HHS Public Access

Author manuscript

Gene. Author manuscript; available in PMC 2017 May 10.

Published in final edited form as:

Gene. 2016 May 10; 582(1): 1–13. doi:10.1016/j.gene.2016.01.006.

TNNT1, TNNT2, and TNNT3: Isoform Genes, Regulation, and Structure-Function Relationships

Bin Wei and **J.-P. Jin**

Department of Physiology, Wayne State University School of Medicine, 540 E. Canfield Street, Detroit, MI, 48201, USA

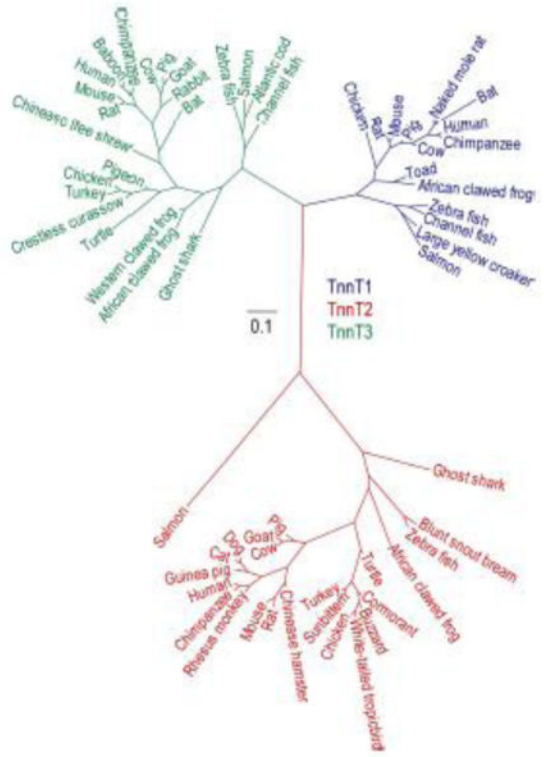
Abstract

Troponin T (TnT) is a central player in the calcium regulation of actin thin filament function and is essential for the contraction of striated muscles. Three homologous genes have evolved in vertebrates to encode three muscle type-specific TnT isoforms: *TNNT1* for slow skeletal muscle TnT, *TNNT2* for cardiac muscle TnT, and *TNNT3* for fast skeletal muscle TnT. Alternative splicing and posttranslational modifications confer additional structural and functional variations of TnT during development and muscle adaptation to various physiological and pathological conditions. This review focuses on the TnT isoform genes and their molecular evolution, alternative splicing, developmental regulation, structure-function relationships of TnT proteins, posttranslational modifications, and myopathic mutations and abnormal splicing. The goal is to provide a concise summary of the current knowledge and some perspectives for future research and translational applications.

Graphical Abstract

Correspondence to: J.-P. Jin, jjin@med.wayne.edu.

The corresponding Gene Wiki entry for this review can be found here:
https://en.wikipedia.org/wiki/TNNT1
https://en.wikipedia.org/wiki/TNNT2
https://en.wikipedia.org/wiki/TNNT3



Keywords

Troponin; striated muscle; isoform; evolution; alternative splicing; restrictive proteolysis; cardiac function; myopathy

1. Introduction

The basic contractile unit of vertebrate striated muscle, *i.e.*, skeletal and cardiac muscles, is the sarcomeres in myofibrils, which consist of overlapping myosin thick filaments and actin thin myofilaments (Tobacman 1996; Gordon, Homsher et al. 2000). Muscle contraction is powered by actin-activated myosin II motor (Berchtold, Brinkmeier et al. 2000), which converts the energy from ATP hydrolysis to a power stroke of myosin head during each cross-bridge cycle. This process is regulated by calcium-mediated protein conformational changes in both thick and thin myofilament of the sarcomere (Tobacman 1996).

The troponin complex associated with the actin thin filament plays a central role in the regulation of muscle contraction and relaxation. The troponin complex has three protein subunits: The calcium binding subunit troponin C (TnC) (Chaudhuri, Mukherjea et al. 2005), the actomyosin ATPase inhibitory subunit troponin I (TnI), and the tropomyosin binding and thin filament anchoring subunit troponin T (TnT). Troponin T interacts with TnI, TnC, tropomyosin and actin to play an organizer role in the calcium regulation of muscle contraction and relaxation (Jin, Zhang et al. 2008).

Discovered in the 1960s (Ebashi 1963; Kato, Negishi et al. 1969), vertebrate TnT is a 30~35kDa myofilament protein. Visualized by electron microscopy, TnT anchors troponin complex on the actin-tropomyosin thin filament at ~37 nm intervals (Otsuki, Masaki et al. 1967). The stoichiometry of TnT binding to F-actin is in a ratio of 1 (TnT) to 7 (actin monomers) (al-Hillawi, Bhandari et al. 1995). During the last two decades, extensive studies revealed that vertebrate TnT is encoded by three homologous genes that specifically expressed in slow skeletal muscle (*TNNT1*), cardiac muscle (*TNNT2*), and fast skeletal muscle (*TNNT3*) (Barton, Townsend et al. 1997) (Table 1).

Early protein sequencing work and mainly later cDNA cloning studies have determined the primary structure of TnT isoforms in multiple species (Jin, Zhang et al. 2008). Due to its extended molecular conformational and allosteric structure (Cabral-Lilly, Tobacman et al. 1997; Wendt, Guenebaut et al. 1997), crystallographic data for the troponin complex only determined the high resolution structure of a C-terminal portion of TnT (Takeda, Yamashita et al. 2003; Vinogradova, Stone et al. 2005), whereas the N-terminal and middle regions and the last 14 amino acids at the C-terminal end of TnT were missing. Therefore, most of the current understanding of TnT structure and its interaction with other thin filament proteins are based on protein binding studies and conformational changes detected using monoclonal antibody (mAb) epitope analysis and fluorescence spectral study.

In addition to anchoring the troponin complex on the thin filament, TnT dynamically interacts with other thin filament regulatory proteins to conduct the calcium signaled excitation-contraction-relaxation cycles of striated muscle (Jin, Zhang et al. 2008). The present review focuses on the molecular evolution of TnT isoform genes, alternative splicing, developmental regulation, posttranslational modifications, structure-function relationship of TnT protein, myopathic mutations, and splicing abnormalities. More detailed information can be found in several previous review articles (Tobacman 1996; Perry 1998; Gordon, Homsher et al. 2000; Jin, Zhang et al. 2008; Sheng and Jin 2014).

2. Molecular evolution of *TNNT* genes encoding muscle type-specific TnT isoforms

Three homologous genes have evolved in vertebrates, encoding the cardiac (*TNNT2*), slow skeletal muscle (*TNNT1*) and fast skeletal muscle (*TNNT3*) isoforms of TnT. The three TnT isoforms are significantly diverged in the N-terminal region but highly conserved in the middle and C-terminal regions that contain binding sites for TnC, TnI and tropomyosin (Figure 1) (Jin, Zhang et al. 2008). While *TNNT1* and *TNNT3* are specifically expressed in slow and fast twitch skeletal muscle fibers, respectively, *TNNT2* is expressed in cardiac muscle and transiently expressed in embryonic and neonatal skeletal muscles, including both slow and fast fiber dominant muscles, in avian and mammalian species (Toyota and Shimada 1983; Cooper and Ordahl 1985; Jin 1996).

Knockout of mouse cardiac TnT gene caused embryonic lethality (Nishii, Morimoto et al. 2008), indicating the lack of compensatory expression of slow or fast skeletal muscle TnT gene in cardiac muscle. Similarly, mutations in *TNNT1* gene resulting a complete loss of slow TnT in slow skeletal muscle also causes severe nemaline myopathy with childhood

lethality (Johnston, Kelley et al. 2000; Jin, Brotto et al. 2003). Therefore, the muscle type-specific TnT isoform genes are non-redundant and differentially expressed in specific type of muscle cells to function in differentiated cellular environments.

While the expression of *TNNT1* is slow skeletal muscle specific in almost all vertebrate species studied, the heart muscle of dry land toad (*Bufo*) utilizes exclusively slow TnT other than cardiac TnT (Feng, Chen et al. 2012). Multiple representative vertebrate species studied from fish to human, including the closely related frog, all express only cardiac TnT in the cardiac muscle. Therefore, the unique utilization of slow TnT in toad heart reflects a unique evolutionary selection, potentially with a value in the adaptation of cardiac function to the dry land habitats of toad, which causes drastic change in blood volume and stress on cardiac function. Supporting this observation, studies of cardiac function have showed that toad heart is significantly more tolerant to increases in afterload than that of frog heart (Feng, Chen et al. 2012).

Troponin T and TnI genes are located in vertebrate genomes in three closely linked pairs: fast TnI-fast TnT, cardiac TnI-slow TnT and slow TnI-cardiac TnT (Chong and Jin 2009; Wei and Jin 2011). Although TnT and TnI are significantly diverged proteins, their close linkage in the vertebrate genomes and protein epitope analysis suggested that TnT and TnI genes have originated from a TnI-like ancestor gene via gene duplication (Figure 2) (Chong and Jin 2009). Structural integration may have preserved the close chromosomal linkage between the paired TnI and TnT genes. For example, deletion of the genomic DNA segment containing cardiac TnI gene also deleted a part of the slow TnT gene promoter and caused decreased transcriptional activity (Huang, Chen et al. 1999) and expression of slow TnT in slow muscle fibers (Feng, Wei et al. 2009).

The cardiac TnI-slow TnT and slow TnI-cardiac TnT gene pairs are apparently not linked in a fiber type-specific manner. However, slow TnI is expressed as the sole TnI together with cardiac TnT in embryonic heart (Saggin, Ausoni et al. 1988; Saggin, Gorza et al. 1989). Therefore, the slow TnI-cardiac TnT gene pair is actually a functionally linked pair. It remains to be investigated whether the cardiac TnI-slow TnT gene pair also has a functional significance.

Sequence analysis and protein epitope studies further revealed the evolutionary linage of the three TnT isoform genes (Figure 2) (Chong and Jin 2009). A novel approach was used to detect ancestor conformations that have been repressed allosterically in the three dimensional structures in present-day TnT proteins by the addition of later modulator structures during evolutionary diversification. These modulator structures served as evolutionary determinants for their structural effect on repressing the ancestral conformation (Chong and Jin 2009). For example, the N-terminal variable region of TnT has been identified as a repressive modulator. Removing this conformational modulator peptide fragment of TnT restored epitopic structures of the origin of an ancestral TnT isoform, which are detectable using mAb probes. The epitope analysis revealed the evolutionary lineage relationships between the muscle fiber type-specific TnT isoform genes as well as between TnI and TnT genes.

Experimental data demonstrated that a fast TnI-like-fast TnT-like gene pair was first emerged from duplication of a TnI-like ancestor gene (Figure 2). The present-day fast TnI-fast TnT gene pair is derived directly from this ancestor gene pair. A slow TnI-like-cardiac TnT-like gene pair emerged by duplication of the original fast TnI-like-fast TnT-like gene pair. The emergence of cardiac TnI-slow TnT gene pair occurred latest from duplication of the slow TnI-like-cardiac TnT-like gene pair (Chong and Jin 2009). The molecular evolution lineage of TnT-TnI gene pairs provides valuable information for understanding the structure-function relationships of TnT.

Studies of primary structures of numerous TnT protein isoforms revealed that each of the muscle type specific TnT isoforms in different vertebrate species is more conserved than the three muscle type specific TnT isoforms in a given species (Figure 3) (Jin, Zhang et al. 2008). This observation suggests that the demands for muscle type specific functional adaptations in cardiac, slow and fast muscles provided the primary selection pressure and have driven the diversification and conservation of TnT isoform genes during evolution (Chong and Jin 2009).

3. Structure-function relationships of TnT

Troponin T is a protein of 30~35kDa in size containing ~220 to 300 amino acids. Amino acid sequence comparison demonstrated that the diversity of TnT isoforms is largely in the N-terminal variable region, whereas the middle and C-terminal regions are highly conserved (Jin, Zhang et al. 2008). The structure-function relationship of TnT has been extensively investigated in protein binding studies using TnT fragments (Figure 1). The chymotryptic fragments T1 and T2 of rabbit fast skeletal TnT (Tanokura, Tawada et al. 1981) were studied for their interactions with other regulatory proteins in the sarcomeric thin filament. The T1 fragment binds to the head-to-tail junction of tropomyosin and the T2 fragment binds to the middle region of tropomyosin and also binds to TnI in the troponin complex (Pato, Mak et al. 1981; Tanokura, Tawada et al. 1983; Tanokura and Ohtsuki 1984; White, Cohen et al. 1987).

High resolution X-ray crystallographic structures have been obtained from human cardiac troponin complex and chicken skeletal muscle troponin complex. The high resolution data revealed only a small portion of the TnT structure but included the regions interacting with TnI and TnC (Takeda, Yamashita et al. 2003; Vinogradova, Stone et al. 2005). Troponin T binds TnI through a helical coiled-coil interface corresponding to Leu_{224}-Val_{274} of human cardiac TnT and Glu_{199}-Gln_{245} of chicken fast skeletal TnT. This interface structure is referred to as the I-T arm region. Amino acid sequences within the I-T arm region are highly conserved in the three muscle type specific TnT isoforms (Jin, Zhang et al. 2008). The 12 amino acids at the very C-terminal end of TnT were missing from the crystal structures (Figure 1). This segment is conserved in vertebrate TnT and its function remains to be investigated.

Not resolved in the high resolution crystal structures, two tropomyosin binding sites of TnT have been identified by protein binding studies. A T1 region tropomyosin binding site has been mapped to a 39-amino acid segment at the N-terminal portion of the middle conserved

region of TnT. A tropomyosin binding site in the T2 fragment was mapped to a 25-amino acid segment at the beginning of the T2 fragment (Jin and Chong 2010). Amino acid sequences of the segments containing these tropomyosin binding sites are also highly conserved in the three muscle type specific TnT isoforms and across vertebrate species (Jin, Zhang et al. 2008).

The structure of the N-terminal region of TnT is hypervariable in different muscle types and regulated during development. The CNBr fragment CB3 of rabbit fast skeletal muscle TnT (amino acids 2–50) representing the N-terminal variable region does not bind any known myofilament proteins (Pearlstone and Smillie 1982; Heeley, Golosinska et al. 1987). More recent studies have demonstrated a role of the N-terminal variable region in the regulation of TnT molecular conformation and interaction with other myofilament regulatory proteins.

Monoclonal antibody epitope analysis demonstrated that the binding of metal ions or structural variations in the N-terminal region alters epitope conformation in the middle and C-terminal regions (Wang and Jin 1998; Jin, Chen et al. 2000). This mechanism was confirmed by spectral analysis of fluorescence of residues in the C-terminal region (Jin and Root 2000). Such modulatory effects reflect that the N-terminal region regulates the molecular conformation of TnT.

The N-terminal variable region based regulation results in functional alterations. Microplate protein binding assays showed that Zn^{2+} binding to the N-terminal variable region of chicken fast skeletal TnT decreased the binding affinity of TnT for tropomyosin (Ogut and Jin 1996; Wang and Jin 1998). Protein binding studies further demonstrated that the N-terminal variable region of TnT modulates the binding affinity of TnT for tropomyosin primarily by reducing the affinity of site 1 in the middle region (Amarasinghe and Jin 2015). Physiological significance of the regulatory effects of the N-terminal variable region of TnT have also been demonstrated *in vivo* in studies using transgenic mice expressing N-terminal modified cardiac TnT in the heart (Biesiadecki, Elder et al. 2002; Feng, Biesiadecki et al. 2008; Wei, Gao et al. 2010).

In vitro studies using reconstituted troponin complex showed that the presence of the N-terminal fragment in TnT potentiated actomyosin ATPase activity (Malnic, Farah et al. 1998). Physiological studies using transgenic mice expressing cardiac TnT with a deletion of the N-terminal 71 amino acids showed moderately decreased contractile velocity but preserved physiological cardiac function and sustained cardiac output against increases of afterload (Feng, Biesiadecki et al. 2008).

These data demonstrate that the diverged N-terminal structure of the muscle type specific TnT isoforms provides a regulatory mechanism to fine tune the function of troponin and muscle contractility, and reflects the differentiated physiological features of the differentiated muscle fiber types. On the other hand, the conserved structures in the middle and C-terminal regions of TnT reflect the similar baseline function of troponin in the calcium regulation of cardiac, slow and fast skeletal muscle contractions.

4. Developmental regulation of TnT isoform genes and alternative pre-mRNA spicing

Expression of the three TnT isoform genes is regulated during development at the transcriptional level as well as via alternative splicing. Slow and fast skeletal muscle TnT genes have their specific expression primarily in slow and fast twitch skeletal muscle fibers, respectively, throughout development. Cardiac TnT gene is the sole TnT isoform expressed in cardiac muscle, and is expressed at significant levels in embryonic skeletal muscle (Jin 1996). Cardiac TnT is also found in extraocular muscles of adult dog and rat (Bicer and Reiser 2013), further indicating its expression in adult animals is not restricted to the heart.

Alternative splicing plays a major role in generating further diversity in TnT structures for fine tuning cardiac and skeletal muscle contractility during development with changes in functional demands and in adaption to pathological conditions. Alternative splicing of TnT pre-mRNA increases the N-terminal variation in all three TnT isoforms, and generates a C-terminal variable region in fast skeletal muscle TnT and avian cardiac TnT (Jin, Zhang et al. 2008; Wei and Jin 2011). With one exception in avian fast skeletal muscle TnT (to be discussed in the following section), a common feature of TnT N-terminal alternative splicing during embryonic and postnatal development is the switch from high molecular weight to low molecular weight and acidic to basic splice forms (Wei and Jin 2011).

4.1. *TNNT3*

Mammalian *TNNT3* gene encoding the fast skeletal muscle isoform of TnT (fsTnT) contains 19 exons. Alternative *TNNT3* pre-mRNA splicing has been observed very early on from the diversity of fsTnT proteins (Wilkinson, Moir et al. 1984). N-terminal coding exons 4, 5, 6, 7 and 8 are alternatively spliced (Wilkinson, Moir et al. 1984; Breitbart and Nadal-Ginard 1986; Wang and Jin 1997). A fetal exon between exon 8 and 9 is also alternatively spliced and exclusively expressed in embryonic fast muscles (Briggs and Schachat 1993). Exons 16 and 17 in the C-terminal region of fsTnT, previously designated as α and β exons respectively, are alternatively spliced in a mutually exclusive manner (Medford, Nguyen et al. 1984).

Avian fsTnT gene has more complex alternative splicing patterns due to the additional alternative exons w, P1-7(x) and y in the N-terminal region (Smillie, Golosinska et al. 1988; Miyazaki, Jozaki et al. 1999; Jin and Samanez 2001). Correspondingly, two-dimensional electrophoresis study of chicken leg muscle protein detected more than 40 different fsTnT protein splice forms (Imai, Hirai et al. 1986).

Expression of *TNNT3* during embryonic and postnatal development undergoes a high molecular weight to low molecular weight splice form switch in both fast and slow fiber-dominant skeletal muscles (Wei, Lu et al. 2014). This switch also represents a transition of low isoelectric point splice forms to high isoelectric point forms (Jin, Zhang et al. 2008). The size and charge changes are produced by alternative inclusions of N-terminal exons that encode mainly acidic amino acids (Wang and Jin 1998).

Alternative splicing of the two mutually exclusive C-terminal exons 16 and 17 is also regulated during development (Wang and Jin 1997). Analysis of cDNA sequences suggested that Exon 17 that has a higher similarity in amino acid sequence to its counterparts in slow skeletal muscle TnT and cardiac TnT is predominantly expressed in embryonic and neonatal fsTnT isoforms and exon 16 was mainly found in adult fsTnT (Wang and Jin 1997; Jin, Chen et al. 1998). Exons 16 and 17 both encode a 14 amino acids peptide fragment, which resides in the interface with TnI and TnC (Wang and Jin 1997). Protein interaction studies revealed that incorporation of exon 17 weakened binding of fsTnT to TnC and tropomyosin (Wu, Jha et al. 1995).

Additional alternative N-terminal coding exons have been found in avian *Tnnt3* gene with unique splicing patterns (Ogut and Jin 1998). In addition to the high molecular weight to low molecular weight switch via combinations of alternative splicing of exons 4–8 as that in the expression of mammalian fsTnT during development, the expression of a Tx segment encoded by the seven P exons located between exon 5 and 6 in the N-terminal variable region of avian fsTnT (Jin and Smillie 1994) is up-regulated in pectoral but not leg muscles during post-hatch development (Ogut and Jin 1998). Around 28 days after hatch, splice-in of the Tx segment is rapidly up-regulated to become predominant in fsTnT expressed in pectoral muscles of adult birds (Jin and Samanez 2001). The P exons in *Tnnt3* gene of avian species in the orders of *Galliformes* and *Craciformes* have evolved to encode repeating pentapeptide motifs of AHH(A/E)A. An intriguing finding is that the inclusion of 7 P exons in chicken *Tnnt3* mRNA encodes a Tx segment containing seven repeating H(A/E)AAH motifs in the N-terminal variable region of fsTnT, which bind transition metal ions Cu(II), Ni(II), Zn(II) and Co(II) (Jin and Smillie 1994).

The Tx segment is negatively charged and its inclusion cancels the developmental size and charge switch produced by various exclusions of exon 4–8. This feature corresponds to a higher tolerance of adult pectoral muscle fsTnT to acidosis (Ogut and Jin 1998; Jin, Zhang et al. 2008). While the physiological function of the high affinity binding to transition metal ions remains to be investigated, the Tx segment of avian fsTnT shows binding to calcium, potentially serves as a calcium reservoir in avian pectoral muscle myofilaments (Zhang, Jin et al. 2004). Supporting this hypothesis, adult chicken breast muscle exhibits higher calcium sensitivity in myofilament force production than that of Tx-negative muscle (Ogut, Granzier et al. 1999).

Deficiency of slow skeletal TnT did not affect the developmental switch of fsTnT splice forms, indicating that the alternative splicing of *TNNT3* pre-mRNA is regulated independent of skeletal muscle fiber types (Wei, Lu et al. 2014). Although the N-terminal variable region of TnT does not bind to any know proteins in the thin filament regulatory system, alternative splicing in the N-terminal segment of fsTnT has shown effects on the overall conformation of TnT and the binding affinities for TnI and tropomyosin (Biesiadecki, Chong et al. 2007).

4.2. *TNNT2*

Mammalian cardiac TnT gene contains 17 exons, of which 3 are alternatively spliced (Jin, Huang et al. 1992). Exon 13 between the conserved middle and C-terminal regions encodes 2–3 amino acids and is alternatively spliced with unknown function and regulation. Exon 4

in the N-terminal region is alternatively spliced in both adult and embryonic hearts (Jin, Wang et al. 1996; Farza, Townsend et al. 1998), of which the functional significance also remains to be investigated.

Exon 5 in the N-terminal region of cardiac TnT is expressed in embryonic heart and excluded from adult cardiac TnT (Jin and Lin 1989). Exon 5 of *TNNT2* gene encodes a 9 or 10 amino acids segment that is highly acidic and negatively charged at physiological pH (Jin, Zhang et al. 2008). Embryonic cardiac TnT with more negative charges in the N-terminal region exerts higher calcium sensitivity of actomyosin ATPase activity and myofilament force production (Gomes and Potter 2004) as compared with that of adult cardiac TnT. This function may also correspond to the effect of TnT isoforms on the tolerance of cardiac muscle to acidosis (Nosek et al., 2004).

When *TNNT2* is transiently expressed in embryonic and neonatal skeletal muscles, its alternative splicing pattern is synchronized to that in the heart (Jin 1996). While the isoform switch from slow to cardiac TnI regulated at the transcriptional level corresponds to functional demands during postnatal heart development, timing of the switching of *TNNT2* alternative splicing may vary in different species (Jin 1996), indicating the regulation by a genetically programmed biological clock, rather than adaptation to changes in contractile function.

4.3. *TNNT1*

The expression of slow skeletal muscle TnT gene involves relatively less complex alternative splicing in comparison to that of cardiac and fast skeletal muscle TnT genes. Among the 14 exons of *TNNT1*, exon 5 encoding an 11-amino acid segment in the N-terminal region is alternatively spliced, generating a high molecular weight and a low molecular weight slow TnT variants (Gahlmann, Troutt et al. 1987; Jin, Chen et al. 1998; Huang, Chen et al. 1999). In addition, splicing at alternative acceptor sites of *TNNT1* pre-mRNA produces a single amino acid difference in the peptide segment encoded by exon 6 (Huang, Chen et al. 1999). The alternative splicing of slow skeletal muscle TnT pre-mRNA has not been found with any correlation to muscle development.

Discussed above, the heart muscle of dry land toad utilizes exclusively slow TnT other than cardiac TnT reflecting a unique evolutionary selection with a value in the adaptation of cardiac function to the drastic changes in blood volume (Feng, Chen et al. 2012). Interestingly, the slow TnT expressed in toad heart is the low molecular weight splice form excluding the exon 5-encoded segment (Feng, Chen et al. 2012). The low molecular weight slow skeletal muscle TnT is also predominantly expressed in overused prior polio muscle and up-regulated in type 1 demyelination, but not type 2, Charcot-Marie-Tooth disease (CMT) (Larsson, Wang et al. 2008). Based on the observation that the N-terminal structural variations alter the molecular conformation and function of the middle and C-terminal regions with functional effects on the interactions with TnI, TnC and tropomyosin, the alternative splicing regulation of *TNNT1* expression may play an important role in modulating muscle contractility in physiological and pathophysiological adaptations.

5. Posttranslational modifications

Posttranslational modifications can rapidly regulate the function of proteins in physiological and pathophysiological conditions. Known posttranslational modifications of TnT include phosphorylation, O-linked GlcNAcylation and proteolytic modifications. Among the three TnT isoforms, posttranslational modifications are most intensely studied in cardiac TnT with an emphasis on the significance in heart diseases.

5.1. Phosphorylation

Phosphorylation is a fundamental mechanism in the regulation of the structure and function of myofilament proteins, including TnT. In both cardiac and skeletal muscle cells, TnT is phosphorylated at multiple Ser and Thr residues. Table 2 summarizes major phosphorylation sites of cardiac TnT and their functional effects on the contractility and pathophysiological adaptation of cardiac muscle.

Ser_2, a residue conserved at the N-terminus of all three TnT isoforms, is constitutively phosphorylated (Perry 1998). Little is known about the kinase and regulatory mechanisms for TnT phosphorylation at Ser_2 or its functional significance. When embryonic cardiac TnT is over-expressed in the adult heart of transgenic mice, Ser_{25} encoded by the embryonic exon 5 is also constitutively phosphorylated (Zhang, Feng et al. 2011). Therefore, the N-terminal structure of TnT appears highly accessible by a highly active kinase at physiological conditions, and potentially resistant to phosphatases. The functional significance of N-terminal phosphorylation of TnT remains to be investigated.

Although protein kinase A (PKA) plays an important role in the phosphorylation regulation of multiple myofilament proteins, such as TnI, myosin binding protein C and titin, neither cardiac nor skeletal muscle isoforms of TnT is a physiological substrate of PKA. Nonetheless, in an *in vitro* study, cardiac TnT was demonstrated as a PKA anchoring protein, tethering PKA to the myofilament to facilitate phosphorylation of other myofilament components (Sumandea, Garcia-Cazarin et al. 2011).

Multiple Ser and Thr residues in the middle and C-terminal regions of cardiac TnT have been reported to be phosphorylated by protein kinase C (PKC), especially PKCα, PKCε and PKCξ (Katoh, Wise et al. 1983; Perry 1998; Dubois-Deruy, Belliard et al. 2015). PKC phosphorylation of cardiac TnT exerted a negative inotropic effect on decreasing the calcium-dependent actomyosin ATPase activity and myofilament force production, which was secondary to decreases in the binding affinity of TnT for tropomyosin-F-actin filament (Noland and Kuo 1992).

Mouse cardiac TnT was phosphorylated by PKC at Thr_{197}, Ser_{201}, Thr_{206}, Ser_{208} and Thr_{287}, and phosphorylation of Thr_{206} alone was sufficient to reduce myofilament calcium sensitivity and force production. In a study using reconstituted myofilaments, substitution of Thr_{206} in cardiac TnT with Glu to mimic the negative charge of phosphorylation resulted in significant decreases of actomyosin Mg-ATPase activity, calcium sensitivity and myofilament cooperativity (Sumandea, Pyle et al. 2003). Phosphorylation of TnT at Ser_{208}

by PKCε enhanced cardiac contractility, which was reduced in heart failure due to decreased level of PKCε (Dubois-Deruy, Belliard et al. 2015).

In the conserved C-terminal region of cardiac TnT, phosphorylation sites were also identified for Apoptosis Signal-Regulating Kinase (ASK1) and Rho-Dependent Kinase 2 (ROCK2). Yeast-two-hybrid screen detected association of ASK1 and cardiac TnT (He, Liu et al. 2003). ASK1 was activated in cardiac muscle under stress conditions by proinflammatory mediators such as TNFα and ROS. Phosphorylation of cardiac TnT at Thr_{194} and Ser_{198}, lead to an inhibition of the shortening and calcium transient in cardiomyocytes (He, Liu et al. 2003).

ROCK2 is a protein kinase associated with cardiac myofilaments. Mass spectrometry determined that ROCK2 phosphorylation of cardiac TnT at Ser_{278} and Thr_{287} (Vahebi, Kobayashi et al. 2005). Using skinned mouse ventricular muscle bundles, ROCK2 phosphorylation of myofilament proteins, especially cardiac TnT and cardiac TnI, reduced myosin ATPase activity and tension development (Vahebi, Kobayashi et al. 2005).

Together with the regulation of kinase activities, protein phosphatase 1 (PP1) (Jideama, Crawford et al. 2006) and protein phosphatase 2A (PP2A) (Dubois-Deruy, Belliard et al. 2015) dephosphorylate cardiac TnT at multiple sites and reverse the effects of phosphorylation on calcium sensitivity and myofilament force production.

5.2. O-linked GlcNAcylation

A recent study identified that Ser_{190} of cardiac TnT is a substrate of O-N-acetylglucosamine modification (Dubois-Deruy, Belliard et al. 2015). O-GlcNAcylation of cardiac TnT was increased in a rat model of heart failure in correlation to a loss of cardiac muscle contractile function. Interestingly, O-GlcNAcylation of TnT at Ser_{190} inhibits phosphorylation of TnT at Ser_{208}, suggesting a crosstalk between the two posttranslational modifications of TnT, possibly through intramolecullar allosteric competition for occupancy in the two closely positioned sites (Dubois-Deruy, Belliard et al. 2015).

5.3. Proteolytic modifications

Proteolytic regulation of TnT plays important roles in striated muscle cells in physiological as well as pathological conditions.

Maintaining normal stoichiometry of myofilament proteins—Troponin T undergoes rapid turnover with a half life of ~3.5 days in cardiac muscle (Martin 1981). When not incorporated in the myofilaments, TnT was rapidly degraded to avoid toxic effects (Jeong, Wang et al. 2009). The fast turnover and removal of surplus TnT protein in muscle cells is critical in maintaining protein stoichiometry of the contractile machinery (Feng, Hossain et al. 2009).

Caspase destruction—In apoptotic rat cardiomyocytes, cardiac TnT was cleaved by caspase 3 to generate a 25-kDa N-terminal truncated fragment, losing the N-terminal hypervariable region and a portion of the middle conserved region (Communal, Sumandea et al. 2002). In canine diaphragm muscle under hypoxia, fast skeletal muscle TnT was also

cleaved by caspase 3, to generate a 28-kDa protein fragment (Simpson, van Eyk et al. 2000). The caspase 3-mediated cleavage of TnT was shown to attenuate myofilament force production by decreasing myosin ATPase activity.

Restrictive N-terminal truncation—The N-terminal variable segment of cardiac TnT can be cleaved by μ-calpain proteolysis during myocardial ischemia-reperfusion (Zhang, Biesiadecki et al. 2006). In contrast to the caspase 3 cleavage discussed above, this structure modification is restrictive and selectively deleting only the entire N-terminal variable region (amino acids1–71) and leave the conserved middle and C-terminal regions intact (Zhang, Biesiadecki et al. 2006). The N-terminal truncated cardiac TnT remains incorporated in the myofilaments and produces a functional impact (Zhang, Biesiadecki et al. 2006). The restrictive N-terminal truncation of cardiac TnT has been found in cardiac muscle in multiple mammalian species including mouse, rat and pig treated with acute ischemia-reperfusion (Zhang, Biesiadecki et al. 2006).

In transgenic mouse hearts expressing both cardiac TnT and fast skeletal TnT, N-terminal truncation can be induced by stress treatment for both TnT isoforms despite their differences in the amino acid sequence at the cleavage site (Zhang, Biesiadecki et al. 2006). Stress of *ex vivo* fatigue contractions resulted in N-terminal truncation of fast skeletal TnT in mouse diaphragm muscle with a higher level under higher duty cycles and in overloaded contractions (Feng et al. 2013). Therefore, N-terminal truncation of TnT may be an early response to myocyte injury when myofilament-associated calpain is activated by intracellular calcium overload.

Ex vivo working hearts of transgenic mice over-expressing N-terminal truncated cardiac TnT in the cardiac muscle exhibited moderately reduced left ventricular systolic velocity, which prolonged the time of left ventricular rapid ejection and increased stroke volume, especially at high afterload (Feng, Biesiadecki et al. 2008). Consistent with the modulatory effect on decreasing contractile velocity, biochemical studies demonstrated that similarly N-terminal truncated cardiac TnT altered TnT's binding affinities for tropomyosin, TnI and TnC but preserved the overall calcium sensitivity and cooperativity of cardiac myofilament with moderately decreased maximum myosin ATPase activity (Pan, Gordon et al. 1991; Biesiadecki, Chong et al. 2007). Therefore, the N-terminal truncation of cardiac TnT by myofilament-associated μ-calpain is a novel regulatory mechanism for fine tuning troponin function in response to stress conditions. It provides an acute adaptation mechanism for the heart to compensate for the loss of systolic function against increased workload and/or in energetic crisis such as myocardial ischemia (Feng, Biesiadecki et al. 2008). Considering TnT's half life of ~3.5 days in cardiomyocytes (Martin and Orlowski 1981), the N-terminal truncated cardiac TnT will be replaced by newly synthesized intact protein in a few days, allowing the regulatory effect to be reversible.

6. Mutations and abnormal expressions of TnT genes in myopathies

Cardiac and skeletal muscle myopathies have been linked to abnormalities in the TnT isoform genes. *TNNT1* mutations result in nemaline myopathy (Jin et al., 2003), *TNNT2* mutations are linked to hypertrophic (HCM) and dilated (DCM) cardiomyopathies

(Thierfelder, Watkins et al. 1994), and *TNNT3* mutations have been found in distal arthrogryposis (DA) (Zhao et al. 2011). Most of these myopathic mutations of TnT are in the conserved middle and C-terminal regions that contain binding sites for tropomyosin, TnI and TnC and presumably do not tolerate structural variations. Table 3 summarizes representative pathogenic mutations and aberrant splicing found in cardiac, slow and fast skeletal TnT.

6.1. *TNNT1*

Mutations in *TNNT1* gene encoding slow skeletal muscle TnT have been identified in recently years with increasing clinical significance.

Point mutations—A nonsense mutation in the exon 11 of *TNNT1* gene at codon E180 was found to cause a recessive form of nemaline myopathy with infantile lethality in the Old Order Amish in Lancaster, Pennsylvania, thus named the Amish Nemaline Myopathy (ANM) (Johnston, Kelley et al. 2000). Deletion of the C-terminal segment of slow skeletal muscle TnT by the E180X mutation causes a loss of the T2 region tropomyosin-binding site 2 (Figure 1). The truncated ssTnT is unable to form troponin complex and incapable of incorporating into the myofilaments (Jin, Brotto et al. 2003). The non-incorporated ssTnT fragment was effectively degraded in muscle cells (Wang, Huang et al. 2005) to avoid cytotoxic effect (Jeong and Jin 2009), which is consistent with the recessive phenotype of ANM patients.

Transgenic mouse models reproduced partial myopathic phenotypes of ANM (Feng, Wei et al. 2009; Wei, Lu et al. 2014). Animal model studies revealed that deficiency of ssTnT significantly decreased the contents of type I slow fibers in diaphragm and soleus muscles, accompanied by hypertrophic growth of type II fibers and increased muscle fatigability (Feng, Wei et al. 2009; Wei, Lu et al. 2014).

More recent case reports have identified another nonsense mutation in *TNNT1* in a non-Amish population, which also causes severe nemaline myopathies. This nonsense mutation of codon S108 in exon 9 of *TNNT1* gene reported in a Hispanic male patient in New York caused ANM-like recessive phenotypes, including severe respiration muscle weakness, type I fiber atrophy and compensatory hypertrophy of type II fibers (Marra, Engelstad et al. 2015). The similar phenotypes of the E180X and S108X mutations of *TNNT1* in causing recessive nemaline myopathies based on their loss of the T2 region tropomyosin-binding site 2 (Figure 1) (Jin and Chong 2010) demonstrate the critical role of the two-site anchoring of troponin on the thin filament in the assembly and function of the thin filament regulatory system.

Aberrant splicing—Mutation in intron sequences may cause error splicing of *TNNT1* pre-mRNA. A recent case report found in a Dutch nemaline myopathy patient that a combination of alleles with aberrant exclusion of exon 8- and exon 14-encoded segments in compound heterozygous mutations caused severe slow skeletal muscle atrophy and weakness (van der Pol, Leijenaar et al. 2014). The patient presented with phenotypes similar to that of Amish Nemaline Myopathy. The deletion of exon 8 segment partially destroys the T1 region tropomyosin-binding site 1 but preserved the high affinity binding site 2 (Jin and Chong 2010), and deletion of the exon 14-encoded C-terminal end segment would not

directly affect either of the tropomyosin-binding sites (Figure 1). Therefore, more investigation is required to understand the pathogenic mechanisms of these aberrant splicing mutations of *TNNT1*.

A rearrangement in *TNNT1* gene (c.574_577 delins TAGTGCTGT) was reported in 9 Palestinian patients from 7 unrelated families with recessively inherited nemaline myopathy (Abdulhaq, Daana et al. 2015). This mutation leads to aberrant splicing that causes a truncation of ssTnT polypeptide at L203. This mutation also presents as a recessive nemaline myopathy with phenotypes similar but not identical to that found in ANM. While ssTnT truncated at L203 would preserve both T1 and T2 region tropomyosin-binding sites (Figure 1), the truncated ssTnT will not be able to bind TnI and TnC (Figure 1). The recessive phenotype of L203 truncation demonstrates that the formation of troponin complex is essential for anchoring on the thin filament, a mechanism that preventing a potentially dominant toxicity of the truncated ssTnT to compete for tropomyosin-binding in the muscle cells of carriers. This hypothesis is worth investigating.

6.2. *TNNT2*

Numerous point mutations and aberrant N-terminal splicing of cardiac TnT have been identified as causes of cardiomyopathies. These abnormalities alter cardiac TnT's interactions within the thin filament regulatory system, myofilament calcium sensitivity, and/or the myosin ATPase activity (Watkins, McKenna et al. 1995; Knollmann and Potter 2001; Harada and Potter 2004; Garcia-Castro, Coto et al. 2009; Lu et al., 2013) (Table 3).

Point mutations—Single amino acid substitutions Ile79Asn, Arg92Gln (Morimoto, Yanaga et al. 1998; Schwartz and Mercadier 2003), Arg92Leu, Arg94Leu (Palm, Graboski et al. 2001) located in the middle region of cardiac TnT were found to increase calcium sensitivity of cardiac myofilament force production and cause dominantly inherited hypertrophic cardiomyopathy in humans. Transgenic mouse cardiac muscle over-expressing cardiac TnT-I79N showed increased myofilament calcium sensitivity, diastolic malfunction, and increased susceptibility to arrhythmia (Baudenbacher, Schober et al. 2008).

Mutations Glu244Asp (Nakaura, Yanaga et al. 1999; Harada and Potter 2004) Lys273Glu (Fujino, Shimizu et al. 2002) and Arg278Cys (Theopistou, Anastasakis et al. 2004) in the C-terminal region of cardiac TnT increase calcium sensitivity and cause dominantly inherited hypertrophic cardiomyopathy.

Cardiac TnT mutations that caused dilated cardiomyopathy were also found in both middle and C-terminal regions of cardiac TnT. For example, single residue deletion of Lys_{210} (Kamisago, Sharma et al. 2000) and Arg141Trp substitution (Li, Czernuszewicz et al. 2001) both lead to calcium desensitization of myofilament force production and cause dilated cardiomyopathy.

Cardiac TnT interacts with TnI through the I-T interface segment spanning Leu_{224} to Val_{274}. Mutations E244D (Tobacman, Lin et al. 1999; Yanaga, Morimoto et al. 1999), K247R, D270N, N271I and K273E in the TnT-TnI interacting coiled-coil region, *i.e.*, the I-T arm, disrupt the stability of troponin complex and lead to cardiomyopathies.

Aberrant splicing—Error splicing of cardiac TnT have been found in animal models to cause cardiomyopathies. For example, continuing expression of the embryonic exon 5 in adult cardiac TnT was found in dog, cat and guinea pig hearts in correlation with dilated cardiomyopathy (Biesiadecki, Elder et al. 2002). Although embryonic cardiac TnT is a normal variant of TnT instead of a mutation, chronic coexistence of embryonic and adult cardiac TnT in adult ventricular muscle generates desynchronized myofilament actions that decreases the energetic efficiency of the heart as a rhythmic pump, imposing pathogenic consequences (Feng, Chen et al. 2012).

Abnormal splice-out of the exon 8-encoded segment of turkey cardiac TnT and the counterpart, exon 7, in dog cardiac TnT are found to cause dilated cardiomyopathy (Biesiadecki and Jin 2002; Biesiadecki, Elder et al. 2002). The causal relationship was confirmed in transgenic mouse hearts over-expressing the equivalent cardiac TnT with exon 7-encoded segment deleted (Wei, Gao et al. 2010).

A cardiac TnI mutation R111C is found in wild turkey cardiac muscle in combination with the exon 8-deleted cardiac TnT (Biesiadecki et al. 2004). Transgenic mouse hearts expressing the equivalent mutation K118C or cardiac TnT with exon 7 deletion showed dominant negative effects on cardiac function (Wei, Gao et al. 2010). However, when cardiac TnI-118C was co-expressed with cardiac TnT exon 7 deletion as that occurs in the heart of wild turkeys, they mutually rescued each other's dominantly negative phenotypes and restored cardiac function to near normality (Wei, Gao et al. 2010). This compound phenotype of mutations in the two subunits of cardiac troponin suggests that the structure-function relationships of TnT and TnI need to be investigated using integrative approaches.

It is worth noting that despite that an increasing number of *TNNT2* mutations have been reported to date, no point mutation has be identified in the N-terminal variable region (Figure 4). This observation may indicate the hypervariable nature of the N-terminal segment of TnT, which would be tolerant to changes introduced by single amino acid substitutions. On the other hand, The N-terminal variable region functions as a regulator to modulate the overall molecular conformation and function of TnT, thus aberrant splicing of the N-terminal segment of cardiac TnT to introduce large structural changes is able to cause cardiomyopathies.

6.3. TNNT3

Fewer pathogenic mutations of fast skeletal muscle TnT gene (*TNNT3*) have been reported. A *TNNT3* mutation (p.R63C) was found in a Chinese family with distal arthrogryposis (DA) phenotypes (Zhao et al., 2011). A recent exomal sequencing study identified another dominant *TNNT3* mutation at the same residue (p.R63H) in 18 members of a large Indian family also with the phenotypes of distal arthrogryposis (Daly et al., 2014). The affected individuals exhibit significant variability in phenotypes, suggesting an interesting model to further understand the structure-function relationship of TnT and the penetration of pathogenic mutations.

The locations of these pathogenic mutations in the polypeptide chains of ssTnT, cTnT and fsTnT are summarized in Figure 4.

7. Summary and perspective remarks

Troponin T plays a central role in the calcium regulation of striated muscle contraction and relaxation. Three homologous genes have evolved in vertebrates to encode muscle type specific TnT isoforms. Alternative splicing and posttranslational modifications provide additional regulation of TnT structure and function during development and in muscle and heart adaptations to physiological and pathological conditions. Over half century of extensive research has provided a strong knowledge base for understanding the gene regulation and structure-function relationship of TnT. Mechanistic insights have been learned from TnT isoform expression, myopathic mutations, and splicing abnormalities, laying a foundation for translational research for the development of new treatment and prevention of cardiac and skeletal muscle diseases.

In the meantime, many important questions regarding the gene regulation and structure-function relationship of TnT remain to be answered. Some examples are: What is the mechanism for the feedback from muscle mechanic properties to the TnT gene regulation and protein modification? What is the functional significance of alternative splicing of specific exons of the TnT genes? What is the functional significance of the mutually exclusive splicing of fast TnT exons 16 and 17? And what is the precise position of the very C-terminus, middle, and especially the N-terminal variable region of TnT in the thin myofilament? Continued research work using advanced technologies will address these questions for ultimately understanding the evolution and regulation of *TNNT* isoform genes and the molecular mechanisms that govern the function of TnT in striated muscle contraction and relaxation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This review and the corresponding Gene Wiki article are written as part of the Cardiac Gene Wiki Review series - a series resulting from a collaboration between the journal *GENE*, the Gene Wiki Initiative, and the BD2K initiative. The Cardiac Gene Wiki Initiative is supported by National Institutes of Health (GM089820 and GM114833). Additional support for Gene Wiki Reviews is provided by Elsevier, the publisher of GENE.

The authors would like to thank Dr. Rong Liu for the sequence analysis and construction of phylogenetic tree shown in Figure 3. The outstanding contributions of the present and past members of the Jin laboratory have formed the foundation of our troponin research. Our research on TnT was supported by grants from the National Institutes of Health (AR048816, HL-078773 and HL098945 to J.-P.J).

Abbreviations

ANM	Amish Nemaline Myopathy
ASK	Apoptosis Signal-Regulating Kinase
ATP	Adenosine triphosphate
CaMKII	Ca^{2+} /calmodulin-dependent protein kinase II

CMT	Charcot-Marie-Tooth disease
cTnT	cardiac troponin T
DCM	dilated cardiomyopathy
fsTnT	fast skeletal troponin T
HCM	hypertrophic cardiomyopathy
PKA	protein kinase A
PKC	protein kinase C
PP1	protein phosphatase 1
PP2A	protein phosphatase 2A
Raf-1	proto-oncogene, serine/threonine kinase
RCM	restricted cardiomyopathy
ROCK	Rho-Dependent Kinase
ROS	reactive oxygen species
ssTnT	slow skeletal troponin T
TnC	troponin C
TNFa	Tumor necrosis factor a
TnI	troponin I
TNNT1	human slow troponin T gene
TNNT2	human cardiac troponin T gene
TNNT3	human fast troponin T gene
TnT	troponin T

References

Abdulhaq UN, Daana M, et al. Nemaline body myopathy caused by a novel mutation in Troponin T1 (TNNT1). Muscle Nerve. 2015

al-Hillawi E, Bhandari DG, et al. The effects of phosphorylation of cardiac troponin-I on its interactions with actin and cardiac troponin-C. Eur J Biochem. 1995; 228(3):962–970. [PubMed: 7737201]

Amarasinghe C, Jin JP. N-Terminal Hypervariable Region of Muscle Type Isoforms of Troponin T Differentially Modulates the Affinity of Tropomyosin-Binding Site 1. Biochemistry. 2015; 54(24): 3822–3830. [PubMed: 26024675]

Barton PJ, Townsend PJ, et al. Localization of the fast skeletal muscle troponin I gene (TNNI2) to 11p15.5: genes for troponin I and T are organized in pairs. Ann Hum Genet. 1997; 61(Pt 6):519–523. [PubMed: 9543552]

Baudenbacher F, Schober T, et al. Myofilament Ca2+ sensitization causes susceptibility to cardiac arrhythmia in mice. J Clin Invest. 2008; 118(12):3893–3903. [PubMed: 19033660]

Berchtold MW, Brinkmeier H, et al. Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev. 2000; 80(3):1215–1265. [PubMed: 10893434]

Bicer S, Reiser PJ. Complex tropomyosin and troponin T isoform expression patterns in orbital and global fibers of adult dog and rat extraocular muscles. J Muscle Res Cell Motil. 2013; 34(3–4):211–231. [PubMed: 23700265]

Biesiadecki BJ, Chong SM, et al. Troponin T core structure and the regulatory NH2-terminal variable region. Biochemistry. 2007; 46(5):1368–1379. [PubMed: 17260966]

Biesiadecki BJ, Elder BD, et al. Cardiac troponin T variants produced by aberrant splicing of multiple exons in animals with high instances of dilated cardiomyopathy. J Biol Chem. 2002; 277(52): 50275–50285. [PubMed: 12377784]

Biesiadecki BJ, Jin JP. Exon skipping in cardiac troponin T of turkeys with inherited dilated cardiomyopathy. J Biol Chem. 2002; 277(21):18459–18468. [PubMed: 11886865]

Biesiadecki BJ, Schneider KL, Yu ZB, Chong SM, Jin JP. An R111C polymorphism in wild turkey cardiac troponin I accompanying the dilated cardiomyopathy-related abnormal splicing variant of cardiac troponin T with potentially compensatory effects. J Biol Chem. 2004; 279(14):13825–32. [PubMed: 14736877]

Breitbart RE, Nadal-Ginard B. Complete nucleotide sequence of the fast skeletal troponin T gene. Alternatively spliced exons exhibit unusual interspecies divergence. J Mol Biol. 1986; 188(3):313–324. [PubMed: 3735424]

Briggs MM, Schachat F. Origin of fetal troponin T: developmentally regulated splicing of a new exon in the fast troponin T gene. Dev Biol. 1993; 158(2):503–509. [PubMed: 8344466]

Cabral-Lilly D, Tobacman LS, et al. Molecular polarity in tropomyosin-troponin T co-crystals. Biophys J. 1997; 73(4):1763–1770. [PubMed: 9336171]

Chaudhuri T, Mukherjea M, et al. Role of the fetal and alpha/beta exons in the function of fast skeletal troponin T isoforms: correlation with altered Ca2+ regulation associated with development. J Mol Biol. 2005; 352(1):58–71. [PubMed: 16081096]

Chong SM, Jin JP. To investigate protein evolution by detecting suppressed epitope structures. J Mol Evol. 2009; 68(5):448–460. [PubMed: 19365646]

Communal C, Sumandea M, et al. Functional consequences of caspase activation in cardiac myocytes. Proc Natl Acad Sci U S A. 2002; 99(9):6252–6256. [PubMed: 11972044]

Cooper TA, Ordahl CP. A single cardiac troponin T gene generates embryonic and adult isoforms via developmentally regulated alternate splicing. J Biol Chem. 1985; 260(20):11140–11148. [PubMed: 2993302]

Daly SB, Shah H, O'Sullivan J, Anderson B, Bhaskar S, Williams S, Al-Sheqaih N, Mueed Bidchol A, Banka S, Newman WG, Girisha KM. Exome Sequencing Identifies a Dominant TNNT3 Mutation in a Large Family with Distal Arthrogryposis. Mol Syndromol. 2014; 55:218–28.

D'Cruz LG, Baboonian C, et al. Cytosine methylation confers instability on the cardiac troponin T gene in hypertrophic cardiomyopathy. J Med Genet. 2000; 37(9):E18. [PubMed: 10978365]

Dubois-Deruy E, Belliard A, et al. Interplay between troponin T phosphorylation and O-N-acetylglucosaminylation in ischaemic heart failure. Cardiovasc Res. 2015; 107(1):56–65. [PubMed: 25916824]

Ebashi S. Third Component Participating in the Superprecipitation of 'Natural Actomyosin'. Nature. 1963; 200:1010.

Farza H, Townsend PJ, et al. Genomic organisation, alternative splicing and polymorphisms of the human cardiac troponin T gene. J Mol Cell Cardiol. 1998; 30(6):1247–1253. [PubMed: 9689598]

Feng HZ, Biesiadecki BJ, et al. Restricted N-terminal truncation of cardiac troponin T: a novel mechanism for functional adaptation to energetic crisis. J Physiol. 2008; 586(14):3537–3550. [PubMed: 18556368]

Feng HZ, Chen X, et al. Toad heart utilizes exclusively slow skeletal muscle troponin T: an evolutionary adaptation with potential functional benefits. J Biol Chem. 2012; 287(35):29753–29764. [PubMed: 22778265]

Feng HZ, Hossain MM, et al. Myofilament incorporation determines the stoichiometry of troponin I in transgenic expression and the rescue of a null mutation. Arch Biochem Biophys. 2009; 487(1):36–41. [PubMed: 19433057]

Feng HZ, Jin JP. Coexistence of cardiac troponin T variants reduces heart efficiency. Am J Physiol Heart Circ Physiol. 2010; 299(1):H97–H105. [PubMed: 20418479]

Feng HZ, Wang Q, et al. Localization and function of Xinalpha in mouse skeletal muscle. Am J Physiol Cell Physiol. 2013; 304(10):C1002–1012. [PubMed: 23485711]

Feng HZ, Wei B, et al. Deletion of a genomic segment containing the cardiac troponin I gene knocks down expression of the slow troponin T gene and impairs fatigue tolerance of diaphragm muscle. J Biol Chem. 2009; 284(46):31798–31806. [PubMed: 19797054]

Forissier JF, Carrier L, et al. Codon 102 of the cardiac troponin T gene is a putative hot spot for mutations in familial hypertrophic cardiomyopathy. Circulation. 1996; 94(12):3069–3073. [PubMed: 8989109]

Fujino N, Shimizu M, et al. Cardiac troponin T Arg92Trp mutation and progression from hypertrophic to dilated cardiomyopathy. Clin Cardiol. 2001; 24(5):397–402. [PubMed: 11346248]

Fujino N, Shimizu M, et al. A novel mutation Lys273Glu in the cardiac troponin T gene shows high degree of penetrance and transition from hypertrophic to dilated cardiomyopathy. Am J Cardiol. 2002; 89(1):29–33. [PubMed: 11779518]

Gahlmann R, Troutt AB, et al. Alternative splicing generates variants in important functional domains of human slow skeletal troponin T. J Biol Chem. 1987; 262(33):16122–16126. [PubMed: 2824479]

Garcia-Castro M, Coto E, et al. Mutations in sarcomeric genes MYH7, MYBPC3, TNNT2, TNNI3, and TPM1 in patients with hypertrophic cardiomyopathy. Rev Esp Cardiol. 2009; 62(1):48–56. [PubMed: 19150014]

Gomes AV, Potter JD. Cellular and molecular aspects of familial hypertrophic cardiomyopathy caused by mutations in the cardiac troponin I gene. Mol Cell Biochem. 2004; 263(1–2):99–114.

Gordon AM, Homsher E, et al. Regulation of contraction in striated muscle. Physiol Rev. 2000; 80(2): 853–924. [PubMed: 10747208]

Gusev NB, Barskaya NV, et al. Some properties of cardiac troponin T structure. Biochem J. 1983; 213(1):123–129. [PubMed: 6615417]

Hanson EL, Jakobs PM, et al. Cardiac troponin T lysine 210 deletion in a family with dilated cardiomyopathy. J Card Fail. 2002; 8(1):28–32. [PubMed: 11862580]

Harada K, Potter JD. Familial hypertrophic cardiomyopathy mutations from different functional regions of troponin T result in different effects on the pH and Ca2+ sensitivity of cardiac muscle contraction. J Biol Chem. 2004; 279(15):14488–14495. [PubMed: 14722098]

Harada K, Takahashi-Yanaga F, et al. Functional consequences of the deletion mutation deltaGlu160 in human cardiac troponin T. J Biochem. 2000; 127(2):263–268. [PubMed: 10731693]

He X, Liu Y, et al. ASK1 associates with troponin T and induces troponin T phosphorylation and contractile dysfunction in cardiomyocytes. Am J Pathol. 2003; 163(1):243–251. [PubMed: 12819028]

Heeley DH, Golosinska K, et al. The effects of troponin T fragments T1 and T2 on the binding of nonpolymerizable tropomyosin to F-actin in the presence and absence of troponin I and troponin C. J Biol Chem. 1987; 262(21):9971–9978. [PubMed: 3611073]

Hershberger RE, Pinto JR, et al. Clinical and functional characterization of TNNT2 mutations identified in patients with dilated cardiomyopathy. Circ Cardiovasc Genet. 2009; 2(4):306–313. [PubMed: 20031601]

Huang QQ, Chen A, et al. Genomic sequence and structural organization of mouse slow skeletal muscle troponin T gene. Gene. 1999; 229(1–2):1–10. [PubMed: 10095098]

Huang QQ, Feng HZ, et al. Co-expression of skeletal and cardiac troponin T decreases mouse cardiac function. Am J Physiol Cell Physiol. 2008; 294(1):C213–222. [PubMed: 17959729]

Imai H, Hirai S, et al. Many isoforms of fast muscle troponin T from chicken legs. J Biochem. 1986; 99(3):923–930. [PubMed: 3711049]

Jaquet K, Fukunaga K, et al. A site phosphorylated in bovine cardiac troponin T by cardiac CaM kinase II. Biochim Biophys Acta. 1995; 1248(2):193–195. [PubMed: 7748902]

Jeong EM, Wang X, et al. Nonmyofilament-associated troponin T fragments induce apoptosis. Am J Physiol Heart Circ Physiol. 2009; 297(1):H283–292. [PubMed: 19395545]

Jideama NM, Crawford BH, et al. Dephosphorylation specificities of protein phosphatase for cardiac troponin I, troponin T, and sites within troponin T. Int J Biol Sci. 2006; 2(1):1–9. [PubMed: 16585947]

Jideama NM, Noland TA Jr, et al. Phosphorylation specificities of protein kinase C isozymes for bovine cardiac troponin I and troponin T and sites within these proteins and regulation of myofilament properties. J Biol Chem. 1996; 271(38):23277–23283. [PubMed: 8798526]

Jin JP. Alternative RNA splicing-generated cardiac troponin T isoform switching: a non-heart-restricted genetic programming synchronized in developing cardiac and skeletal muscles. Biochem Biophys Res Commun. 1996; 225(3):883–889. [PubMed: 8780706]

Jin JP, Brotto MA, et al. Truncation by Glu180 nonsense mutation results in complete loss of slow skeletal muscle troponin T in a lethal nemaline myopathy. J Biol Chem. 2003; 278(28):26159–26165. [PubMed: 12732643]

Jin JP, Chen A, et al. Three alternatively spliced mouse slow skeletal muscle troponin T isoforms: conserved primary structure and regulated expression during postnatal development. Gene. 1998; 214(1–2):121–129. [PubMed: 9651500]

Jin JP, Chen A, et al. Conformational modulation of slow skeletal muscle troponin T by an NH(2)-terminal metal-binding extension. Am J Physiol Cell Physiol. 2000; 279(4):C1067–1077. [PubMed: 11003587]

Jin JP, Chong SM. Localization of the two tropomyosin-binding sites of troponin T. Arch Biochem Biophys. 2010; 500(2):144–150. [PubMed: 20529660]

Jin JP, Huang QQ, et al. Complete nucleotide sequence and structural organization of rat cardiac troponin T gene. A single gene generates embryonic and adult isoforms via developmentally regulated alternative splicing. J Mol Biol. 1992; 227(4):1269–1276. [PubMed: 1433301]

Jin JP, Lin JJ. Isolation and characterization of cDNA clones encoding embryonic and adult isoforms of rat cardiac troponin T. J Biol Chem. 1989; 264(24):14471–14477. [PubMed: 2760070]

Jin JP, Root DD. Modulation of troponin T molecular conformation and flexibility by metal ion binding to the NH2-terminal variable region. Biochemistry. 2000; 39(38):11702–11713. [PubMed: 10995238]

Jin JP, Samanez RA. Evolution of a metal-binding cluster in the NH(2)-terminal variable region of avian fast skeletal muscle troponin T: functional divergence on the basis of tolerance to structural drifting. J Mol Evol. 2001; 52(2):103–116. [PubMed: 11231890]

Jin JP, Smillie LB. An unusual metal-binding cluster found exclusively in the avian breast muscle troponin T of Galliformes and Craciformes. FEBS Lett. 1994; 341(1):135–140. [PubMed: 8137914]

Jin JP, Wang J, et al. Expression of cDNAs encoding mouse cardiac troponin T isoforms: characterization of a large sample of independent clones. Gene. 1996; 168(2):217–221. [PubMed: 8654947]

Jin JP, Zhang Z, et al. Isoform diversity, regulation, and functional adaptation of troponin and calponin. Crit Rev Eukaryot Gene Expr. 2008; 18(2):93–124. [PubMed: 18304026]

Johnston JJ, Kelley RI, et al. A novel nemaline myopathy in the Amish caused by a mutation in troponin T1. Am J Hum Genet. 2000; 67(4):814–821. [PubMed: 10952871]

Kamisago M, Sharma SD, et al. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. N Engl J Med. 2000; 343(23):1688–1696. [PubMed: 11106718]

Kaski JP, Syrris P, et al. Idiopathic restrictive cardiomyopathy in children is caused by mutations in cardiac sarcomere protein genes. Heart. 2008; 94(11):1478–1484. [PubMed: 18467357]

Kato K, Negishi T, et al. Effect of calcium ion transported into uterine muscular cell through cell membrane on actomyosine-ATPase of uterine muscle of rat. Nihon Heikatsukin Gakkai Zasshi. 1969; 5(3):166–169. [PubMed: 4260548]

Katoh N, Wise BC, et al. Phosphorylation of cardiac troponin inhibitory subunit (troponin I) and tropomyosin-binding subunit (troponin T) by cardiac phospholipid-sensitive Ca2+-dependent protein kinase. Biochem J. 1983; 209(1):189–195. [PubMed: 6303300]

Knollmann BC, Potter JD. Altered regulation of cardiac muscle contraction by troponin T mutations that cause familial hypertrophic cardiomyopathy. Trends Cardiovasc Med. 2001; 11(5):206–212. [PubMed: 11597833]

Koga Y, Toshima H, et al. Clinical manifestations of hypertrophic cardiomyopathy with mutations in the cardiac beta-myosin heavy chain gene or cardiac troponin T gene. J Card Fail. 1996; 2(4 Suppl):S97–103. [PubMed: 8951566]

Larsson L, Wang X, et al. Adaptation by alternative RNA splicing of slow troponin T isoforms in type 1 but not type 2 Charcot-Marie-Tooth disease. Am J Physiol Cell Physiol. 2008; 295(3):C722–731. [PubMed: 18579801]

Li D, Czernuszewicz GZ, et al. Novel cardiac troponin T mutation as a cause of familial dilated cardiomyopathy. Circulation. 2001; 104(18):2188–2193. [PubMed: 11684629]

Lin D, Bobkova A, et al. Altered cardiac troponin T in vitro function in the presence of a mutation implicated in familial hypertrophic cardiomyopathy. J Clin Invest. 1996; 97(12):2842–2848. [PubMed: 8675696]

Lu QW, Morimoto S, et al. Cardiac troponin T mutation R141W found in dilated cardiomyopathy stabilizes the troponin T-tropomyosin interaction and causes a Ca2+ desensitization. J Mol Cell Cardiol. 2003; 35(12):1421–1427. [PubMed: 14654368]

Lu QW, Wu XY, Morimoto S. Inherited cardiomyopathies caused by troponin mutations. J Geriatr Cardiol. 2013; 10(1):91–101. [PubMed: 23610579]

Malnic B, Farah CS, et al. Regulatory properties of the NH2- and COOH-terminal domains of troponin T. ATPase activation and binding to troponin I and troponin C. J Biol Chem. 1998; 273(17): 10594–10601. [PubMed: 9553120]

Marian AJ, Zhao G, et al. Expression of a mutant (Arg92Gln) human cardiac troponin T, known to cause hypertrophic cardiomyopathy, impairs adult cardiac myocyte contractility. Circ Res. 1997; 81(1):76–85. [PubMed: 9201030]

Marra JD, Engelstad KE, et al. Identification of a novel nemaline myopathy-causing mutation in the troponin T1 (TNNT1) gene: a case outside of the old order Amish. Muscle Nerve. 2015; 51(5): 767–772. [PubMed: 25430424]

Martin AF. Turnover of cardiac troponin subunits. Kinetic evidence for a precursor pool of troponin-I. J Biol Chem. 1981; 256(2):964–968. [PubMed: 7451483]

Martin AF, Orlowski J. Molecular cloning and developmental expression of the rat cardiac-specific isoform of troponin I. J Mol Cell Cardiol. 1991; 23(5):583–588. [PubMed: 1886137]

Medford RM, Nguyen HT, et al. A novel mechanism of alternative RNA splicing for the developmentally regulated generation of troponin T isoforms from a single gene. Cell. 1984; 38(2):409–421. [PubMed: 6205765]

Mirza M, Marston S, et al. Dilated cardiomyopathy mutations in three thin filament regulatory proteins result in a common functional phenotype. J Biol Chem. 2005; 280(31):28498–28506. [PubMed: 15923195]

Miyazaki J, Jozaki M, et al. The structure of the avian fast skeletal muscle troponin T gene: seven novel tandem-arranged exons in the exon x region. J Muscle Res Cell Motil. 1999; 20(7):655–660. [PubMed: 10672513]

Mogensen J, Murphy RT, et al. Severe disease expression of cardiac troponin C and T mutations in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol. 2004; 44(10):2033–2040. [PubMed: 15542288]

Montgomery DE, Chandra M, et al. Transgenic incorporation of skeletal TnT into cardiac myofilaments blunts PKC-mediated depression of force. Am J Physiol Heart Circ Physiol. 2001; 280(3):H1011–1018. [PubMed: 11179042]

Moolman JC V, Corfield A, et al. Sudden death due to troponin T mutations. J Am Coll Cardiol. 1997; 29(3):549–555. [PubMed: 9060892]

Morimoto S, Nakaura H, et al. Functional consequences of a carboxyl terminal missense mutation Arg278Cys in human cardiac troponin T. Biochem Biophys Res Commun. 1999; 261(1):79–82. [PubMed: 10405326]

Morimoto S, Yanaga F, et al. Ca2+-sensitizing effects of the mutations at Ile-79 and Arg-92 of troponin T in hypertrophic cardiomyopathy. Am J Physiol. 1998; 275(1 Pt 1):C200–207. [PubMed: 9688851]

Nakajima-Taniguchi C, Matsui H, et al. Novel missense mutation in cardiac troponin T gene found in Japanese patient with hypertrophic cardiomyopathy. J Mol Cell Cardiol. 1997; 29(2):839–843. [PubMed: 9140840]

Nakaura H, Yanaga F, et al. Effects of missense mutations Phe110Ile and Glu244Asp in human cardiac troponin T on force generation in skinned cardiac muscle fibers. J Biochem. 1999; 126(3):457–460. [PubMed: 10467159]

Nishii K, Morimoto S, et al. Targeted disruption of the cardiac troponin T gene causes sarcomere disassembly and defects in heartbeat within the early mouse embryo. Dev Biol. 2008; 322(1):65–73. [PubMed: 18671960]

Noland TA Jr, Kuo JF. Protein kinase C phosphorylation of cardiac troponin T decreases Ca(2+)-dependent actomyosin MgATPase activity and troponin T binding to tropomyosin-F-actin complex. Biochem J. 1992; 288(Pt 1):123–129. [PubMed: 1445257]

Noland TA Jr, Raynor RL, et al. Identification of sites phosphorylated in bovine cardiac troponin I and troponin T by protein kinase C and comparative substrate activity of synthetic peptides containing the phosphorylation sites. J Biol Chem. 1989; 264(34):20778–20785. [PubMed: 2584239]

Nosek TM, Brotto MA, Jin JP. Troponin T isoforms alter the tolerance of transgenic mouse cardiac muscle to acidosis. Arch Biochem Biophys. 2004; 430(2):178–84. [PubMed: 15369816]

Ogut O, Granzier H, et al. Acidic and basic troponin T isoforms in mature fast-twitch skeletal muscle and effect on contractility. Am J Physiol. 1999; 276(5 Pt 1):C1162–1170. [PubMed: 10329966]

Ogut O, Jin JP. Expression, zinc-affinity purification, and characterization of a novel metal-binding cluster in troponin T: metal-stabilized alpha-helical structure and effects of the NH2-terminal variable region on the conformation of intact troponin T and its association with tropomyosin. Biochemistry. 1996; 35(51):16581–16590. [PubMed: 8987993]

Ogut O, Jin JP. Developmentally regulated, alternative RNA splicing-generated pectoral muscle-specific troponin T isoforms and role of the NH2-terminal hypervariable region in the tolerance to acidosis. J Biol Chem. 1998; 273(43):27858–27866. [PubMed: 9774396]

Otsuki I, Masaki T, et al. Periodic distribution of troponin along the thin filament. J Biochem. 1967; 61(6):817–819. [PubMed: 4863038]

Palm T, Graboski S, et al. Disease-causing mutations in cardiac troponin T: identification of a critical tropomyosin-binding region. Biophys J. 2001; 81(5):2827–2837. [PubMed: 11606294]

Pan BS, Gordon AM, et al. Deletion of the first 45 NH2-terminal residues of rabbit skeletal troponin T strengthens binding of troponin to immobilized tropomyosin. J Biol Chem. 1991; 266(19):12432–12438. [PubMed: 1829457]

Pato MD, Mak AS, et al. Fragments of rabbit striated muscle alpha-tropomyosin. II. Binding to troponin-T. J Biol Chem. 1981; 256(2):602–607. [PubMed: 7451463]

Pearlstone JR, Smillie LB. Binding of troponin-T fragments to several types of tropomyosin. Sensitivity to Ca2+ in the presence of troponin-C. J Biol Chem. 1982; 257(18):10587–10592. [PubMed: 7107628]

Peddy SB, Vricella LA, et al. Infantile restrictive cardiomyopathy resulting from a mutation in the cardiac troponin T gene. Pediatrics. 2006; 117(5):1830–1833. [PubMed: 16651346]

Perry SV. Troponin T: genetics, properties and function. J Muscle Res Cell Motil. 1998; 19(6):575–602. [PubMed: 9742444]

Pfleiderer P, Sumandea MP, et al. Raf-1: a novel cardiac troponin T kinase. J Muscle Res Cell Motil. 2009; 30(1–2):67–72. [PubMed: 19381846]

Pinto JR, Parvatiyar MS, et al. A troponin T mutation that causes infantile restrictive cardiomyopathy increases Ca2+ sensitivity of force development and impairs the inhibitory properties of troponin. J Biol Chem. 2008; 283(4):2156–2166. [PubMed: 18032382]

Robinson P, Griffiths PJ, et al. Dilated and hypertrophic cardiomyopathy mutations in troponin and alpha-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res. 2007; 101(12):1266–1273. [PubMed: 17932326]

Saggin L, Ausoni S, et al. Troponin T switching in the developing rat heart. J Biol Chem. 1988; 263(34):18488–18492. [PubMed: 2973462]

Saggin L, Gorza L, et al. Troponin I switching in the developing heart. J Biol Chem. 1989; 264(27): 16299–16302. [PubMed: 2777792]

Schwartz K, Mercadier JJ. Cardiac troponin T and familial hypertrophic cardiomyopathy: an energetic affair. J Clin Invest. 2003; 112(5):652–654. [PubMed: 12952912]

Sheng JJ, Jin JP. Gene regulation, alternative splicing, and posttranslational modification of troponin subunits in cardiac development and adaptation: a focused review. Front Physiol. 2014; 5:165. [PubMed: 24817852]

Shimizu M, Ino H, et al. Autopsy findings in siblings with hypertrophic cardiomyopathy caused by Arg92Trp mutation in the cardiac troponin T gene showing dilated cardiomyopathy-like features. Clin Cardiol. 2003; 26(11):536–539. [PubMed: 14640471]

Simpson JA, van Eyk JE, et al. Hypoxemia-induced modification of troponin I and T in canine diaphragm. J Appl Physiol (1985). 2000; 88(2):753–760. [PubMed: 10658047]

Smillie LB, Golosinska K, et al. Sequences of complete cDNAs encoding four variants of chicken skeletal muscle troponin T. J Biol Chem. 1988; 263(35):18816–18820. [PubMed: 3198600]

Sumandea CA, Garcia-Cazarin ML, et al. Cardiac troponin T, a sarcomeric AKAP, tethers protein kinase A at the myofilaments. J Biol Chem. 2011; 286(1):530–541. [PubMed: 21056973]

Sumandea MP, Burkart EM, et al. Molecular and integrated biology of thin filament protein phosphorylation in heart muscle. Ann N Y Acad Sci. 2004; 1015:39–52. [PubMed: 15201148]

Sumandea MP, Pyle WG, et al. Identification of a functionally critical protein kinase C phosphorylation residue of cardiac troponin T. J Biol Chem. 2003; 278(37):35135–35144. [PubMed: 12832403]

Sumandea MP, Vahebi S, et al. Impact of cardiac troponin T N-terminal deletion and phosphorylation on myofilament function. Biochemistry. 2009; 48(32):7722–7731. [PubMed: 19586048]

Takeda S, Yamashita A, et al. Structure of the core domain of human cardiac troponin in the Ca(2+)-saturated form. Nature. 2003; 424(6944):35–41. [PubMed: 12840750]

Tamura K, Peterson D, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011; 28(10):2731–2739. [PubMed: 21546353]

Tanokura M, Ohtsuki I. Interactions among chymotryptic troponin T subfragments, tropomyosin, troponin I and troponin C. J Biochem. 1984; 95(5):1417–1421. [PubMed: 6746613]

Tanokura M, Tawada Y, et al. Chymotryptic subfragments of troponin T from rabbit skeletal muscle. Interaction with tropomyosin, troponin I and troponin C. J Biochem. 1983; 93(2):331–337. [PubMed: 6841341]

Tanokura M, Tawada Y, et al. Primary structure of chymotryptic subfragments from rabbit skeletal troponin T. J Biochem. 1981; 90(1):263–265. [PubMed: 7287682]

Theopistou A, Anastasakis A, et al. Clinical features of hypertrophic cardiomyopathy caused by an Arg278Cys missense mutation in the cardiac troponin T gene. Am J Cardiol. 2004; 94(2):246–249. [PubMed: 15246915]

Thierfelder L, Watkins H, et al. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. Cell. 1994; 77(5):701–712. [PubMed: 8205619]

Tobacman LS. Thin filament-mediated regulation of cardiac contraction. Annu Rev Physiol. 1996; 58:447–481. [PubMed: 8815803]

Tobacman LS, Lin D, et al. Functional consequences of troponin T mutations found in hypertrophic cardiomyopathy. J Biol Chem. 1999; 274(40):28363–28370. [PubMed: 10497196]

Toyota N, Shimada Y. Isoform variants of troponin in skeletal and cardiac muscle cells cultured with and without nerves. Cell. 1983; 33(1):297–304. [PubMed: 6380757]

Vahebi S, Kobayashi T, et al. Functional effects of rho-kinase-dependent phosphorylation of specific sites on cardiac troponin. Circ Res. 2005; 96(7):740–747. [PubMed: 15774859]

van der Pol WL, Leijenaar JF, et al. Nemaline myopathy caused byTNNT1 mutations in a Dutch pedigree. Mol Genet Genomic Med. 2014; 2(2):134–137. [PubMed: 24689076]

Van Driest SL, Ackerman MJ, et al. Prevalence and severity of “b”enign” mutations in the beta-myosin heavy chain, cardiac troponin T, and alpha-tropomyosin genes in hypertrophic cardiomyopathy. Circulation. 2002; 106(24):3085–3090. [PubMed: 12473556]

Varnava A, Baboonian C, et al. A new mutation of the cardiac troponin T gene causing familial hypertrophic cardiomyopathy without left ventricular hypertrophy. Heart. 1999; 82(5):621–624. [PubMed: 10525521]

Villar-Palasi C, Kumon A. Purification and properties of dog cardiac troponin T kinase. J Biol Chem. 1981; 256(14):7409–7415. [PubMed: 7251602]

Vinogradova MV, Stone DB, et al. Ca(2+)-regulated structural changes in troponin. Proc Natl Acad Sci U S A. 2005; 102(14):5038–5043. [PubMed: 15784741]

Wang J, Jin JP. Primary structure and developmental acidic to basic transition of 13 alternatively spliced mouse fast skeletal muscle troponin T isoforms. Gene. 1997; 193(1):105–114. [PubMed: 9249073]

Wang J, Jin JP. Conformational modulation of troponin T by configuration of the NH2-terminal variable region and functional effects. Biochemistry. 1998; 37(41):14519–14528. [PubMed: 9772180]

Wang X, Huang QQ, et al. Cellular fate of truncated slow skeletal muscle troponin T produced by Glu180 nonsense mutation in amish nemaline myopathy. J Biol Chem. 2005; 280(14):13241–13249. [PubMed: 15665378]

Watkins H, McKenna WJ, et al. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. N Engl J Med. 1995; 332(16):1058–1064. [PubMed: 7898523]

Wei B, Gao J, et al. Mutual rescues between two dominant-negative mutations in cardiac troponin I and cardiac troponin T. J Biol Chem. 2010

Wei B, Gao J, et al. Mutual rescues between two dominant negative mutations in cardiac troponin I and cardiac troponin T. J Biol Chem. 2010; 285(36):27806–27816. [PubMed: 20551314]

Wei B, Jin JP. Troponin T isoforms and posttranscriptional modifications: Evolution, regulation and function. Arch Biochem Biophys. 2011; 505(2):144–154. [PubMed: 20965144]

Wei B, Lu Y, et al. Deficiency of slow skeletal muscle troponin T causes atrophy of type I slow fibres and decreases tolerance to fatigue. J Physiol. 2014; 592(Pt 6):1367–1380. [PubMed: 24445317]

Wendt T, Guenebaut V, et al. Structure of the Lethocerus troponin-tropomyosin complex as determined by electron microscopy. J Struct Biol. 1997; 118(1):1–8. [PubMed: 9087910]

White SP, Cohen C, et al. Structure of co-crystals of tropomyosin and troponin. Nature. 1987; 325(6107):826–828. [PubMed: 3102969]

Wilkinson JM, Moir AJ, et al. The expression of multiple forms of troponin T in chicken-fast-skeletal muscle may result from differential splicing of a single gene. Eur J Biochem. 1984; 143(1):47–56. [PubMed: 6468390]

Wu QL, Jha PK, et al. Overproduction and rapid purification of human fast skeletal beta troponin T using Escherichia coli expression vectors: functional differences between the alpha and beta isoforms. Gene. 1995; 155(2):225–230. [PubMed: 7721095]

Yanaga F, Morimoto S, et al. Ca2+ sensitization and potentiation of the maximum level of myofibrillar ATPase activity caused by mutations of troponin T found in familial hypertrophic cardiomyopathy. J Biol Chem. 1999; 274(13):8806–8812. [PubMed: 10085122]

Zhang J, Zhang H, et al. Phosphorylation, but not alternative splicing or proteolytic degradation, is conserved in human and mouse cardiac troponin T. Biochemistry. 2011; 50(27):6081–6092. [PubMed: 21639091]

Zhang Z, Biesiadecki BJ, et al. Selective deletion of the NH2-terminal variable region of cardiac troponin T in ischemia reperfusion by myofibril-associated mu-calpain cleavage. Biochemistry. 2006; 45(38):11681–11694. [PubMed: 16981728]

Zhang Z, Feng HZ, et al. Structure of the NH2-terminal variable region of cardiac troponin T determines its sensitivity to restrictive cleavage in pathophysiological adaptation. Arch Biochem Biophys. 2011; 515(1–2):37–45. [PubMed: 21924234]

Zhang Z, Jin JP, et al. Binding of calcium ions to an avian flight muscle troponin T. Biochemistry. 2004; 43(9):2645–2655. [PubMed: 14992602]

Zhao N, Jiang M, Han W, Bian C, Li X, Huang F, Kong Q, Li J. A novel mutation in TNNT3 associated with Sheldon-Hall syndrome in a Chinese family with vertical talus. Eur J Med Genet. 2011; 54(3):351–3. [PubMed: 21402185]

Highlights

- Troponin T (TnT) is a regulator of striated muscle contraction
- 3 homologous genes have evolved in vertebrates encoding muscle type TnT isoforms
- Alternative splicing and posttranslational modifications add variations of TnT
- TnT gene expression is regulated during development and adaptations
- This review summarizes the current knowledge and perspectives of TnT research

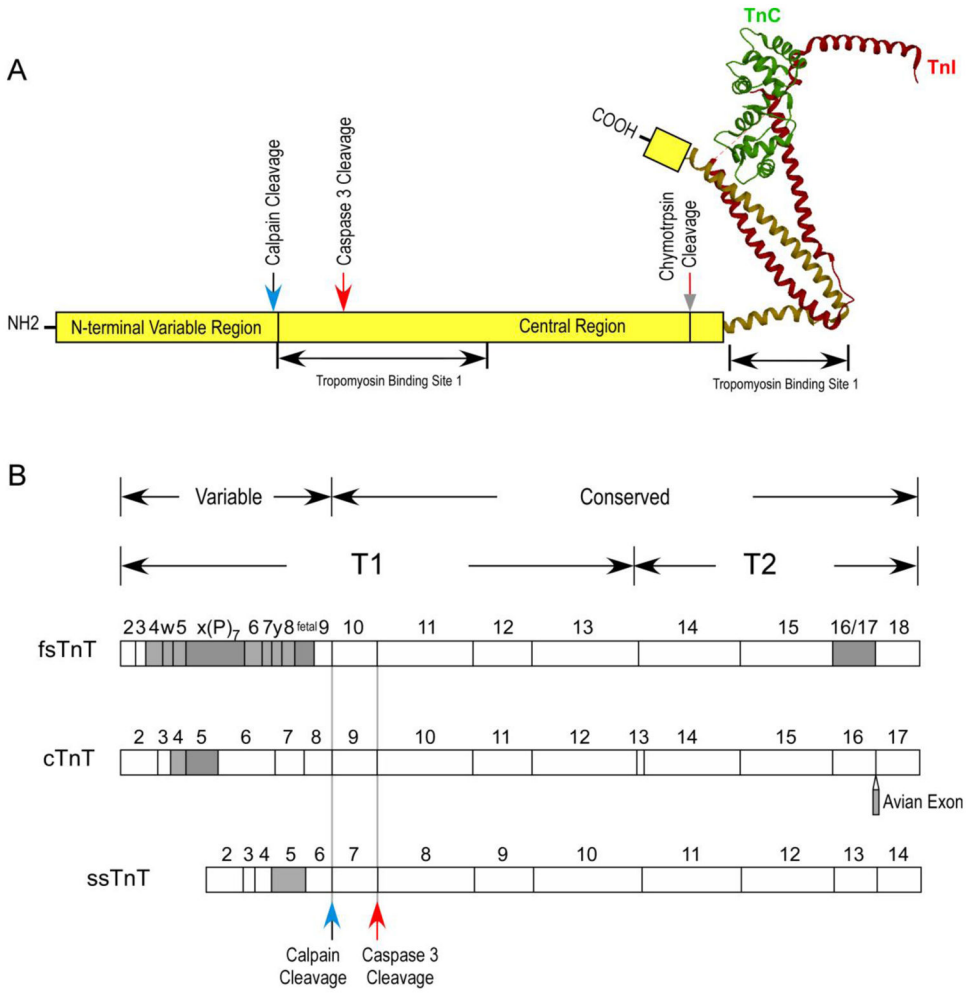


Figure 1. Structural and functional domains and alternatively spliced exons of the three TnT isoforms

A. Partial crystallography structure of troponin complex was modified from published data of cardiac troponin (Takeda, Yamashita et al. 2003) using UCSF chimera software. The calpain cleavage site, caspase 3 cleavage sit, chymotryptic cleavage site between TnT T1 and T2 fragments, and the two tropomyosin-binding sites are indicated. B. The linear structure alignment shows that the N-terminal region of TnT is highly variable in cardiac, fast and slow skeletal muscle isoforms and regulated via alternative splicing, whereas the middle and C-terminal regions are highly conserved. The filled boxes indicate alternative spliced exons, of which the solid boxes represent those under developmental regulation. The alternatively spliced exons w, x (P) and y in the fast TnT exon map are only found in avian species. The T1 and T2 fragments generated by limited chymotryptic digestion are outlined. The calpain and caspase cleavage sites in cardiac TnT are also indicated.

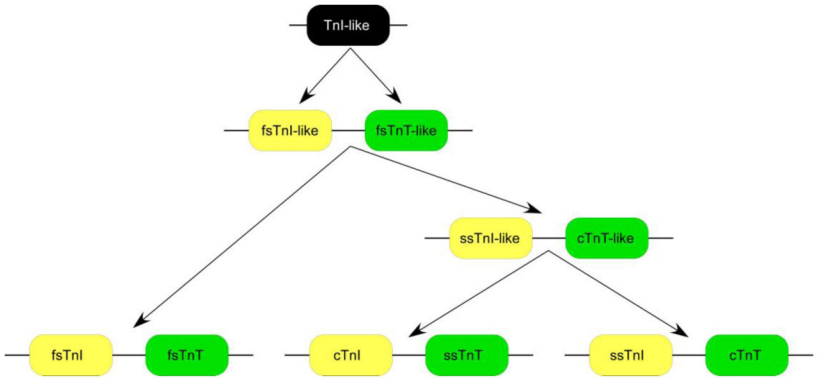


Figure 2. Molecular evolution lineage of vertebrate TnT isoform genes

Modified from Chong and Jin (2009), the illustration shows the evolutionary lineage of the three closely linked pairs of muscle type specific TnT and TnI genes. Genes encoding TnT and TnI were likely originated from a TnI-like ancestor gene. A fsTnI-like-fsTnT-like gene pair was first emerged by duplication and diversified from the original TnI-like ancestor gene and further gave rise to a ssTnI-like-cTnT-like gene pair. Further duplication and diversification of the cTnI like-cTnT-like gene pair occurred as a later event form the three present-day muscle type specific pairs of TnT and TnI isoform genes in vertebrates.

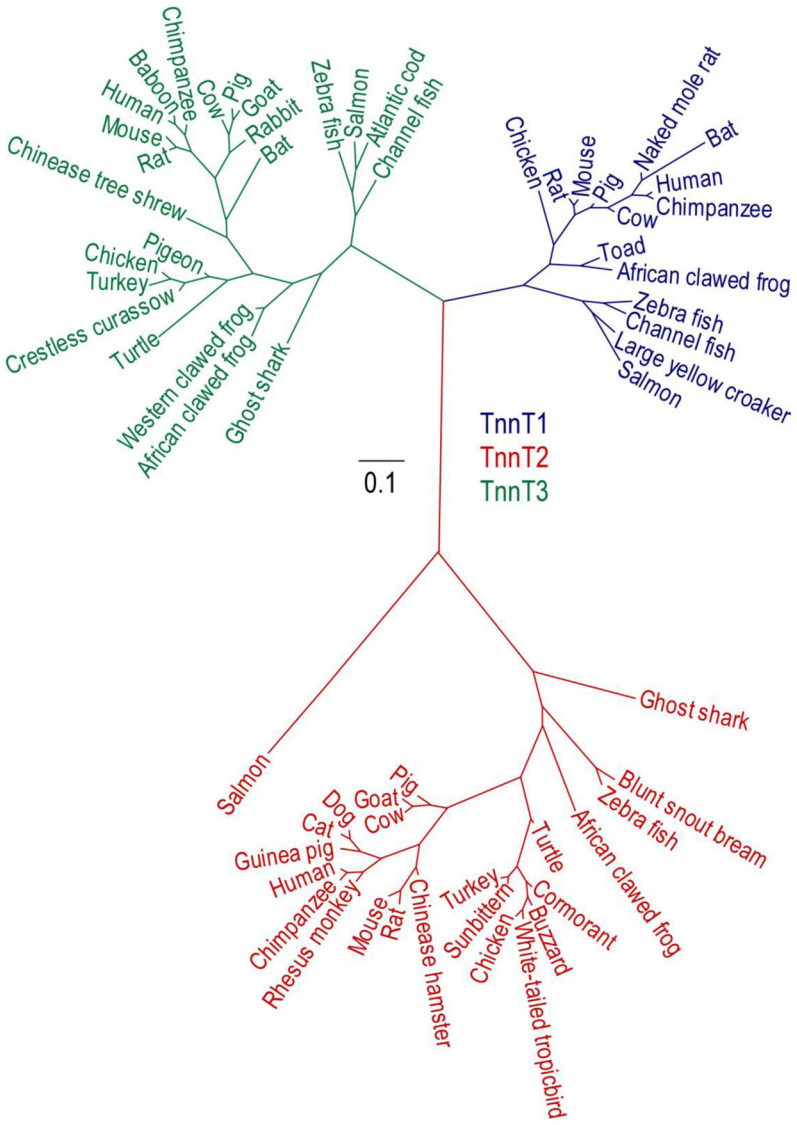


Figure 3. Phylogenic tree of vertebrate TnT isoforms

A phylogenetic tree of vertebrate *Tnnt* genes is derived from protein sequence alignment performed with the Clustal V method using the MegAlign computer program (Lasergene; DNASTAR, lnc, Madison, WI). The degree of divergence is indicated with the length of the lineage lines. *Tnnt1, 2 and 3* isoforms are marked in blue, red and green, respectively. The NCBI database accession numbers for the sequences analyzed are: African clawed frog *Tnnt1*, NP_001086207.1; African clawed frog *Tnnt2*, AAO33405.1; African clawed frog *Tnnt3*, NP_001080403.1; Atlantic cod *Tnnt3*, AAM21701.1; baboon *Tnnt3*, NP_001162538.1; bat *Tnnt1*, ELK09186.1; bat *Tnnt3*, ELK13205.1; blunt snout bream *Tnnt2*, bovine *Tnnt1*, NP_776899.1; bovine *Tnnt2*, NP_777196.1; bovine *Tnnt3*, BAB92975.1; buzzard *Tnnt2*, KFP58299.1; cat *Tnnt2*, AAO13362.1; channel fish *Tnnt1*, AHH42303.1; channel fish *Tnnt3*, AHH42694.1; chicken *Tnnt1*, BAD06455.1; chicken *Tnnt2*, NP_990780.1; chicken *Tnnt3*, NP_990253.1; chimpanzee *Tnnt1*, JAA42790.1; chimpanzee *Tnnt2*, JAA36029.1; chimpanzee *Tnnt3*, JAA19567.1; Chinese hamster *Tnnt2*,

EGW02189.1; Chinese tree shrew *Tnnt3*, ELW71918.1; Cormorant *Tnnt2*, KFW92284.1; crestless curassow *Tnnt3*, AAF81014.1; dog *Tnnt2*, NP_001003012.2; ghost shark *Tnnt2*, AFP07845.1; ghost shark *Tnnt3*, AFP05162.1; goat *Tnnt2*, NP_001301119.1; goat *Tnnt3*, NP_001301139.1; Guinea pig *Tnnt2*, NM_001172863.1; human *TNNT1*, CAA09752.1; human *TNNT2*, AAK92231.1; human *TNNT3*, AAF21629.1; large yellow croaker *Tnnt1*, KKF32449.1; mouse *Tnnt1*, AAD00730.1; mouse *Tnnt2*, AAA85350.1; mouse *Tnnt3*, AAF01502.1; naked mole rat *Tnnt1*, EHB13469.1; pig *Tnnt1*, NP_998913.1; pig *Tnnt2*, NP_001244282.1; pig *Tnnt3*, NP_001001863.1; pigeon *Tnnt3*, EMC88041.1; rabbit *Tnnt3*, GU944669.1; rat *Tnnt1*, NM_134388.2; rat *Tnnt2*, NP_036808.1; rat *Tnnt3*, NM_001270665.1; Rhesus monkey *Tnnt2*, NM_001247991.1; salmon *Tnnt1*, ACM09392.1; salmon *Tnnt2*, NP_001140134.1; salmon *Tnnt3*, ACM08284.1; sun bittern *Tnnt2*, KFW09657.1; toad *Tnnt1*, AY773671.2; turkey *Tnnt2*, NM_001303165.1; turkey *Tnnt3*, NP_001290143.1; turtle *Tnnt2*, EMP40998.1; turtle *Tnnt3*, EMP32847.1; KC556826.1; white-tailed tropicbird *Tnnt2*, KFQ82867.1; western clawed frog *Tnnt3*, NP_989143.1; zebra fish *Tnnt1*, NP_001122167.1; zebra fish *Tnnt2*, AAL06279.1; zebra fish *Tnnt3*, AAF78472.1.

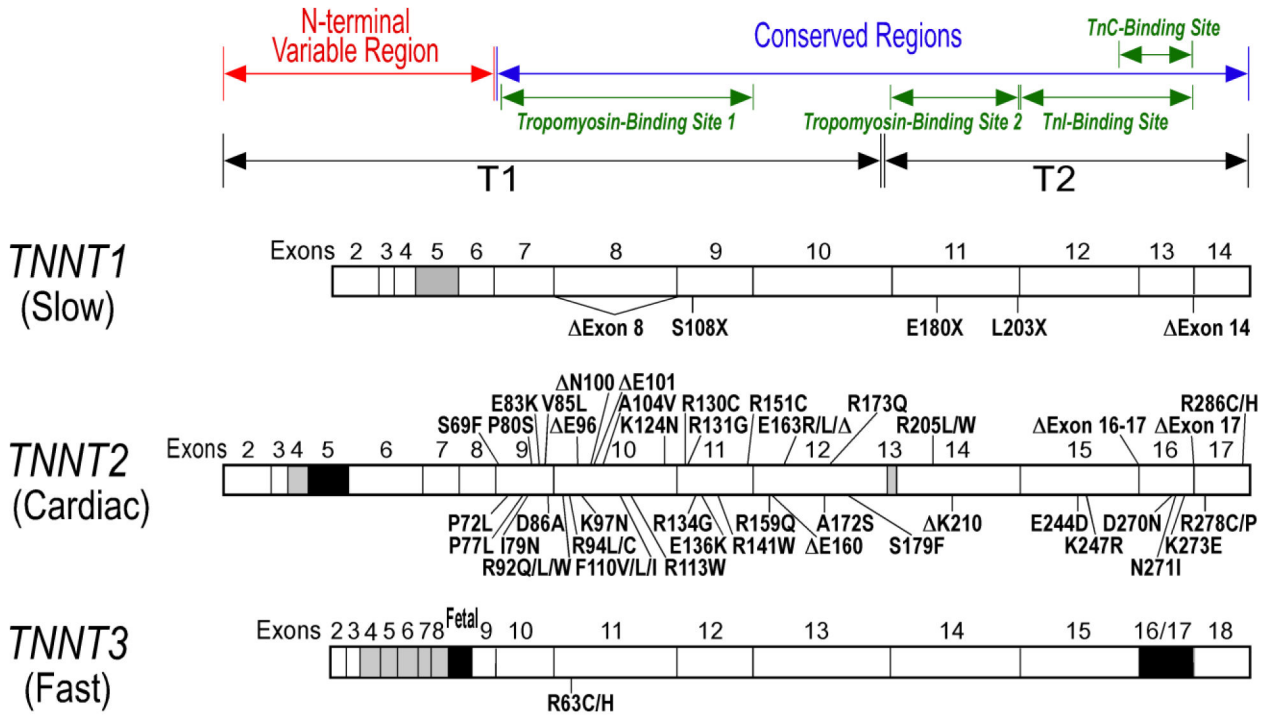


Figure 4. Pathological mutations of TnT

Locations of single amino acid substitutions and deletions, as well as exon deletions and C-terminal truncations found in inherited human cardiac and skeletal muscle diseases are indicated on the linear maps of cardiac, slow and fast skeletal muscle TnT. The filled boxes indicate alternatively spliced exons, among which the developmentally regulated ones are in solid black. The pathological phenotypes some representative mutations are listed in Table 3. Majority of these mutations are located in the conserved middle region of TnT corresponding the to tropomyosin binding site 1. Despite the large number of pathogenic mutations reported to date, none has been reported in the N-terminal hypervariable region of TnT.

Table 1

Human TnT isoform genes and tissue-specific expression

Protein isoforms	Slow Skeletal Muscle TnT	Cardiac TnT	Fast skeletal Muscle TnT
Gene	*TNNT1*	*TNNT2*	*TNNT3*
Chromosomal Location	19q13.4	1q32	11p15.5
Number of Exons	14	17	19
Number of Amino Acids	261	297	268
Relative Molecular Mass	32948.15	35923.49	30596.19
Isoelectric Point	5.95	4.88	6.21
Tissue Specificity	Slow-twitch fibers	Heart Embryonic skeletal muscle	Fast-twitch fibers

Molecular mass and isoelectric point of human TnT isoforms were calculated using the following protein sequences: *TNNT1*, NM_003283; *TNNT2*, NM_001276345; and *TNNT3*, NM_006757.

Table 2

Phosphorylation sites in TnT

Phosphorylation site			Kinases	Function	Reference
cTnT	ssTnT	fsTnT			
Ser_2	**C**	**C**	Unknown	Unknown	(Villar-Palasi and Kumon 1981; Gusev, Barskaya et al. 1983; Zhang, Zhang et al. 2011)
Thr_{204}	**N**	**N**	PKC	Reduce Myosin ATPase activity, myofilament force production and Ca^{2+} sensitivity	(Noland, Raynor et al. 1989; Jideama, Noland et al. 1996; Montgomery, Chandra et al. 2001; Sumandea, Pyle et al. 2003)
Thr_{204}	**N**	**N**	CaMK II	Unknown	(Jaquet, Fukunaga et al. 1995)
Thr_{204}	**N**	**N**	ASK I	Reduce cardiomyocyte contractility	(He, Liu et al. 2003)
Ser_{208}	**N**	**N**	PKC	Reduce Myosin ATPase activity, alter myofilament Ca^{2+} sensitivity	(Jideama, Noland et al. 1996; Montgomery, Chandra et al. 2001; Sumandea, Vahebi et al. 2009)
Ser_{208}	**N**	**N**	ASK I	Reduce cardiomyocyte contractility	(He, Liu et al. 2003)
Thr_{213}	**C**	**C**	PKC	Reduce Myosin ATPase activity, myofilament force production and Ca^{2+} sensitivity	(Noland, Raynor et al. 1989; Jideama, Noland et al. 1996; Sumandea, Pyle et al. 2003; Sumandea, Burkart et al. 2004; Sumandea, Vahebi et al. 2009)
Thr_{213}	**C**	**C**	Raf-1	Unknown	(Pfleiderer, Sumandea et al. 2009)
Ser_{285}	**N**	**C**	PKC	Reduce Myosin ATPase activity, myofilament force production and Ca^{2+} sensitivity	(Sumandea, Vahebi et al. 2009)
Ser_{285}	**N**	**C**	ROCK-II	Reduce myofilament force development, Myosin ATPase activity and Ca^{2+} sensitivity	(Vahebi, Kobayashi et al. 2005)
Thr_{294}	**N**	**N**	PKC	Reduce Myosin ATPase activity, myofilament force production and Ca^{2+} sensitivity	(Noland, Raynor et al. 1989; Jideama, Noland et al. 1996; Montgomery, Chandra et al. 2001; Sumandea, Vahebi et al. 2009)
Thr_{294}	**N**	**N**	ROCK-II	Reduce myofilament force development, myosin ATPase activity and Ca^{2+} sensitivity	(Vahebi, Kobayashi et al. 2005)

Amino acid residues in cardiac TnT identified with phosphorylation regulations are summarized. The residue numbers are that in the sequence of human cardiac TnT with the first methionine counted. The phosphorylation of cardiac TnT at each of the residues is compared with its counterparts in fast TnT and slow TnT. C, conserved and N, non-conserved phosphorylatable residue in skeletal muscle TnT. Kinases responsible for each phosphorylation, potential functional effects, and references are also summarized.

Table 3

Representative TnT gene abnormalities found in cardiac and skeletal myopathies

TnT abnormality	Phenotype	References
Cardiac TnT (*TNNT2*)		
Ile79Asn	HCM	(Thierfelder, Watkins et al. 1994; Lin, Bobkova et al. 1996; Palm, Graboski et al. 2001)
Arg92Gln	HCM	(Thierfelder, Watkins et al. 1994; Marian, Zhao et al. 1997)
Arg92Leu	HCM	(Forissier, Carrier et al. 1996; Palm, Graboski et al. 2001)
Arg92Trp	HCM	(Moolman, Corfield et al. 1997; Fujino, Shimizu et al. 2001; Shimizu, Ino et al. 2003)
Arg94Leu	HCM	(Varnava, Baboonian et al. 1999; Palm, Graboski et al. 2001)
Arg94Cys	HCM	(D'Cruz, Baboonian et al. 2000)
Ala104Val	HCM	(Nakajima-Taniguchi, Matsui et al. 1997)
Arg130Cys	HCM	(Koga, Toshima et al. 1996)
ΔGlu160	HCM	(Harada, Takahashi-Yanaga et al. 2000)
Glu163Arg	HCM	(Koga, Toshima et al. 1996)
Glu163Lys	HCM	(Watkins, McKenna et al. 1995)
Ser179Phe	HCM	(Van Driest, Ackerman et al. 2002)
Glu244Asp	HCM	(Watkins, McKenna et al. 1995)
Exon 17 deletion (Δ14)	HCM	(Thierfelder, Watkins et al. 1994)
Exon 16–17 deletions (Δ28+7)	HCM	(Thierfelder, Watkins et al. 1994)
Phe110Ile	DCM	(Watkins, McKenna et al. 1995; Nakaura, Yanaga et al. 1999)
Arg141Trp	DCM	(Mogensen, Murphy et al. 2004; Mirza, Marston et al. 2005)
Arg141Trp	DCM	(Li, Czernuszewicz et al. 2001; Lu, Morimoto et al. 2003)
Arg205Leu	DCM	(Mogensen, Murphy et al. 2004)
ΔLys210	DCM	(Kamisago, Sharma et al. 2000; Hanson, Jakobs et al. 2002; Hershberger, Pinto et al. 2009)
Glu244Asp	DCM	(Nakaura, Yanaga et al. 1999)
Asp270Asn	DCM	(Robinson, Griffiths et al. 2007)
Lys273Glu	DCM	(Fujino, Shimizu et al. 2002)
Arg278Cys	DCM	(Watkins, McKenna et al. 1995; Morimoto, Nakaura et al. 1999)
ΔE96	RCM	(Peddy, Vricella et al. 2006; Pinto, Parvatiyar et al. 2008)
E136K	RCM	(Kaski, Syrris et al. 2008)
Slow skeletal muscle TnT (*TNNT1*)		
ΔExon8	NM	(van der Pol, Leijenaar et al. 2014)
S108X	NM	(Marra, Engelstad et al. 2015)
Glu180X	NM	(Johnston, Kelley et al. 2000; Jin, Brotto et al. 2003)
L203X	NM	(Abdulhaq, Daana et al. 2015)
ΔExon14	NM	(van der Pol, Leijenaar et al. 2014)
Fast skeletal muscle TnT (*TNNT3*)		
Arg63Cys	DA	(Zhao et al. 2011)
Arg63His	DA	(Dale et al. 2014)

Representative mutations in human cardiac TnT that cause hypertrophic (HCM), dilated (DCM) and restrictive (RCM) cardiomyopathies, and mutations in human slow and fast skeletal muscle TnT that cause nemaline myopathy (NM) and distal arthrogryposis (DA) are summarized.