

# Cardiac titin, the giant sarcomeric protein in health and disease

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## INTRODUCTION

The last decade has witnessed exciting results of extensive investigations into the structure and function of the giant sarcomere protein, titin. This has opened up new perspectives of diagnosis and treatment of heart failure (HF), as well as of investigations into the genetic background of some cardiac diseases. This review briefly presents the results of basic investigations and describes how they have been used recently to solve some clinical problems. Excellent, extensive reviews have been recently published describing

the results of investigations into titin. These will be referred to in the text.

## THE LOCATION OF TITIN IN THE HEART AND ITS STRUCTURE

Titin is located in the sarcomere, an elementary force generating unit of the striated muscles. Sarcomere's boundaries are two Z discs (Fig. 1A). The thick myosin filaments are positioned in the centre of the sarcomere. Their spatial arrangement is secured by proteins which form an M-band running across the centre of the sarcomere. The ends of myosin filaments are connected to the Z discs by connecting filaments build up of titin. The thin actin filaments are anchored to the Z discs and partly interdigitated with the myosin filaments. The part of the sarcomere formed by actin filaments alone is marked as I (isotropic) band and the part containing myosin filaments is marked as A (anisotropic) band (Fig. 1A). The myosin filaments generate force by pulling on the actin filaments upon the myocyte excitation, so myosin and actin are commonly called the contractile proteins. Sarcomere is also built up by a large number of other proteins of many functions. Titin is the third (after contractile proteins) most abundant protein of the sarcomere.

Titin was first isolated from the connecting filaments of sarcomeres in 1976 by Maruyama [1] and originally called connectin. In 1985, Wang [2] found that connectin extends also along the myosin filaments, thus spanning all the half of the sarcomere between the Z disc and the M-band (Fig. 1A). Because of the gigantic size of the connectin molecule (it is the largest protein of the body), he called it titin.

Titin is encoded by a single gene (TTN), but its many isoforms may be expressed due to alternative splicing. The molecular mass of these isoforms ranges between ~3.0 and ~4.2 MDa. Titin consists of a total of 38,138 amino-acid residues [3, 4]. The C-terminal of titin is anchored to the M-band of the sarcomere and its N-terminal is anchored to the Z disc.

The fragment of titin molecule spanning the I-band consists of tandemly arranged immunoglobulin (Ig)-like domains (Fig. 1B). In adult humans, titin is expressed in two isoforms: N2B and N2BA. In the N2B isoform, the Ig-like domains of the I-band titin are interspersed with the N2B element and reach in proline (P), glutamate (E), valine (V) and lysine (K) residues (PEVK) sequence. In the isoform N2BA, the I-band part of titin molecule contains additionally the element N2A positioned between element N2B and the PEVK sequence (Fig. 1B) [4, 5]. All these titin components function as the springs [3].

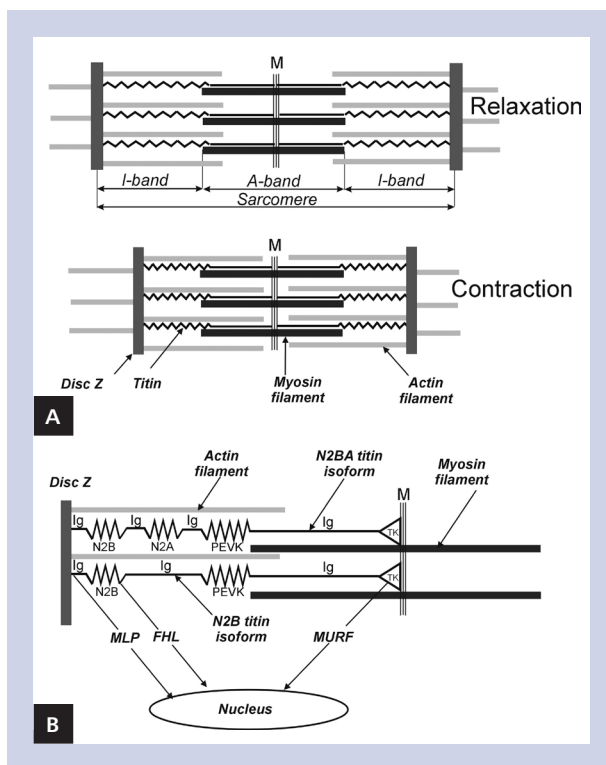
The part of the titin molecule spanning the A-band is built of the Ig and fibronectin-like domains mostly arranged in a super-repeat pattern of six or 11 superdomains [3].

Isoform N2BA is less stiff (more compliant) than isoform N2B [5, 6]. So the relative abundance of the isoforms is one factor determining the stiffness of titin in physiological and clinical settings. In a normal left ventricle (LV) of the human heart, the N2BA/N2B ratio ranges 0.4–0.6. However, it differs in cardiac chambers. Generally, the right ventricle (RV) expresses more N2BA isoform than the LV, and atria express more of it than ventricles [7].

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**Figure 1.** Schematic presentation of a sarcomere of cardiac muscle (A), titin molecule structure (B), its localisation in the sarcomere and the main signalling pathways of titin mechanosensing; MLP — muscle LIM protein; FHL — four and a half LIM protein; MURF — RING finger protein; TK — titin kinase

## ROLE OF TITIN IN THE HEART

### The role of titin in the ventricular haemodynamic cycle

The C-terminal of titin is anchored in the M-band and its N-terminal is anchored in the Z disc. The part of the titin molecule spanning the I-band is compressed below the slack length during myocyte contraction (Fig. 1A), generating the recoil force that contributes at relaxation to restoration of the myocyte diastolic length [7, 8]. When the sarcomere is stretched beyond the slack length, titin generates passive tension.

The sub-segments in the I-band titin contribute differently to generation of the passive tension of titin. Upon stretching of cardiac myocyte from its slack length, at first, the Ig domains region extends mainly due to its unfolding. When its distensibility is exhausted, the PEVK sequence and next N2A and N2B units extend. The passive tension increases more steeply when PEVK and N2B are stretched than upon stretching of the Ig domains region [3].

In the *in situ* working LV, titin is compressed during the phases of isovolumic contraction and ejection. Its recoil force contributes to restoration of the diastolic length of the myocytes at the phase of isovolumic relaxation between the closure of the aortic valves and the opening of the mitral valve. There-

fore the rate of the drop in LV wall tension and the drop in LV pressure at this phase of the haemodynamic cycle is related to titin's recoil force. When the ventricular pressure drops below that in the left atrium, and the volume of the ventricle increases due to the diastolic blood influx, titin is stretched beyond the slack length which results in generation of its passive tension.

### *Titin as a determinant of diastolic stiffness of the heart muscle*

Because in the resting myocyte the myosin and actin filaments slide past each other with minimal resistance, the tension of titin sets the tension of sarcomere, i.e. of myocyte. Tension of titin at the given length of sarcomere depends on its stiffness. There are two main factors contributing to the passive tension of the heart muscle at the given diastolic volume of the ventricle: the stiffness of myocyte set by titin, and the stiffness of collagen nets in extracellular matrix (ECM). According to the results of Granzier and Irving [9] obtained in single isolated cells and in trabeculae, over the working range of sarcomeres (1.9–2.2  $\mu\text{m}$ ), passive tension set by titin dominates at shorter lengths and collagen dominates at longer lengths. Similar results have been obtained in isolated, intact hearts. Diastolic pressure of the LV remains in equilibrium with the passive wall tension. Chung and Granzier [10] found that in Langendorff-perfused LV of the mouse heart, titin contributes ~80% of passive pressure within the working sarcomere length 1.8–2.2  $\mu\text{m}$ . ECM contribution may exceed that of titin at sarcomere lengths > 2.2  $\mu\text{m}$ .

### *Mechanical properties of the A-band titin and its role in structure and function of sarcomere*

The part of the titin molecule spanning the A-band is tightly bound to myosin filaments, so it does not change its length during contraction or passive stretch of sarcomere. Nevertheless, it is very important for the sarcomere mechanics. Anchored firmly to the M-band, A-band titin provides support for the I-band part of titin, enabling development of its passive tension. The A-band part of titin is subjected to both active (during contraction) and passive (during diastolic stretch) forces. These forces can activate titin-related signalling pathways regulating expression of contractile proteins and myocyte hypertrophy (see the section below entitled 'The role of titin in heart hypertrophy — mechanosensing'). Moreover, A-band titin is essential for the proper structure and function of the sarcomere. Its C-terminal interacts with myomesin, and obscurin, the proteins involved in anchoring myosin filaments to other proteins in the M-band. The complex of titin, myomesin, obscurin and myosin is important for the stability of the M-band [3]. The A-band titin interacts also with the big cardiac myosin binding protein-C (cMyBP-C) associated with the myosin of the thick filaments. The titin-cMyBP-C complex is important for the assembly of the myosin thick filaments and their proper positioning in the sarcomere [3, 11].

### **Regulation of the mechanical properties of titin**

Myocyte stiffness may be modulated by the change in the ratio of titin isoforms (isoform N2BA is less stiff than isoform N2B). This is a long process lasting for days. However, titin stiffness may be modulated also rapidly by phosphorylation of its elements by protein kinases.

**PKA-dependent phosphorylation.** N2B element is phosphorylated by cyclic adenosine monophosphate (cAMP) dependent protein kinase A (PKA) activated upon stimulation of  $\beta$ -adrenergic receptors. Phosphorylation results in a decrease of the N2B stiffness, which enhances the diastolic filling of the ventricles at the heart rate increased by adrenergic stimulation [5, 12].

**CaMKII-dependent phosphorylation.** Both the N2B element and the PEVK sequence are phosphorylated also by  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II delta (CaMKII $\delta$ ). Phosphorylation results in a decrease in stiffness of N2B, and an increase in stiffness of PEVK [13, 14]. Since CaMKII $\delta$  phosphorylates preferentially the N2B element, the authors feel that at the physiological concentrations of the kinase in sarcoplasm, the phosphorylation of the PEVK sequence is not significant [13]. CaMKII $\delta$  is activated by increased  $\text{Ca}^{2+}$  concentration during adrenergic stimulation. Since, in humans, phosphosites of CaMKII $\delta$  in N2B do not overlap with the sites targeted by PKA [13], the effects of activation of these kinases upon adrenergic stimulation may add up. The resulting decrease in myocytes stiffness would enhance diastolic filling upon increased heart rates.

**PKC-dependent phosphorylation.** The PEVK sequence is also phosphorylated by protein kinase C alpha (PKC $\alpha$ ) [15] activated by stimulation of Gq proteins coupled receptors (for instance AT-1 receptors for angiotensin II, ET1 receptors for endothelin and  $\alpha$ -adrenergic receptors). Phosphorylation of PEVK by PKC increases titin stiffness [16]. This has been proved in experiments in mice in which the PEVK sequence has been removed by means of genetic manipulation [17]. Increased activation of PKC in HF due to excessive stimulation of the Gq-coupled receptors may contribute to an increase in diastolic stiffness of heart muscle.

**PKG-dependent phosphorylation.** The element N2B, and to a lesser degree N2BA, is also phosphorylated by protein kinase G (PKG) activated by cyclic guanylyl monophosphate (cGMP) [18], which results in a decrease of their stiffness.

The cGMP is produced by guanylyl cyclase (GC) expressed in two forms: soluble (sGC) and sarcolemma-associated particular (pGC). The sGC is activated in sarcoplasm by nitrogen oxide (NO) produced by its endothelial synthase (eNOS). It is very sensitive to the oxidative stress: oxidation renders it insensitive to NO. The sarcolemma-associated pGC is expressed in two forms: A, being a catalytic domain of the natriuretic peptide A receptor (NPR-A) and B, being a catalytic domain of the natriuretic peptide B receptor (NPR-B). Thus, pGC is activated by both atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). In cardiac myocytes, NPR-A dominates [19–21]. The activators of GC enhance diastolic compliance of the heart

muscle. NO and stimulation of eNOS accelerate LV relaxation and increase its distensibility [20, 22]. A similar effect of acute BNP administration has also been reported [20, 23].

The cGMP is hydrolysed by phosphodiesterases (PDEs). At least four PDEs are expressed in cardiac myocytes (1, 2, 3, and 5). PDE1, PDE2 and PDE3 are the dual substrate enzymes hydrolysing both cGMP and cAMP. PDE5 is cGMP specific and represents ~20% of total cGMP-esterase activity in cardiac myocytes. The activity of PDE5 is inhibited by sildenafil [19]. Thus sildenafil increases the concentration of cGMP which activates PKG. Besides phosphorylating N2B, PKG regulates diverse cellular functions in the cardiovascular system. It is antihypertrophic, relaxes the smooth muscle cells in systemic and pulmonary circulation, and is cardioprotective in hypoxic and toxic heart injury [19]. Because of this diversity in PKG actions, its activation by means of sildenafil has been used as a therapeutic strategy in various clinical settings (reviewed in [24, 25]).

### **DEFICIENT TITIN PHOSPHORYLATION IN HEART FAILURE**

The disturbances in the rate and degree of diastolic filling are important factors in the mechanism of HF, especially of its diastolic form. They result from the changes in mechanical properties of ventricular walls due to remodelling of ECM and deviations in stiffness of cardiomyocytes. The later depend almost exclusively on titin. As reviewed above, the stiffness of titin may be changed by altering the ratio of its N2BA to N2B isoforms and by phosphorylation of its N2B, N2BA, and PEVK components.

In systolic HF, the N2BA/N2B ratio has been found to be increased to 0.65–1.0, which should result in decreased myocyte stiffness [20, 26–28]. However, the effects of shifts in expression of titin's isoforms may be modified by the level of phosphorylation of N2B element and PEVK sequence. Borbely et al. [29] found that the N2BA/N2B ratio was increased in the hearts of patients with dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) compared to controls, which should result in decreased myocytes stiffness. However, the stiffness of myocytes isolated from these hearts was higher than in controls in both groups. In these hearts, phosphorylation of the N2B element was reduced, and treatment of their isolated myocytes with PKA or PKG restored their passive tension to normal. These results suggest that the increase in the myocytes stiffness resulted from hypophosphorylation of the N2B unit overcompensating the effect of isoforms shift.

In diastolic HF, in contrast to systolic HF, the N2BA/N2B ratio was decreased, which should result in increased diastolic stiffness. As in systolic HF, titin has been found to be hypophosphorylated [20]. In these patients, the effects of isoforms shift and hypophosphorylation add up, resulting in increased myocyte stiffness and impeded diastolic LV filling [7, 30, 31].

Hamdani et al. [14] found that expression of protein kinase CaMKII is increased in human end stage failing hearts. This results in an increase in phosphorylation of N2B and hyperphos-

phorylation of PEVK. The passive stiffness of human myocytes has not been measured in this study. However, the stiffness of skinned myocytes isolated from the CaMKII KO mice was largely increased and brought to the level of those from the WT mice by incubation with this kinase. Additionally, overexpression of CaMKII set the myocyte stiffness below that in the WT mice in this study. So, in their conclusions, the authors propose that the overall effect of increase in expression of CaMKII in the human myocytes would be decreased myocytes stiffness.

Hamdani et al. [32] studied myofilament phosphorylation and function in the old, hypertensive dog model of diastolic HF. The activity of PKG was reduced, whereas the activity of PKC $\alpha$  was increased in the myocardium of the old dogs compared to controls. Accordingly, phosphorylation of N2B and N2BA units was decreased, whereas that of PEVK was increased. The skinned myocytes isolated from the LV of the old dogs revealed increased passive stiffness which could be normalised by incubation with PKA or PKG. Previously this group [24] found that sildenafil and BNP increased the plasma cGMP levels and phosphorylation of N2B and N2BA titin isoforms. The increased stiffness of the skinned cardiomyocytes isolated from the hearts of old dogs was reduced in myocytes isolated from the dogs receiving sildenafil or BNP. Augmentation of cGMP was associated with enhanced LV diastolic distensibility reflected in an increase in end diastolic volume without an increase in LV end-diastolic pressure and in a decrease in the mean left atrial pressure.

Taken together, the results of investigations in animal models and in clinical settings strongly suggest that hypophosphorylation of titin is a common finding in HF, being an important factor in the mechanism of derangement of the LV diastolic function. This leads to the assumption that the correction of titin phosphorylation might be of benefit to patients suffering HF, especially its diastolic form.

### TARGETING TITIN PHOSPHORYLATION IN HEART FAILURE

Two important mechanisms of deficient titin phosphorylation by PKG in failing hearts have been proposed: (1) the expression of PDE5 is close to nil in the myocytes of normal hearts, but it is largely increased in myocytes of failing human hearts. PDE5 expression and activity was also increased in hypertrophied mice hearts, the increase being positively related to the degree of oxidative stress; (2) the bioavailability of NO in HF is decreased by oxidative stress by a number of mechanisms [reviewed in [20]], which results in a decrease in the activity of soluble GC. Both mechanisms lead to decreased PKG activity [33]. Sildenafil may correct this deficiency by PDE5 inhibition.

The logical consequence of the above findings is that attempts should be made to use sildenafil for the correction of the impaired diastolic mechanics of the heart muscle, especially in patients suffering diastolic HF. This is under current intensive investigation.

### *Diastolic heart failure*

Guazzi et al. [34] published results of a double-blind, randomised, placebo controlled study on treatment with sildenafil (one year, 50 mg thrice per day) of a group of 44 patients with HF with preserved ejection fraction and pulmonary artery systolic hypertension complicated by RV dysfunction.

There was a significant improvement in most of the haemodynamic parameters studied, as well as an improvement in life comfort. Mean pulmonary arterial pressure decreased, tricuspid annular systolic excursion and ejection rate increased, whereas right atrial pressure decreased, proving an improvement in the RV function. There was also a marked improvement in the LV function as evidenced by a decrease in the wedge pulmonary pressure, deceleration time, isovolumic relaxation time, and increased septal mitral annulus velocity. These changes suggest a marked improvement of the LV diastolic function and its diastolic compliance by the treatment with sildenafil. Improvement in the LV function could ameliorate pulmonary arterial hypertension. However, since the drop in pulmonary arterial resistance was greater than the drop in the pulmonary wedge pressure, an additional direct effect of sildenafil on the pulmonary circulation as a component of the beneficial outcome of the therapy seems likely.

Recently, the RELAX Trial investigating the effect of sildenafil in 216 patients with HF with preserved ejection fraction recruited in 26 centres in North America has been completed [35]. Unfortunately, the results are much less encouraging than those of the Guazzi group [34]. The drug study group received sildenafil at 20 mg three times daily for 12 weeks with complete re-examination at the end of this period. Then the dose was increased to 60 mg three times a day for the next 12 weeks. The primary end-point was maximal oxygen consumption and one of the secondary end-points was six-minute walk distance. The effects of sildenafil on LV hypertrophy and function estimated by cardiac magnetic resonance imaging, on Doppler estimated diastolic function parameters, and on pulmonary artery systolic pressure, were investigated. No significant differences between these end-points in the sildenafil and placebo group were found. Discussing the reasons for the difference between the outcome of their study and that of Guazzi et al. [34], the authors pointed out that the patients of the Guazzi group were generally more severely ill, having greater LV hypertrophy, advanced RV failure, and higher arterial pulmonary pressure. The authors refer to the experiments in mice with pressure overload heart hypertrophy in which the beneficial effect of sildenafil was the stronger the more severely the hearts were damaged [36].

Negative results were also published by Andersen et al. [37] who tested the effect of sildenafil (40 mg thrice a day for nine weeks) in 70 patients with diastolic dysfunction and preserved ejection fraction after myocardial infarction. The pulmonary wedge pressure and pulmonary arterial pressure at rest and during exercise did not differ between the

sildenafil and placebo groups, while cardiac index and LV diastolic volume index increased at rest and at exercise. The authors concluded that sildenafil did not decrease the filling pressure in their patients. So it probably did not affect the LV diastolic compliance.

The general conclusion of the RELAX Trial study and other studies is that more multicentre studies on the effects of inhibition of PDE5 in the diastolic HF are needed. Recently Cooper et al. [38] published a project of multicentre investigation into the effect of sildenafil in 210 patients with HF and group 2 pulmonary hypertension.

### ***Systolic heart failure***

LV diastolic dysfunction is a common finding both in diastolic and systolic HF. This has prompted investigators to test the effects of sildenafil also in systolic HF. The results obtained by various authors and published up to September 2011 were reviewed by Cvelich et al. [39], with the general conclusion that sildenafil can improve markers of cardiovascular and pulmonary function in patients with HF.

The recent trial of Guazzi et al. [40] included 45 patients in NYHA class II–III with ischaemic, idiopathic, or hypertensive cardiomyopathy. They received sildenafil vs. placebo as an adjuvant to the stabilised basic therapy consisting of renin–angiotensin–aldosterone system inhibition. Sildenafil was administered 50 mg three times daily. Complete examination of patients was repeated after six and 12 months of treatment. The results are in favour of sildenafil improving LV relaxation, as shown by an increase in early diastolic tissue velocities at the mitral lateral and septal annuli as well as reverse remodelling of the left atrial volume index. LV ejection fraction increased and LV mass index decreased. This latter effect could depend on the direct effect of inhibition of PDE5 on myocardial hypertrophy [41]. The changes in haemodynamic indices were accompanied by improved exercise performance, ventilation efficiency, and quality of life.

Positive results were obtained also by Reichenbach et al. [42] in patients with advanced, pre-transplant HF and severe pulmonary hypertension followed up for 349 days of treatment with sildenafil. Pulmonary vascular resistance and transpulmonary gradient were reduced, and cardiac output increased, without a change in ventricular filling pressure. There was also an improvement in NYHA class. The peri-transplant survival was better in patients receiving sildenafil.

The results of the above studies suggest that PDE5 inhibitors may be therapeutic options for patients who cannot tolerate standard therapy for HF or who remain symptomatic with standard therapy.

## **THE ROLE OF TITIN IN HEART HYPERTROPHY — MECHANOSENSING**

The muscles of the ventricular walls of the heart generate tension coping with the varying afterload to ensure forward

pumping of the blood. The afterload depends on the diastolic pressure in the pulmonary artery or aorta and on the shape and volume of the ventricles. Prolonged increase in afterload, for instance by the aortic banding in experimental animals or in human pulmonary or systemic arterial hypertension, leads to initially adaptive hypertrophy of cardiomyocytes enabling generation of force matching increased afterload. Myocytes hypertrophy results from the increased expression of the contractile and accompanying proteins of sarcomeres. So the myocyte genome must be somehow informed on the changes in mechanical conditions of the heart work in order to answer the needs. Titin is an important mechanosensor in sarcomere, able to initiate transmission of information on increased load to the nucleus. Being anchored in the M-band and the Z disc, spanning all the half of the sarcomere, titin is subjected to the forces developed by the sarcomere as well as forces developed within ECM and transmitted to the myocyte skeleton. On the other hand, titin interacts with a number of proteins involved in pathways transmitting the signals to the nucleus. Therefore titin may play a role of mechanosensor transforming mechanical signals into chemical messengers. Three fragments of titin are mainly involved in mechanosensing: the N-terminus anchored in the Z disc, the N2B element and PEVK sequence in the I-band, and the C-terminus and A-band titin close to the M-band (titin kinase [TK] domain) (Fig. 1B).

### ***Mechanosensing of N-terminus of titin in the Z disc***

The thin actin filaments anchor in the Z disc, so it is exposed to the active tension generated by the sarcomere. On the other hand, the Z disc is connected to a number of proteins transmitting the mechanical signals from the myocyte to the ECM, and from the ECM to the myocyte. The ends of the actin filaments anchored in the Z disc are interconnected by  $\alpha$ -actinin forming a network called a small square lattice [43] which changes its pattern when the tension of sarcomere increases. Titin is integrated with this network, with protein theletonin, and with CapZ, into a putative mechanosensor complex [7]. This complex also contains muscle LIM protein (MLP). In response to mechanical stress, MLP translocates to the nucleus (Fig. 1B) where it may increase sarcomeric proteins expression [7, 44]. The Z disc titin is also tied by several proteins to the calcineurin/NFAT pro-hypertrophic pathway [45].

### ***Mechanosensing of I-band titin***

Two members of the four and a half LIM protein family, FHL-1 and FHL-2, are expressed in the heart and bind to N2B element. Under the increased tension sensed by the N2B element, they can shuttle to the nucleus (Fig. 1B) and act as transcriptional co-activators [7]. Moreover, they may function as transmitters linking various signalling pathways to transcriptional regulation. The cardiac atrophy and decreased FHL levels have been found in N2B KO mice in which the N2B element is absent [5, 46] and FHL deficiency has been

proven to protect the myocardium from pathological hypertrophy [5, 47].

Removal of PEVK in KO mice results in upregulation of FHL-1 and FHL-2 and cardiac hypertrophy [5, 48], which may be interpreted as PEVK signalling having antihypertrophic activity.

### ***Mechanosensing of M/A-band titin***

Titin contains a single autoinhibitory kinase domain (TK) located near the C-terminal anchored to the M-band of sarcomere (Fig. 1B). The catalytic domain of the TK contains the autoinhibitory serine residue preventing its activation. Phosphorylation of this residue initiates activation of the enzyme [49]. In the relaxed myocyte, the C-terminal tail wraps around the catalytic domain and occludes the ATP binding site and its access to the autoinhibitory tyrosine residue preventing its phosphorylation. Therefore in the relaxed myocyte, TK remains inhibited [50]. The M-band is relatively compliant, therefore the proteins embedded in this sarcomere component are exposed to the stress developed by interaction of myosin and actin filaments in the activated sarcomere. Experiments with single-molecule force spectroscopy suggest that the exposure of TK domain to stretch force causes the unfolding of the inhibitory C-terminal opening the access of ATP to its binding site and phosphorylation of the autoinhibitory tyrosine [49–51].

The activated TK binds a number of proteins, among them RING finger protein — MURF2, forming a signalosome. In the relaxed myocyte, the signalosome dissociates, and MURF2 translocates to the nucleus where it suppresses the SRF-dependent expression of genes encoding the contractile proteins [50, 52]. So TK activated by the strain developed by activated sarcomere enhances expression of the myocyte proteins by keeping MURF2 out of the nucleus. It is very likely that this is one mechanism of myocardial hypertrophy in response to the myocardial overload. The signalosome has also links to other pathways regulating protein turnover, reviewed in detail in [50].

## **TITIN RELATED INHERITED CARDIOMYOPATHIES**

The mutations located throughout the length of the titin molecule have been identified by many authors and linked to various heart pathologies. Here we review more recent results enabling physicians to relate the mutations to the DCM and arrhythmogenic RV cardiomyopathy (ARVC). DCM is of familial aetiology in approximately 30–50% of cases, the inheritance being generally autosomal dominant with age dependent penetrance. ARVC is an inherited disease predisposing to cardiac arrhythmias and sudden death.

### ***Titin truncated at the A-band***

Gerull et al. [53] reported heterozygous mutation in TTN with a guanine deletion in position 62890 (c.62890delG)

that cosegregated with DCM in a large Australian family. The mutation causes a frameshift generating a truncated A-band titin due to premature stop codon and the addition of ten novel amino acid residues.

Yoskovitz et al. [54] analysed TTN mutations in 43 members of an Arab family. Of these, 13 were clinically affected with DCM. They identified a mutation in exon 326 which with an adenine insertion in position 58,880 (c.58880insA) causes a frameshift creating a stop codon and titin truncation after 19,628 amino acids. The mutation was identified in all 13 clinically affected patients and in six out of 30 healthy members of the family. The mutation was not found in other Arab families.

Herman et al. [55] analysed TTN in 312 subjects with DCM, 231 subjects with HCM, and 249 controls. They identified 72 unique mutations (25 nonsense, 23 frameshift, 23 splicing and one tandem insertion). The mutations were found in approximately 25% of familial cases and in 18% of sporadic cases of DCM, but only in 1% of cases with HCM and in 3% of healthy controls. Mutations were located in the A-band titin, but not in the I- or M-band or in the Z discs, resulting in truncation of the titin molecule. The results of Hermann et al. [55] support the hypothesis that mutations in the TTN gene have an important role in DCM.

The mutations identified by Gerull et al. [53], Yoskovitz et al. [54] and Hermann et al. [55] resulted in expression of titin truncated at the A-band part of its molecule which has a number of important functions. It anchors in the M-band providing mechanical support for the resting tension generation. It binds numerous proteins (as described above), forming a complex securing the stability of the M-band and proper alignment of myosin filaments, and it contains the TK. The truncated titin molecules are incorporated into the sarcomere and anchor in the Z disc. However, deprived of the anchorage of their C-terminal in the M-band, they cannot contribute properly to the myocytes resting tension nor control alignment of sarcomere filaments and function. Moreover, mechanosensing linked to TK is lost and mechanosensing linked to N2B unit is deficient because of deficient mechanical tension.

Thus incorporation of truncated titin molecules into the sarcomeres is very probably importantly contributing to the changes in diastolic mechanical properties, remodelling and decrease of contractility in the hearts of DCM patients.

### ***Titin mutations in the I-band***

The genetic variation located in the I-band titin has been identified in patients suffering from ARVC. Taylor et al. [56] sequenced 312 titin exons and the complete 3' untranslated region in 38 ARVC families. In seven families, the authors identified eight unique TTN variants, the missense mutation causing substitution of the threonine in position 2896 by isoleucine (THR2896Ile), showing complete segregation with the ARVC phenotype in one large family. This mutation maps

within the Ig10 domain of the N2B (spring) region of I-band titin. The authors proved that the mutant domain is more vulnerable to proteolysis and degradation. They proposed that the mutation lowers domain stability, leading to titin degradation which initiates the pathological process eventually leading to ARVC.

Roncardi et al. [57] used whole-exome sequencing to investigate the causes of clinical variability in an extended DCM family of 41 persons, 14 of whom were affected by a range of cardiological syndromes. Four of the affected members of the family showed particularly severe manifestations of cardiomyopathy requiring heart transplantation in early adulthood. A variant of the substitution of the adenine in position 656 by the cytosine (c.656A>C) in the gene for lamin was identified in all affected subjects. In the four most severely affected patients, an additional variant of the substitution of the cytosine in position 14563 by the thymine in TTN (TTN:c.14563C>T) was identified. The variant TTN:c.14563C>T affects N2BA unit of the I-band titin.

Since two family members from whom the TTN:c.14563C>T variant could have come lived beyond the age of 70, the authors hypothesise that it does not in itself cause DCM, but in combination with lamin mutation might have a synergistically deleterious effect.

### SUMMARY AND PERSPECTIVES

Titin has a surprisingly large number of functions in the heart: (1) It is important for the assembling of the myosin filaments and it is a ruler securing the proper alignment of the contractile filaments during the contraction-relaxation cycle; (2) Due to its elastic properties, it generates the recoil force at contraction restoring the resting length of myocyte at its relaxation. When the myocyte is stretched beyond the slack length, titin develops tension setting the passive tension of the cell. These properties of titin are of great importance for the mechanics of LV diastole and its diastolic filling. Their disturbances play an important role in HF; (3) Sensing of the forces developed by sarcomere, titin controls expression of the contractile proteins of myocytes in response to the changing afterload.

So it is titin which is responsible for initiating myocytes hypertrophy in response to the myocardial overload. Mutations affecting mechanical properties of titin and its mechanosensing play a considerable role in cardiac diseases such as DCM or ARVC.

So titin is emerging as a promising target of therapeutic measures. Presently, these are limited to attempts to improve therapy of patients suffering HF by means of sildenafil, and the results warrant further extensive investigation. Considering the extensive investigations into the basic properties of titin and its role in cardiac diseases, it is very likely that titin-related new therapeutic approaches will emerge in the not too distant future.

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**Note added in the proof:** *The Reader is advised to consult also the recent review by M. LeWinter and H. Granzier "Cardiac titin and heart disease", Journal of Cardiovascular Pharmacology, 2013 (published ahead of print), available in PUBMED.*

**Conflict of interest:** none declared

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