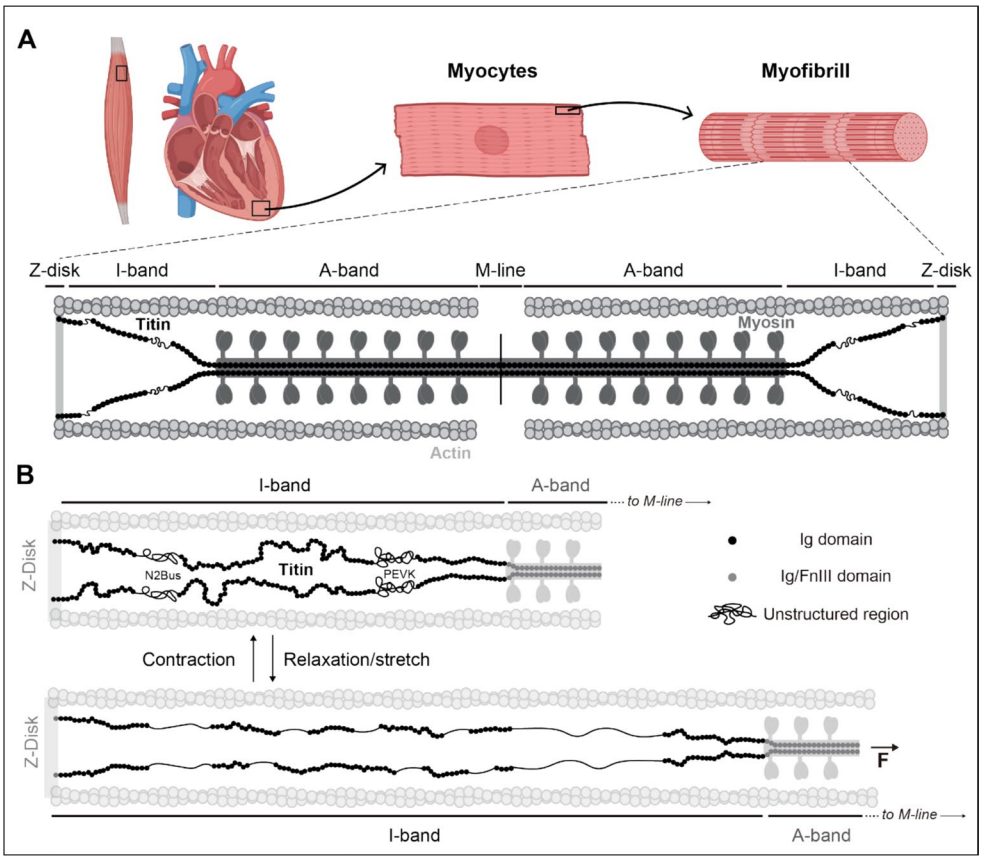

Fig. 1 Titin structure and mechanics. **A** Schematic representation of a cardiac sarcomere and its contextualized location in muscle tissues (black boxes, not to scale). The main protein components of the sarcomere are indicated. Created with BioRender.com. **B** Schematic representation of half a sarcomere (not to scale) showing the I-band (black) and A-band (dark gray) regions of titin (N2BA isoform). Immunoglobulin-like domains (Ig), fibronectin III-like domains (FnIII), and unstructured regions are shown. During muscle relaxation, mechanical force induces the straightening of the interdomain linkers and the unstructured regions, and the unfolding of the Ig domains

development of disease, as demonstrated recently by our group (Martinez-Martin et al. 2023; López-Unzu et al., 2025; Silva-Rojas et al. 2025) and others (Rees et al. 2021, 2023; Freundt et al. 2025). Building on excellent previous exhaustive review papers, including Linke and Hamdani (2014) and H. L. Granzier and Labeit (2025), here we will summarize the current knowledge on titin function in the sarcomere (for less studied non-sarcomeric roles of titin, the reader is referred to (S. Labeit et al. 2006; Zastrow et al. 2006; Toffali et al. 2023)) and the mechanisms that regulate titin-based stiffness under both physiological and pathophysiological conditions. Given the strong association between titin and disease, we will provide an overview of recently described genetic variants in the titin gene (*TTN*) and their link to cardiac and musculoskeletal disease. We will also reflect on biophysical mechanisms that can contribute to pathogenicity in these genetic conditions and describe recent developments that place titin as a causative factor also in acquired cardiac disease. Finally, we will build on the current unknowns regarding the integrative functions of titin to suggest future work to better comprehend the fundamental roles of titin in health and disease, with the ultimate goal of finding effective therapies for a range of human diseases.

Structure and mechanics of titin

Titin from vertebrates is a giant, long-lived protein of up to 4.2 MDa that extends along the whole length of half a sarcomere (Maruyama 1976; Wang et al. 1979; Bang et al. 2001; Douvdevany et al. 2024) (Fig. 1A). Structurally, human titin is composed of up to ~ 170 immunoglobulin-like (Ig) and ~ 130 fibronectin III-like (FnIII) domains arranged in series, which can be divided into different regions depending on its localization in the sarcomere. How such a large polypeptide can be integrated in sarcomeres remains controversial, although mechanisms may be different in embryonic and adult cardiomyocytes (da Silva Lopes et al. 2011; Rudolph et al. 2019; Douvdevany et al. 2024). Several independent lines of evidence demonstrate that properly tethered titin is required to stabilize sarcomeres (Radke et al. 2019; Swist et al. 2020; Pricolo et al. 2025; Silva-Rojas et al. 2025).

According to the current annotation of titin in Uniprot (accession number Q8WZ42), the N-terminal region of the protein consists of up to 7 Z-repeats and 9 Ig domains that anchor it to the Z-disk of the sarcomere (Gautel and Djinović-Carugo 2016), where titin strongly interacts with alpha-actinin (Grison et al. 2017) and telethonin (Bertz

et al. 2009). On the opposite side of the protein, the C-terminal region is integrated into the M-band, although recent structural data on intact sarcomeres suggest that only half of the titin molecules reach this region (Tamborrini et al. 2023). When present, the M-band region of titin comprises the titin kinase domain, a pseudokinase suggested to participate in mechanosensing and degradation mechanisms (Lange et al. 2005; Puchner et al. 2008; Bogomolovas et al. 2014, 2021; Tamborrini et al. 2023), and 10 Ig domains that interact with myomesin and obscurin (Fukuzawa et al. 2008; Pernigo et al. 2010), among others (Gautel and Djinović-Carugo 2016). The I-band part of titin is the most variable region of the protein, since it is heavily affected by alternative splicing (Savarese et al. 2018). This region consists of several Ig domains and mostly unstructured segments like the PEVK and the N2Bus stretches, which can respond to intracellular calcium levels (D. Labeit et al. 2003). Interestingly, recent work has identified a folded and mechanically weak globular structure within the cardiac-specific N2Bus region (Sun et al. 2024). Finally, the A-band of titin is composed of super-repeats of Ig and FnIII domains whose periodicity matches that of the myosin crowns (S. Labeit et al. 1992; Dutta et al. 2023; Tamborrini et al. 2023). Because of this A-band arrangement, titin has been proposed as a ruler for thick filament assembly (H. Granzier et al. 2014; Linke and Hamdani 2014; Tskhovrebova et al. 2015; Tonino et al. 2017, 2019; Bennett et al. 2020; Linke 2023).

The mechanical function of titin resides mainly in the I-band, which is therefore considered the mechanically active region of the protein (Linke et al. 1998; Linke and Hamdani 2014). When a force is applied to titin, the interdomain linkers and the unstructured regions of the protein stretch, providing entropic elasticity to the sarcomere (H. Li et al. 2002; Von Castelmur et al. 2008; Lanzicher et al. 2020). At increased sarcomere lengths, the forces experienced by titin molecules also induce the unfolding of Ig domains in the I-band of titin (M. Kellermayer et al. 1997; Rief et al. 1997; Tskhovrebova et al. 1997), a process that can be particularly relevant at physiological temperatures (Yu et al. 2021). Termed the sequential titin-extension hypothesis, the series of extension and unfolding events sets titin as a molecular spring driven primarily by entropic elasticity yet also featuring enthalpic components that contribute to its viscoelastic behavior (Minajeva et al. 2001; H. Li et al. 2002; Linke 2023). Once the force ceases, the elastic regions recoil elastically whereas the Ig domains can regain their folded structure (Fig. 1B). The mechanical energy released during these events has been proposed to contribute to the early phase of sarcomere active shortening (Opitz et al. 2003). Indeed, recent studies have shown that domain refolding generates mechanical work that can act cooperatively with myosin motors to generate force (Rivas-Pardo et al. 2016, 2020; Mártonfalvi et al. 2017; Eckels et al. 2019). According to these observations, titin may not only play a role in the passive forces of muscle but would also contribute to active contraction (Eckels et al. 2018). Titin mechanics has also been proposed to contribute to the Frank-Starling law of the heart (Ait-Mou et al. 2016), potentially through the modulation of both thick and thin filament structures, as well as to residual force enhancement in myofibers (Hessel et al. 2024). Furthermore, force transmission across titin is required for the stability of the A-band in contracting sarcomeres (Y. Li et al. 2020). It has also been proposed that interactions between the thin filament and titin can modulate the mechanical properties of the I-band of the protein, impacting the overall stiffness of cardiomyocytes particularly during active contraction (Linke et al. 2002; Squarci et al. 2023; Desai et al. 2025).

Regulation of titin-based stiffness

The mechanical properties of titin can be finely tuned by a variety of biological processes. The best-established mechanism is the alternative splicing of titin mRNA, which mainly affects the I-band region of the protein, generating several isoforms of different lengths and domain composition. Titin is encoded by the *TTN* gene, which contains 363 and 347 exons in humans (ENST00000589042.5) and in mice (ENSMUST00000099981.10), respectively. Alternative splicing of the titin mRNA or usage of an alternative promoter in the case of the Cronos isoform yields the different titin isoforms expressed in muscles (Fig. 2). The main cardiac isoforms are N2BA, N2B, Cronos, and Novex 1–3, while skeletal muscles express mostly N2A isoforms lacking the N2B segments and Cronos. Cronos and Novex are suggested to have rather structural and regulatory roles that might be important for sarcomere assembly but have low relevance in the mechanics of titin (Bang et al. 2001; Zou et al. 2015; D. Kellermayer et al. 2017; Zaunbrecher et al. 2019).

The contribution of titin splicing to muscle physiology has been extensively demonstrated, at very least at the correlation level. Differences in titin isoform content are seen between species and even between heart compartments within the same species, correlating with myofibrillar passive stiffness (Cazorla et al. 2000; Neagoe et al. 2003; Lahmers et al. 2004). The isoform profile, including splice variants of the N2BA and N2A isoforms, also changes dramatically during heart and skeletal muscle development (Opitz et al. 2004; Warren et al. 2004; Opitz and Linke 2006; Ottenheijm et al. 2009). In the heart, the ratio between the longer and compliant N2BA isoform and the shorter and stiffer N2B correlates with the ventricular stiffness (Freiburg et al. 2000) (Fig. 3A). The switch between these two isoforms is controlled, at least partially, by the RNA binding

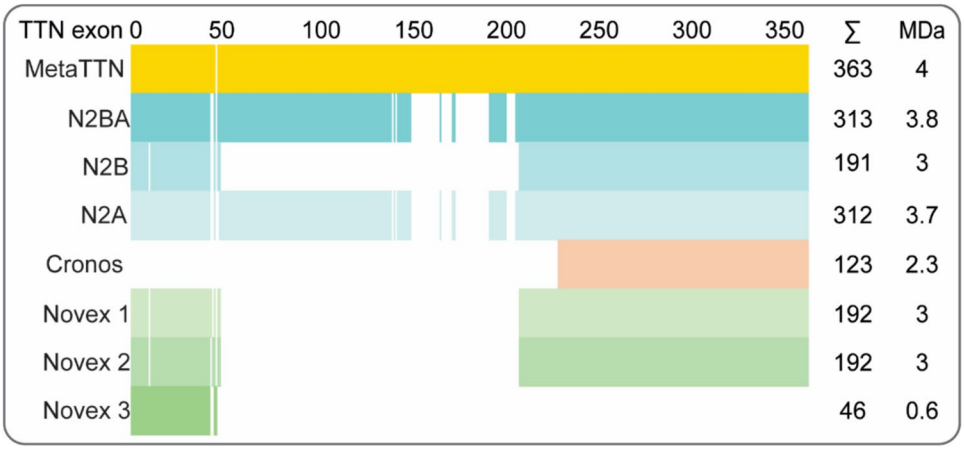

Fig. 2 Exon inclusion in the different titin isoforms described in adult human muscles. Please note that there are exons in the metatranscript of titin that are not expressed in any of the detected isoforms. Total size of isoforms in terms of number of included exons and size of the isoform are indicated. Adapted from https://www.cardiodb.org/titin/ and Zaunbrecher et al. (2019)

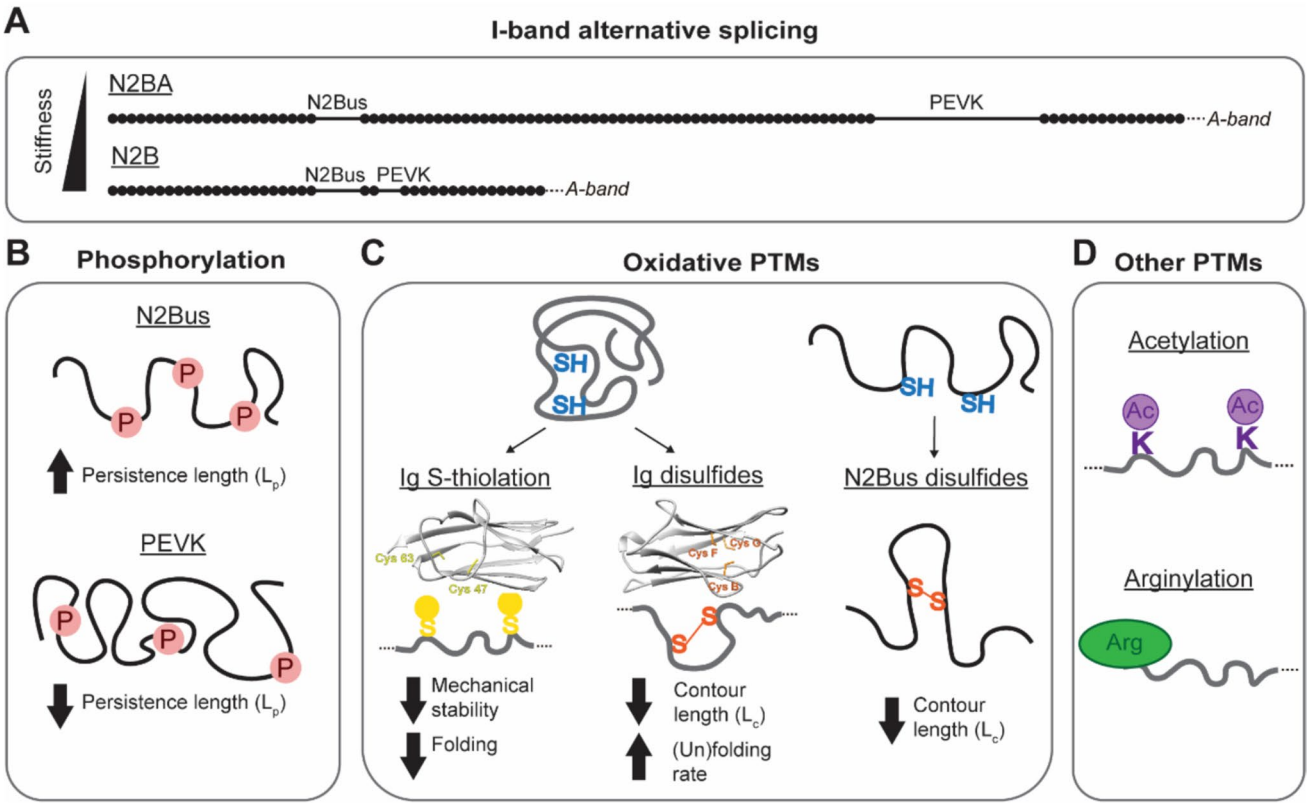

Fig. 3 Regulation of titin-based stiffness. **A** Domain architecture of the I-band region of the main titin isoforms in cardiac muscle. Ig domains are represented as black circles and unstructured regions as black lines. The ratio between the compliant N2BA and the stiffer N2B isoforms modulates titin-based stiffness. **B** Phosphorylation (represented as pink circles) of the unstructured regions of titin modulates titin mechanical properties. Phosphorylation of the N2Bus increases titin L_p while phosphorylation of the PEVK reduces it. **C** Oxidation of titin cysteines in Ig domains (left and middle) and N2Bus (right) modulates titin mechanics. S-thiolation (yellow circles) of titin Ig domains decreases the mechanical stability of titin domains and their folding, while disulfide formation (orange lines) reduces the contour length (Lc) of the targeted regions but increases the (un) folding rates of titin domains. Reduced cysteines are shown in blue. Cartoon representations of protein domains indicate the position of the structurally conserved cysteines in titin that have been shown to be targets of S-thiolation (left, yellow, represented in the structure of domain I27 (PDB ID: 1TIT)) or disulfide bonds (middle, orange, represented in the structure of domain I74 (homology model of positions 9079–9168 of Uniprot Q8WZ42 entry)). **D** Acetylation (top, purple) and arginylation (bottom, green) of titin also target titin molecules, although their mechanical effects are less studied

motif protein 20 (RBM20) (Guo et al. 2012), which promotes the expression of the N2B isoform by repressing the splicing-in of I-band exons (S. Li et al. 2013). The mechanisms that regulate titin isoform switching are still not completely understood, although biochemical pathways including those elicited by insulin and thyroid hormone 3 have been shown to affect RBM20 activity (C. Zhu et al. 2015, 2017). Remarkably, the N2BA:N2B ratio has been observed to increase in cases of disease such as ischemia (Neagoe et al. 2002), dilated cardiomyopathy (DCM) (Makarenko et al. 2004; Nagueh et al. 2004) and in Marfan syndrome (D. Kellermayer et al. 2025). In contrast, the N2BA:N2B ratio decreases during volume overload of the heart, at least in experimental mice (Hutchinson et al. 2015).

Regulation of titin mechanics can also be achieved by posttranslational modifications (PTMs), which are potent regulators of protein nanomechanics (Alegre-Cebollada 2021). The best-studied modification in terms of titin stiffness modulation is the phosphorylation of the unstructured regions N2Bus and PEVK. Phosphorylation of the N2Bus segment by protein kinase A (PKA) (Yamasaki et al. 2002), protein kinase G (PKG) (Krüger et al. 2009), calcium/calmodulin-dependent protein kinase II delta (CaMKIIδ), or extracellular signal-regulated kinase 2 (ERK2) (Hamdani et al. 2013b; Perkin et al. 2015) has been shown to increase the persistence length (L_p) of titin, correlating with reduced cardiac stiffness. In contrast, phosphorylation of the PEVK region of titin by protein kinase C (PKC) decreases L_p and induces increased titin-based passive force (Hidalgo et al. 2009; Anderson et al. 2010) (Fig. 3B). Notably, as further discussed below, altered titin phosphorylation is commonly found in cardiac (Koser et al. 2019) and musculoskeletal (Ottenheijm et al. 2012) disease, but can also be the result of physiological adaptation (Müller et al. 2014). From a mechanistic point of view, it remains unclear how phosphorylation of a few sites can have such dramatic effects on the L_p of long polypeptides, and more so on the mechanics and structure of the whole titin filament (Balogh-Molnár et al. 2025). In this regard, the potential role of Ig domain phosphorylation has not been addressed so far even though multiple potentially relevant phospho-sites at Ig domains have been detected (Hamdani et al. 2017). Also, it is possible that mechanical effects downstream of phosphorylation are contributed by modulation of protein-protein interactions (Raskin et al. 2012).

A more recently studied mechanism for the modulation of titin-based stiffness is the modification of the protein by redox PTMs (Herrero-Galán et al. 2019). The elastic I-band of titin contains > 100 cysteine residues that can be targeted by oxidative modifications. Although these cysteines are generally cryptic, they can be exposed and modified upon unfolding of the domain (Alegre-Cebollada et al. 2014). In fact, recent work has shown that a fraction of them can be found as oxidized in titin molecules in vivo either in physiological (Kramer et al. 2018; Herrero-Galán et al. 2022) or in pathophysiological conditions (Loescher et al. 2020). The location of the cysteines within the fold of titin domains determines the modifications that can target them. Many titin domains contain cysteines that are clustered together at distances and orientations that enable formation of disulfide bonds (Mayans et al. 2001; Giganti et al. 2018). Other domains contain cysteines that are too distant from each other to be crosslinked by disulfide bonds but can be modified by alternative non-crosslinking modifications such as S-glutathionylation (Alegre-Cebollada et al. 2014) (Fig. 3C). The distinction between disulfide bonds and other modifications is fundamental, as they have different effects on titin stiffness. S-glutathionylation of cryptic cysteines in titin Ig domains increases titin compliance by reducing the mechanical stability of the domains and by impairing domain folding (Alegre-Cebollada et al. 2014). In contrast, the formation of disulfide bonds between cysteines belonging to a triad of structurally conserved cysteines increases titin stiffness by reducing the contour length of the unfolded domain (Giganti et al. 2018). Disulfide bonds also accelerate both unfolding and refolding of titin domains, with opposing effects on overall stiffness (Giganti et al. 2018; Eckels et al. 2019). To add further complexity, the unstructured region N2Bus can also be crosslinked by disulfide bonds, which increases titin stiffness (Grützner et al. 2009). Monte Carlo–based computer simulations predict that the overall effect of disulfide bonds is to stiffen titin, although at low forces and for long titin isoforms, they could result in softening through the induction of protein unfolding (Herrero-Galán et al. 2022) (Fig. 3C).

Titin residues can also be targeted by other PTMs such as acetylation (Abdellatif et al. 2021; Koser et al. 2022) or arginylation (Leite et al. 2016), both resulting in stiffening of muscle tissue. It is important to note that while phosphorylation, oxidation, and acetylation have been detected in the mechanically active I-band region of titin, arginylation is only detected in the A-band, and its stiffening effect has been suggested to originate from changes in protein-protein interactions rather than from the alteration of the elastic properties of titin (Fig. 3D).

The role of titin in mechanosignaling

Beyond the mechanostructural roles discussed above, emerging evidence places titin as a central hub for mechanosensing in (cardio)myocytes (Rudolph et al. 2020). For instance, the protein CCDC141 has been identified as an interactor of A-band titin that also binds to nesprin-1, adapting cardiomyocytes to mechanical stress (Hanashima et al. 2025). However, a direct role of titin-based mechanosignaling in

the function of CCDC141 remains to be demonstrated. In addition, a titin-based force-feedback system described in *Drosophila* has been proposed to regulate the length of the thick filament, although the underlying molecular mechanisms remain incompletely understood (Loreau et al. 2025).

At the molecular level, distinct regions of titin can act as hubs for mechanosensing and mechanotransduction, allowing muscle cells to respond to mechanical stress. At the Z-disk, titin participates in a mechanosensory complex with MLP and T-cap (Knöll et al. 2002). At the I-band region, force-dependent modulation of protein-protein interactions involving titin can contribute to mechanosensing and mechanotransduction, although experimental validation remains scarce. A notable exception is the demonstration that the binding affinity between FHL2 and the N2Bus region of titin is activated by force (Sun et al. 2024), which can be important for mechanotransduction during volume overload of the heart (Strom et al. 2024). Other titin interactors have been suggested to participate in titin-mediated mechanosensing. For instance, MARP proteins have been shown to regulate passive force by "locking" titin to thin filaments, potentially protecting myofibrils from damage under excessive stretch (R. J. van der Pijl et al. 2021; Zhou et al. 2021). Interestingly, MARPs proteins limit longitudinal hypertrophy in a unilateral diaphragm denervation model used to study titin-stiffness-dependent muscle hypertrophy (R. van der Pijl et al. 2018, 2025). Similarly, a titin/FHL-1 complex has been proposed to mediate response to mechanical stress in cardiomyocytes (Sheikh et al. 2008). Furthermore, molecular chaperones such as αB-crystallin and Hsp27 bind titin under stress conditions (Golenhofen et al. 2002; Bullard et al. 2004), limiting the extension of unstructured regions and Ig domains (Y. Zhu et al. 2009) and preventing aggregation-induced stiffening (Kötter et al. 2014). Finally, the M-line region has also been implicated in mechanosignaling via its pseudokinase domain. Mechanical stress induces conformational changes within this domain, enabling its ubiquitination by MuRF1 and MuRF2 (Puchner et al. 2008; Bogomolovas et al. 2014). This modification leads to the recruitment of Nbr1 and p62, receptors involved in autophagy and sarcomere turnover (Bogomolovas et al. 2021).

Titin in genetic disease

The importance of titin for striated muscle physiology is further supported by the association of the protein with disease. Indeed, variants in the *TTN* gene cause multiple forms of skeletal (Oates et al. 2018; Savarese et al. 2020; Coppens et al. 2025) and cardiac disease (LeWinter and Granzier 2014; Weston et al. 2024). While the development of skeletal muscle disease requires that pathogenic *TTN* variants are present in homozygosis, compound heterozygosis (Rees et al. 2021) or in combination with pathogenic variants in other genes (Töpf et al. 2024), variants associated with cardiomyopathies exhibit a dominant phenotype and are pathogenic already in heterozygosis (Gerull et al. 2002; Hastings et al. 2016; Domínguez et al. 2023). These heart-disease-causing *TTN* variants are most probably incompatible with life when present in homozygosis or compound heterozygosity (Radke et al. 2019; M. wei Li et al. 2024).

DCM is the best-known example of a genetic disease caused by variants in titin; in most cases, DCM-causing variants generate premature stop codons (i.e., truncating titin variants or *TTN*tvs) (Herman et al. 2012). Besides DCM, *TTN*tvs have also been associated with peripartum cardiomyopathy (Van Spaendonck-Zwarts et al. 2014; Ware et al. 2016; Spracklen et al. 2021), primary myocardial fibrosis (Junttila et al. 2018), atrial fibrillation (Ahlberg et al. 2018; Choi et al. 2018), chemotherapy-induced cardiomyopathy (Garcia-Pavia et al. 2019), and with several muscle pathologies that often involve cardiac phenotypes (Rees et al. 2021). Indeed, the presence of *TTN*tvs has also been shown to increase the risk of several different cardiac conditions, generally in combination with other factors (Shetty et al. 2024a). For example, *TTN*tvs have been shown to favor cardiac remodeling (Schafer et al. 2017) and to be associated with a higher risk of arrhythmias and heart failure (Haggerty et al. 2019; Shetty et al. 2024b). In addition, DCM patients carrying *TTN*tvs in heterozygosis can also have mild skeletal muscle involvement (Skriver et al. 2023). It remains unknown why seemingly equivalent *TTN*tvs can lead to different cardiac conditions. In this regard, a recent report has proposed that the proportion of non-truncating transcripts *TTN*tvs may influence whether carriers develop cardiac conduction disease or DCM (Ishikawa et al. 2025).

Missense variants in titin, which result in single amino acid substitutions, can also contribute to skeletal muscle and heart disease. For instance, pathogenic titin missense variants have been reported in cases of DCM (Gerull et al. 2002; Itoh-Satoh et al. 2002; Hastings et al. 2016; Akinrinade et al. 2019; Meurs et al. 2019; Domínguez et al. 2023), skeletal muscle disease (Hedberg et al. 2014; Rees et al. 2021), arrhythmogenic cardiomyopathy (ACM) (Taylor et al. 2011), restrictive cardiomyopathy (Peled et al. 2014), and atrial fibrillation (Pavel et al. 2025). Missense variants in titin have also been linked to the development of hypertrophic cardiomyopathy (HCM) (Satoh et al. 1999); however, the association of titin with HCM remains controversial (Weston et al. 2024).

In the following sub-sections, we further expand on the available information on the well-established causative role of *TTN* variants in dilated cardiomyopathy, as well as on the emerging role played by *TTN* variants in musculoskeletal diseases.

Titin variants in dilated cardiomyopathy

Among the various heart diseases related to titin, DCM stands out because of its strong association with *TTN* variants. DCM is characterized by left ventricle dilatation and reduced ventricle contractility potentially leading to serious complications such as heart failure and arrhythmias. Indeed, DCM is the most common indication for heart transplantation (Weintraub et al. 2017; Reichart et al. 2019; Schultheiss et al. 2019). Early diagnosis of the disease is important for optimal patient care, but it is hindered by the asymptomatic nature of the early stages of the disease (Japp et al. 2016).

Pathogenic variants linked to DCM have been detected in > 30 genes, including those targeting sarcomere proteins (Reichart et al. 2019). Among them, *TTN*tvs account for ~ 25% of the familial and ~ 18% of the sporadic cases of the disease, making *TTN*tvs the most common cause of DCM (Herman et al. 2012). Most DCM-associated *TTN*tvs originate from variants located in the A-band region of the protein followed by counterparts in the constitutively expressed exons of the I-band (Herman et al. 2012; Schafer et al. 2017). In contrast, *TTN*tvs found in healthy individuals are randomly distributed throughout the titin sequence (Schafer et al. 2017). Titin's extensive alternative splicing likely contributes to this site-dependent effect, as mutations in spliced-out regions may impact less on cardiomyocyte fitness. Another proposed explanation for the variable clinical severity of *TTN*tvs is that the expression of the C-terminal titin isoform Cronos could potentially compensate for truncations in the I-band region, but not for A-band variants (Zou et al. 2015). Finally, it is also possible that inefficient translation termination of certain *TTN*tvs contributes to explaining the absence of pathological consequences in specific cases (van Heesch et al. 2019).

At the protein level, pathomechanisms induced by *TTN*tv appear to stem both from reduced full-length titin levels (haploinsufficiency) and from new toxic properties in truncated titin peptides (Fomin et al. 2021; McAfee et al. 2023), which eventually cause sarcomere deficiency (Hinson et al. 2015). These truncated titin molecules have been suggested to form aggregates that can disrupt the protein quality control system of the cell (Fomin et al. 2021) and to induce sarcomere disarrangement affecting sarcomere organization and mechanosensing (McAfee et al. 2023; D. Kellermayer et al. 2024). In this regard, truncated titin molecules can bear load, but their attachment to the A-band of sarcomeres is less stable, causing recoiling of the protein towards the Z-disk under stress (McAfee et al. 2023). A similar recoiling has been observed when titin cleavage is induced in skeletal muscle (Silva-Rojas et al. 2025). Interestingly, titin cleavage in cardiac muscle induces a fast reactive fibrotic response, suggesting that fibrosis typically found in *TTN*tv-induced DCM is contributed by the toxicity of unloaded titin molecules (López-Unzu et al. 2025). Nevertheless, the fact that increasing both full-length and truncated titin levels leads to improved contractile function of engineered heart tissues carrying *TTN*tvs suggests that titin haploinsufficiency may be more relevant than truncated titins for the pathophysiology of the disease (Ghahremani et al. 2024). It will be interesting to test though whether this scenario also holds in models of disease that can recapitulate DCM-associated fibrosis. Considering the pathogenicity associated with titin haploinsufficiency, a gene variant in a titin intronic enhancer has been linked to DCM (Kim et al. 2024).

Missense variants in titin are also frequently observed in DCM patients (Herman et al. 2012; Akinrinade et al. 2019), but determining whether they are pathogenic or not is complicated by the fact that this class of variants as a whole is also found in the general population at a frequency that is not compatible with DCM prevalence (Begay et al. 2015). As a result, most titin missense variants remain classified as benign or as variants of uncertain significance (VUS) (Ware and Cook 2018; Akinrinade et al. 2019; Morales et al. 2020). To date, only three titin missense variants have been proposed to cause DCM based on strong cosegregation with disease: p.A178D (Hastings et al. 2016; H. Jiang et al. 2021), p.W976R (Gerull et al. 2002; Hinson et al. 2015), and p.C3575S (Domínguez et al. 2023). The pathogenic mechanisms of missense variants may be more variable than those proposed for *TTN*tv, since they can affect any of the > 30,000 residues of the protein. On that note, p.A178D disturbs the interaction between titin and telethonin (Hastings et al. 2016), which accumulates and initiates a proteotoxic response (H. Jiang et al. 2021), while p.W976R and p.C3575S result in destabilization of the hydrophobic core of the parent domain that correlates with reduced contractility of affected cardiomyocytes (Gerull et al. 2002; Hinson et al. 2015; Domínguez et al. 2023; Martinez-Martin et al. 2023). Remarkably, missense titin variants destabilizing the hydrophobic core of titin domains are specifically enriched in DCM patients for which no other genetic cause has been identified (Martinez-Martin et al. 2023). How destabilization of a single titin domain out of hundreds causes DCM remains unknown.

Titin variants in skeletal myopathies

Together with *DMD* and *RYR1*, *TTN* is among the most commonly mutated genes in myopathies (Savarese et al. 2016; Dowling et al. 2021). Myopathies can be classified according to their disease onset, affected muscles, and histopathological hallmarks. For instance, muscle dystrophies are characterized by membrane fragility and myofiber death, while congenital myopathies generally show structural abnormalities. Remarkably, variants in the *TTN* gene cause both muscle dystrophies and congenital myopathies

and the more general term "titinopathies" has been coined to encompass all muscle conditions resulting from mutations in titin, which typically need to occur in homozygosis or compound heterozygosis for disease to develop (Table 1). While *TTN*tvs have historically been the primary focus for geneticists interested in inherited muscle disease, missense variants can also be pathogenic (Rees et al. 2021), which has opened a *Pandora's box* in titin genetics. As for DCM, further developments are needed to fully apprehend the pathogenicity potential of missense variants targeting titin. Similarly, how specific titin variants drive the different titinopathies remains incompletely understood. As discussed below, generation of animal models of titinopathies could be highly informative in this regard.

Among the dystrophic titinopathies, the less severe cases are tibial muscle dystrophy (TMD) and limb girdle muscle dystrophy (LGMD R10 titin-related) (Udd et al. 1991, 2005; Partanen et al. 1994; Hackman et al. 2002). These conditions share a clear hotspot in the C-terminal zone of titin near the M-line, where truncating variants or in-frame deletions enhance the predisposition to cleavage by ubiquitous calpains (Charton et al. 2015). Similar mutations also cause Emery-Dreifuss-like muscular dystrophy (EDMD) phenotypes without cardiomyopathy, where joints are also affected (De Cid et al. 2015). In all these cases, the affected titin molecules are not expected to reach the M-line, likely compromising sarcomere stability. This instability could be behind the various forms of protein aggregation observed by electron microscopy, including cores or disarrays.

Congenital titinopathies caused by titin mutations include centronuclear myopathy (CNM) and autosomal recessive multi-minicore disease with heart disease (MmD-HD) (Ceyhan-Birsoy et al. 2013; Chauveau et al. 2014; Palmio et al. 2019). Both conditions are caused by homozygous or compound heterozygous mutations in *TTN* that introduce premature stop codons at different regions of the protein, with predicted consequences on titin anchoring to both ends of the sarcomere. Cores in electron micrographs also appear in this context and, given the predicted effect of mutations on titin, they presumably arise from sarcomere breakdown due to defective titin scaffolding (Ávila-Polo et al. 2018). Sarcomeric disarrays are also observed in patients with C-terminal variants in *TTN* that are associated with hereditary myopathy with early respiratory failure (HMERF) (Chinnery et al. 2001; Pfeffer et al. 2012; Hedberg et al. 2014).

Another condition recently linked to titin mutations is arthrogryposis multiplex congenita (AMC) (Fernández-Marmiesse et al. 2017; Oates et al. 2018; Bryen et al. 2020; Di Feo et al. 2024), a disorder characterized by prenatal-onset of muscle hypotonia and joint contractures (arthrogryposis). AMC patients typically exhibit hypotonia from prenatal stages, and the disease course depends on the usage of the mutated exons. Notably, cardiac defects are not commonly reported (Di Feo et al. 2024). Most of the AMC-causative mutations are autosomal recessive truncating variants in homozygosity or compound heterozygosity affecting at least one metatranscript-only exon (i.e., exons 158–201) (Pérez-Vidarte et al. 2025). In Fig. 4, we show percent-spliced-in (PSI) values of *TTN* exons, which shows that expression of metatitin exons is almost absent in adult tissues compared to fetal counterparts. The highest expression of metatitin exons is found in fetal skeletal muscle, which may explain the absence of cardiac defects in AMC-*TTN* patients (Di Feo et al. 2024).

Current challenges in the clinical interpretation of titin variants

As discussed above, the central role of titin in striated muscle physiology is reflected in the strong association of the protein with muscle and heart disease (LeWinter and Granzier 2014; Ahlberg et al. 2018; Choi et al. 2018; Weston et al. 2024). However, the presence of titin variants in healthy individuals complicates the evaluation of the pathogenicity

Table 1 Features of skeletal muscle diseases caused by *TTN* variants. *NR*, not reported

Condition feature	TMD/LGMD	EDMD	CNM/MmD-HD	HMERF	AMC
Onset	Adult	Young	Adult	Young	Prenatal
Region	C terminal	C terminal	All through	C terminal	Metatranscript exons
Muscles affected	Anterior part of lower legs	Upper and lower limb muscles and stiff joints	Cranial, axial, proximal, and distal muscles. Joint contractures and cardiac involvement	Upper and lower limb and respiratory muscles	Generalized hypotonia and contractures
Fiber size variability	Yes	Yes	Yes	Yes	Yes
Central nuclei	Yes	Yes	Yes	Yes	Yes
Fibrosis	Yes	Yes	Yes	Yes	Yes
Cytoplasmic bodies	Yes	Yes	NR	Yes	Yes
Vacuoles	Yes	Yes	No	Yes	No
Stiff joints/contractures	Yes	Yes	Yes	Yes	Yes

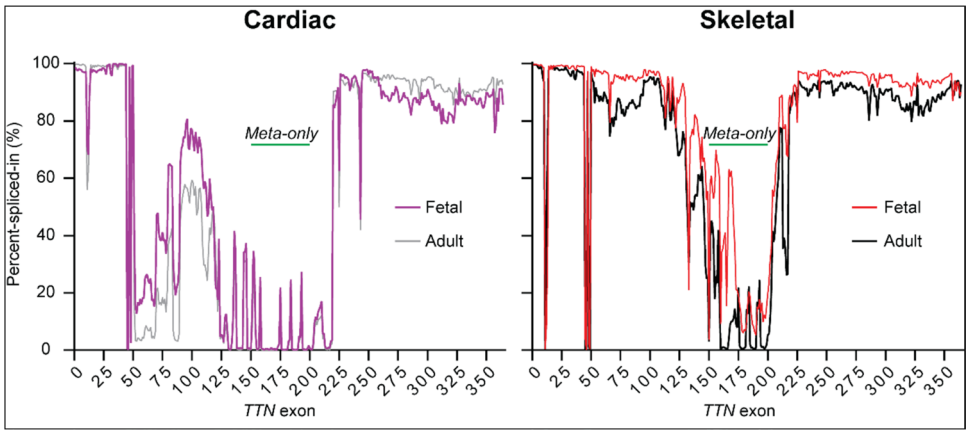

Fig. 4 Expression of *TTN* exons in adult and fetal muscles. Bulk RNAseq analysis of fetal and adult tissues reveals differences in the percent-spliced-in values of *TTN* exons. Cardiac samples show lower expression of exons 50–150 than skeletal muscles. This region corresponds to PEVK-expressing exons, which are incorporated into the N2BA cardiac isoform (representing 30–40% of the titin molecules expressed in cardiac tissue (Neagoe et al. 2002)) and the N2A skeletal isoform. Metatranscript-only *TTN* exons correspond to exons 158–201 (labelled as "Meta-only"), with some exons only expressed in fetal skeletal muscle. The represented data are from Di Feo et al. (2024).

of newly discovered variants. Even *TTN*tvs, which are typically categorized as pathogenic, are detected in 0.5–1% of the general population, a frequency significantly higher than the prevalence of DCM (Norton et al. 2013; Walsh et al. 2017; Bourfiss et al. 2022). This situation is even worse for missense variants. Indeed, most titin missense variants identified in patients are classified as VUS, a genetic category that has little or no use in the clinic (Richards et al. 2015). This situation emphasizes the need for thorough validation when associating variants with disease (Herman et al. 2012; Roberts et al. 2015; Ware and Cook 2018).

The initial approach to assessing the pathogenicity of a newly discovered variant relies on genetic criteria. For instance, variants prevalent in the general population are less likely to be pathogenic, whereas those with low allele frequencies are more likely to be disease-causing. However, for a protein the size of titin, it is not uncommon to find rare missense variants in healthy individuals (Laddach et al. 2017; Weston et al. 2025). Hence, genetic studies aim for cosegregation analysis of the variant of interest with the disease in affected families (Hastings et al. 2016; Domínguez et al. 2023), an approach that is typically inconclusive due to the limited number of relatives and the incomplete penetrance of the disease. Alternatively, geneticists can look for variant enrichment in large cohorts of patients (Herman et al. 2012).

Considering the scarcity of detailed genetics data for many variants, especially missense ones, functional analysis of their damaging effect appears as useful complementary information in pathogenicity assessment (Richards et al. 2015). Classical in vitro methods have attempted to assess changes in ligand binding (Satoh et al. 1999; Rudloff et al. 2015; Hastings et al. 2016), protein structure (Bogomolovas et al. 2016), protein stability (Rudloff et al. 2015; Rees et al. 2021; Domínguez et al. 2023), or mechanical properties (Anderson et al. 2013; Zuo et al. 2021) resulting from titin variants. The main limitation of these methods is that full-length titin cannot be recombinantly expressed due to its size and complexity, and experiments are based on single domains or short fragments of the molecule, which can hinder the characterization of pathogenicity mechanisms. As an alternative, cellular models incorporating titin variants can be used. Although 2D cell cultures of human induced pluripotent stem cells (hiPSC)–derived cardiomyocytes can provide useful information to address structural and functional defects resulting from titin alterations (Fomin et al. 2021; Domínguez et al. 2023), generation of microtissue structures might be needed to fully uncover the pathogenic effects of the variant (Hinson et al. 2015). Functional studies can also be based on animal models (most typically fish and mice) engineered to carry the genetic variant of interest (Marcello et al. 2022). One of the challenges of this approach is that the phenotype developed by the animals does not always resemble the one observed in humans. It is not uncommon that variants causing disease in human patients in heterozygosis require expression in homozygosis in animal models to show phenotypes (H. Jiang et al. 2021), or that even in this case, they exhibit only mild phenotypes (Bogomolovas et al. 2016). Sometimes this challenge can be overcome by stressing the mutant animals, which may show increased sensitivity to the stressor (H. Jiang et al. 2021).

It should be noted that the fact that titin variants have been identified in different pathologies, including cardiac and skeletal conditions, suggests that the disease phenotype

is influenced by mutation-dependent downstream mechanisms. For example, variants targeting Ig or FnIII domains may have different consequences. Similarly, a missense variant affecting exposed regions is more likely to perturb biomolecular interactions (Hastings et al. 2016), whereas another one disrupting the domain hydrophobic core probably results in reduced protein stability (Gerull et al. 2002; Rees et al. 2021; Domínguez et al. 2023; Martinez-Martin et al. 2023). Another obvious consequence of variant localization is its inclusion in specific isoforms. For example, cardiac and skeletal muscle express different isoforms including tissue-specific exons (Laddach et al. 2017) (Fig. 4), which can explain why a given pathogenic variant affects or not specific muscles. Furthermore, recent discovery of AMC-related *TTN* has demonstrated the relevance of prenatal titin isoforms and exon PSI during variant pathogenicity assessment (Di Feo et al. 2024; Vatta et al. 2025). Another factor that needs to be considered is the biochemical environment of the specific region targeted by a given variant. This can explain why very similar variants can result in different phenotypes. For instance, *TTN*-p.A178D has been shown to impair binding to telethonin leading to DCM and left ventricular non compaction (Hastings et al. 2016), while the equivalent variant in an adjacent domain (p.A82D) causes congenital fiber type disproportion with no cardiac involvement (Rees et al. 2021; Weston et al. 2024). Finally, characterizing the effects of titin variants is further complicated by the fact that their associated phenotype may require digenic inheritance with variants targeting other genes (Töpf et al. 2024) or a "second hit" such as pregnancy (Restrepo-Córdoba et al. 2024) or alcoholism, as it has been observed with some *TTN*tv (Ware et al. 2018).

Titin in acquired disease

Recent advances in genetic studies and genetically engineered models have enabled a better understanding of titin biology, firmly establishing the protein as a major genetic determinant of cardiac and muscle disease. In contrast, the role of titin in acquired disease remains less clear, although growing evidence suggests an important contribution of titin in pathological remodeling, particularly in the heart.

The mechanisms linking titin to acquired disease are diverse and not completely understood. For example, dysregulation of the mechanisms that control titin-based stiffness may be a cause of disease. As described above, alterations in titin isoform ratios are present in many forms of cardiac pathology, although it remains unclear whether they serve as a compensatory mechanism for the changes in myocardial stiffness or whether they contribute directly to disease progression. In this context, it is interesting that pathogenic variants targeting RBM20 have been linked to DCM, suggesting a causative role of alterations of titin splicing in disease (Brauch et al. 2009; D. Li et al. 2010; Beqqali et al. 2016). Changes in titin PTMs have also been associated with several diseases (Loescher et al. 2022). Hyperphosphorylation of the PEVK segment and hypophosphorylation of the N2Bus region have been detected in heart failure, diabetes, and diastolic dysfunction correlating with increased myocardial stiffness (Borbély et al. 2009; Hudson et al. 2011; Hamdani et al. 2013a; Kötter et al. 2013; Hopf et al. 2018). These alterations in titin phosphorylation might originate from the aberrant activity of protein kinases observed in pathological conditions (Bowling et al. 1999; van Heerebeek et al. 2012), or by the expression of phosphatases such as protein phosphatase 5, whose levels are increased in heart failure and diastolic dysfunction (Krysiak et al. 2018). Targeting altered titin phosphorylation has been proposed as a therapeutic strategy in heart failure (Bishu et al. 2011; Furukawa et al. 2024; Vahle et al. 2025). The increase in oxidative stress characteristic of several forms of muscle pathology has also been suggested to alter sarcomere function via changes in oxidative PTMs (Avner et al. 2012). Indeed, increased titin oxidation has been detected in failing (Tomin et al. 2021) and ischemic hearts (Loescher et al. 2020; Töpf et al. 2024) and has been proposed to contribute to altered cardiomyocyte mechanics. A similar scenario has been proposed for skeletal muscle subject to immobilization (Watanabe et al. 2025).

Finally, titin integrity is compromised in several pathological contexts including ischemic disease, anthracycline-induced cardiotoxicity, and atrial fibrillation (Ali et al. 2010; Kötter et al. 2016; Chan et al. 2021; Cizauskas et al. 2024), which are conditions in which a cleaved form of titin (known as T2) has been detected. Interestingly, the inhibition of matrix metalloprotease 2 (MMP-2) prevented both cleavage of titin and myocardial fibrosis in experimental models of anthracycline-induced cardiotoxicity (Chan et al. 2021). However, the extent to which titin cleavage directly contributes to myocardial remodeling remains to be fully addressed, given that MMP-2 also targets other sarcomeric and non-sarcomeric proteins. As discussed in the next section, new precise models that selectively compromise titin integrity are beginning to clarify this issue.

Models of titin genetic disease: pathophysiology and therapeutic strategies

Despite the limitations of functional assays outlined in the "Current challenges in the clinical interpretation of titin variants" section, animal and in vitro models have provided insights into the physiopathology and potential treatment strategies for conditions induced by *TTN* variants. For instance, in vitro experiments using hiPSCs-derived

cardiomyocyte models containing DCM-associated *TTN*tvs variants showed that CRISPR-mediated reading frame repair provides functional recovery (Romano et al. 2022) and that CRISPR-directed activation of the *TTN* promoter rescues sarcomere deficits in hiPSC-derived models of DCM (Ghahremani et al. 2024). Gramlich et al. (2015) also showed that antisense oligonucleotide-mediated exon skipping that avoids a titin frameshift mutation improved sarcomeric ultrastructure and cardiac performance in human and murine models. Zebrafish models facilitated the discovery of novel titin isoforms, including Cronos, that allowed a better understanding of titin-driven cardiomyopathies (Zou et al. 2015; Deo 2016), as subsequently confirmed in human cardiomyocytes (Zaunbrecher et al. 2019). Also, models that delete specific regions of titin have been valuable in elucidating titin's role in cardiac physiology and mechanosignaling as well as its contribution to myocyte passive stiffness (Weinert et al. 2006; Radke et al. 2007, 2019; H. L. Granzier et al. 2009; Chung et al. 2013; Charton et al. 2016; Biquand et al. 2021; Methawasin et al. 2022). Regarding acquired heart disease, preclinical studies have shown that the inhibition of RBM20 and subsequent generation of longer titin isoforms can be used to ameliorate symptoms in heart failure models (Bull et al. 2016; Hinze et al. 2016; Methawasin et al. 2016; Radke et al. 2021; Celik et al. 2025).

In the context of skeletal muscle titinopathies, murine models of TMD/LGMD R10 titin-related myopathies carrying the FinMaj mutation, which increases titin's susceptibility to calpain cleavage, (Charton et al. 2015), have shown that reducing calpain-3 expression attenuates dystrophic histological features (Charton et al. 2010). Similarly, mdm mice carrying a spontaneous in-frame deletion of 83 out of the 104 amino acids in the N2A segment of titin show a severe form of muscular dystrophy with early death that correlates with altered protein-protein interactions (Garvey et al. 2002; Witt et al. 2004; Huebsch et al. 2005). Other regional knockouts also result in reduced muscle performance, muscle atrophy, and myonuclei internalization (Gotthardt et al. 2003; Peng et al. 2005; Buck et al. 2014; H. Granzier et al. 2014; Charton et al. 2016; Brynnel et al. 2018; Radke et al. 2019).

Collectively, the mouse models presented above are not only a tool to develop therapeutic strategies for titinopathies, but they also identify titin as a major regulator of myocyte function. However, these models do not yet resolve whether the observed pathophysiological effects arise from the perturbation of titin mechanical function or from non-mechanical signaling roles. This uncertainty has been recently addressed with emerging experimental models that perturb titin integrity in vitro and in vivo by specifically cleaving the protein upon heterologous expression of Tobacco Etch Protease (TEVp) (Rivas-Pardo et al. 2020; Freundt et al. 2025; López-Unzu et al. 2025; Pricolo et al. 2025; Silva-Rojas et al. 2025). Remarkably, these models can offer new mechanistic insights of the contribution of cleaved titin molecules in episodes of cardiac ischemia (Ali et al. 2010; Kötter et al. 2016), anthracycline-derived cardiotoxicity (Chan et al. 2021), atrial fibrillation (Cizauskas et al. 2024), pulmonary hypertension (López-Unzu et al. 2025), metabolic syndrome (Cuijpers et al. 2021), and, potentially, ACM (Taylor et al. 2011; Anderson et al. 2013). Collectively, studies exploiting TEVp-mediated severing of titin in living myocytes demonstrate that titin cleavage induces sarcomere disassembly and drives both skeletal muscle and cardiac disease. In skeletal muscle, fibers containing cleaved titin remain viable, but undergo atrophy and myonuclei internalization (Silva-Rojas et al. 2025). In cardiac muscle, cleavage of ~ 30% of titin triggers a rapid fibrotic response, accompanied by reduced cell-cell connections between cardiomyocytes (López-Unzu et al. 2025; Pricolo et al. 2025). Importantly, cleaved titin molecules may elicit the same mechanosignaling as truncated molecules observed in genetic diseases since both conditions involve titin recoiling towards the Z-disk (McAfee et al. 2023; Silva-Rojas et al. 2025).

Perspectives

The field of titin has made a long way since the initial reports on the existence of a very large protein in muscle samples (Maruyama 1976; Wang et al. 1979). Now we know the fundamental functions of the protein and its association with genetic and acquired skeletal muscle and heart disease, but we are still lacking full mechanistic understanding on how the integrative roles of the protein are perturbed by mutations or posttranslational processing. Emerging models of titin dysfunction are expected to improve our understanding of titinopathies and cardiac conditions involving titin; these models should also be useful to test novel therapies before translation to human patients. Unfortunately, murine models of *TTN*tvs have limited value since they show little to no basal phenotype in heterozygosis but are lethal in homozygosis (Gramlich et al. 2009; Schafer et al. 2017). Alternative inducible KO and knock-down models may not recapitulate toxicity of truncated titins; indeed, differently from carriers of *TTN*tvs (Ahlberg et al. 2018; Junttila et al. 2018; Verdonschot et al. 2018), these models applied to cardiac titin result in heart disease without myocardial fibrosis (Liao et al. 2019; Radke et al. 2019). In contrast, murine titin-cleavage models do trigger fibrosis (Freundt et al. 2025; López-Unzu et al. 2025; Silva-Rojas et al. 2025). In this regard, hiPSC-derived cardiomyocytes and their incorporation into engineered tissues together with stromal cells offers an enticing opportunity to study the role of *TTN*tvs in human cells overcoming limitations of mouse models and to provide proof-of-concept of potential therapies. Future research will also need to address why clinical manifestations of pathogenic

TTN variants vary from no disease to DCM (Herman et al. 2012), primary fibrosis (Junttila et al. 2018) or atrial fibrillation (Ahlberg et al. 2018; X. Jiang et al. 2024). Is this variation dependent on the specific location of the variant? Can the same class of variants cause all these different forms of disease? Do environmental factors play a role in phenotype specification? There also remains an urgent need for the development of methods that can guide pathogenicity assessment of the thousands of titin variants (particularly missense ones and those affecting intronic regions of *TTN* (Kim et al. 2025)) found in patients. Related to this, it is conceivable that mechanisms of pathogenicity of missense titin variants do not fully overlap with those induced by *TTN*tvs, potentially requiring tailored therapeutic interventions. Undoubtedly, mending all possible *Achilles heels* of titin (let them be mutations, sites of proteolysis or aberrant posttranslational modifications) will require the whole warfare of contemporary (personalized) pharmacology (Ware and Cook 2018; Javed and Halliday 2023).

Acknowledgements JAC acknowledges funding from Ministerio de Ciencia, Innovación y Universidades (MCIU, MICIU/AEI/10.13039/501100011033) through grants PID2023-147683NB-I00 and RED2022-134242-T, the Regional Government of Madrid (*Bases Reguladoras* 2402/202; Convocatoria 3177/2024; TecNanoBio TEC-2024/TEC-158), and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. [101002927]). CNIC is supported by the Instituto de Salud Carlos III (ISCIII), the MCIU, and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MCIU). IMM was the recipient of a Doctoral INPhINIT fellowship from 'la Caixa' Foundation (ID100010434, fellowship code LCF/BQ/DR20/11790009). RSR acknowledges funding from the European Molecular Biology Organization and AFM-Téléthon (Postdoctoral fellowships: EMBO ALTF 417-2022 and AFM-Téléthon 29507; Trampolin Research Grant AFM Téléthon 28755). We thank all members of the Molecular Mechanics of the Cardiovascular System Laboratory for feedback and support.

Author Contribution All authors participated in the drafting of the manuscript and figures

Funding 'la Caixa' Foundation, EMBO, AFM Téléthon, MICIU Regional Government of Madrid, European Research Council

Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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