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Consequences of Mutations in Genes Encoding Cardiac Troponin C, T and I – Molecular Insights

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1. Introduction

Cardiac troponin is the main regulatory protein of the thin filament and mediates the Ca^{2+}-sensitivity of the actin-myosin interaction. Troponin forms a heterotrimeric complex composed of the tropomyosin binding subunit (cTnT), the inhibitory subunit (cTnI) and the Ca^{2+}-binding subunit (cTnC). A complex interplay between the cardiac troponin subunits and other thin filament proteins, as tropomyosin (Tm) and actin, is essential to regulate muscle contraction, which can be described by cross bridge cycling on the molecular level. Troponin is located on both sides of the thin filament with a stagger of about 27 Angstroms between two adjacent troponin molecules (Ebashi, 1972; Paul et al., 2009). In the thin filament each troponin binds to one tropomyosin, which covers 7 actin monomers. It is no surprise that mutations in genes encoding proteins, which participate in crossbridge cycling and its regulation, derange interactions and lead to contractile dysfunction and disease. In all three cardiac troponin subunits, changes in amino acid sequence have been identified in families with hypertrophic (HCM), restrictive (RCM) and dilated cardiomyopathy (DCM). Therefore knowledge of structure, function and interactions of the proteins is a prerequisite to understand dysfunction in disease.

1.1 Cardiac troponin T (cTnT)

One of the main tasks of cardiac troponin T (30-35kDa) is to fix the troponin complex to the thin filament. Furthermore cTnT participates in conferring calcium sensitivity to actin/myosin (Tobacman, 1988). Tobacman also showed that the N-terminal half of cTnT, TnT1 (amino acids 1-158, skeletal muscle numbering), was able to keep the thin filament in the blocked state without TnI. In the blocked state of the thin filament no interaction between actin and myosin is possible, i.e. no force production occurs. Thus cTnT plays an active role in inhibition of actin/myosin interaction in the resting state. It further promotes tropomyosin polymerization and binding of tropomyosin to actin.
Structural information on cTnT is poor. According to EM analysis of thin filaments and low resolution co-crystallization of Tm in complex with cTnT, cTnT is highly asymmetric. It is a 180-202 nm long comma –shaped molecule, with the N-terminal rod like part arranged along the thin filament and a C-terminal more globular domain (Ohtsuki, 1979, Flicker et al., 1982, White et al., 1987). The high resolution crystal structure available for the core troponin complex contains only the less flexible C-terminal part of cTnT, which binds the other two

troponin subunits, cTnI and cTnC (Takeda et al., 2003). There is strong evidence from latest single particle reconstruction studies of the thin filament by Paul et al., (2009), that the N-terminal tail of cTnT points to the M-band of the sarcomere, whereas the core domain of the troponin complex is oriented versus the Z-band.

The N-terminal tail fraction of cTnT contains a hypervariable N-terminal part and a highly conserved region, which is located in the central region of the cTnT molecule (Fig. 1). This conserved region contains the main interaction site for tropomyosin (Biesiadecki et al., 2007, Perry, 1998) comprising 39 amino acids (residues 98-136 (human cardiac sequence)) (Jin & Chong, 2010). The Tm interaction site forms a helix according to Murakami et al., (2008) and binds to the overlapping region of two tropomyosin molecules (Jin & Chong, 2010). Crystal structure of the tropomyosin overlap region and the Tm binding helix reveals the formation of a four helix bundle between tropomyosin ends and cTnT (Murakami et al., 2008). The hypervariable region of the cTnT tail fraction does not bind to tropomyosin and can be truncated without losing binding ability of the rest of the molecule (Zhang et al., 2006). Phylogenetically the hypervariable region might be added to the conserved core region of cTnT (Conserved tail region and C-terminal domain) (Jin & Samanez, 2001; Biesiadecki et al., 2007). Its function is not completely elucidated, but the hypervariable region may play a role as modulator of the core molecule thus subtly affecting binding affinities of Tm and cTnT (Biesiadecki et al, 2007; Feng et al., 2008).

A second interaction site for tropomyosin is located at the beginning of the C-terminal half of cTnT and has lately been analysed by Jin & Chong (2010) using antibodies. They showed that highly conserved amino acid sequences are involved comprising amino acid residues 197-236 (human sequence). This interaction site binds to the middle part of tropomyosin near Cys190. Earlier studies (Pearlstone et al., 1983; Morris & Lehrer, 1984) propose the second interaction with tropomyosin at the very C-terminus of cTnT involving residues 272-288. Whichever amino acids are included in the cTnT/Tm interactions, in the presence of cTnC binding of the TnT-C-Terminus to tropomyosin near Cys190 is Ca^{2+} dependent (Chong & Hodges, 1982). Ca^{2+} weakens its binding to tropomyosin though the molecular mechanism, i.e. conformational changes which lead to alteration in Tm/TnT -binding, is not known yet. The TnT-C-Terminus also contains the binding site for the other two troponin subunits, TnI and TnC. According to the 3 D structure of the core cardiac troponin complex, the helix in the C-terminal region of cTnT (residues 226-271), which is highly conserved (Jin et al., 2008) forms a rigid coiled coil with a cTnI-helix (residues 90-136) and is part of a rigid structure within the troponin complex, called the IT-arm (Takeda et al., 2003). At the C-terminal end of the coiled coil the interaction site for cTnC is located and comprises amino acid residues 256- 270. The organisation of cTnT is summarized in Fig.1.

In cardiac muscle the mammalian cTnT- gene (TNNT2) is composed of 17 exons. Exon 5 is absent in adult cTnT (Cooper & Ordahl, 1985). Exon 5 encodes a 10 amino acid long region within the hypervariable N-terminus of cTnT. This sequence contains several acidic residues and contributes to a more negatively charged cTnT. Such a charge difference may modulate function. Indeed fetal cTnT, which contains exon 5, exhibits a higher Ca^{2+}-sensitivity compared to adult cTnT and shows a higher tolerance towards acidic pH (Gomes et al., 2002). Mainly 4 variants of human cTnT ($cTnT_{1-4}$) have been described due to alternative splicing of exons 4 and 5, whereby TnT_1 and TnT_3 are the major isoforms present in fetal and in the adult human cardiac muscle, respectively (Townsend et al., 1995; Anderson et al., 1991). The expression pattern of cTnT isoforms seems to be altered in heart failure and correlates with changes in Ca^{2+}- sensitivity. The modification in Ca^{2+}- sensitivity however, seems not to be caused by an

alteration in the isoform expression pattern. Differences in the phosphorylation status of sarcomeric proteins, especially of myosin light chain 2 (MLC-2) may be decisive (van der Velden et al., 2003). Also cTnT itself is a phospho protein (Fig. 1) which is constitutively phosphorylated at Ser1 in several species inclusive men due to the action of casein kinase- 2 (Gusev et al., 1980; Risnik & Gusev, 1984, Swiderek et al., 1990). The function of this phosphorylation is not known up to date. It might prevent degradation and/or interaction of the hypervariable region. At least one further reversible phosphorylation site for PKC is located in the C-terminal region of cTnT near the second Ca^{2+}- dependent interaction site for Tm and thus may affect Ca^{2+}- sensitivity of the actin/myosin interaction. Indeed, according to Sumandea et al., reversible phosphorylation of Thr206 (mouse sequence) is critical for function (Sumandea et al., 2003). I*n vitro* experiments showed that phosphorylation by PKCα decreases maximal tension, myofilament Ca^{2+} -sensitivity, actomyosin ATPase activity and cooperativity (Sumandea et al., 2003). Other protein kinases than PKC, as for example ROCKII (Vahebi et al., 2005), phosphorylate cTnT *in vitro*. The physiological role of cTnT phosphorylation by different protein kinases is not yet clarified. cTnT, however, is not a target of cAMP dependent protein kinase (PKA), which is activated upon ß-adrenergic stimulation. But as recently described by Sumandea et al., (2011) cTnT forms an AKAP for PKA with either regulatory subunit I or II (PKA-RI, PKA-RII) and thus provides a platform for sarcomeric protein phosphorylation upon ß-adrenergic stimulation. Binding of PKA might occur within the amino acid region 202-226 which forms an amphiphilic helix needed for the docking of PKA RI and—II (Feliciello et al., 2001). The interaction site for PKA-R would then be located just near the second interaction site with tropomyosin described by (Jin & Chong, 2010) and the PKC phosphorylation site. This implies that PKA-R binding might be affected by PKC phosphorylation and vice versa.

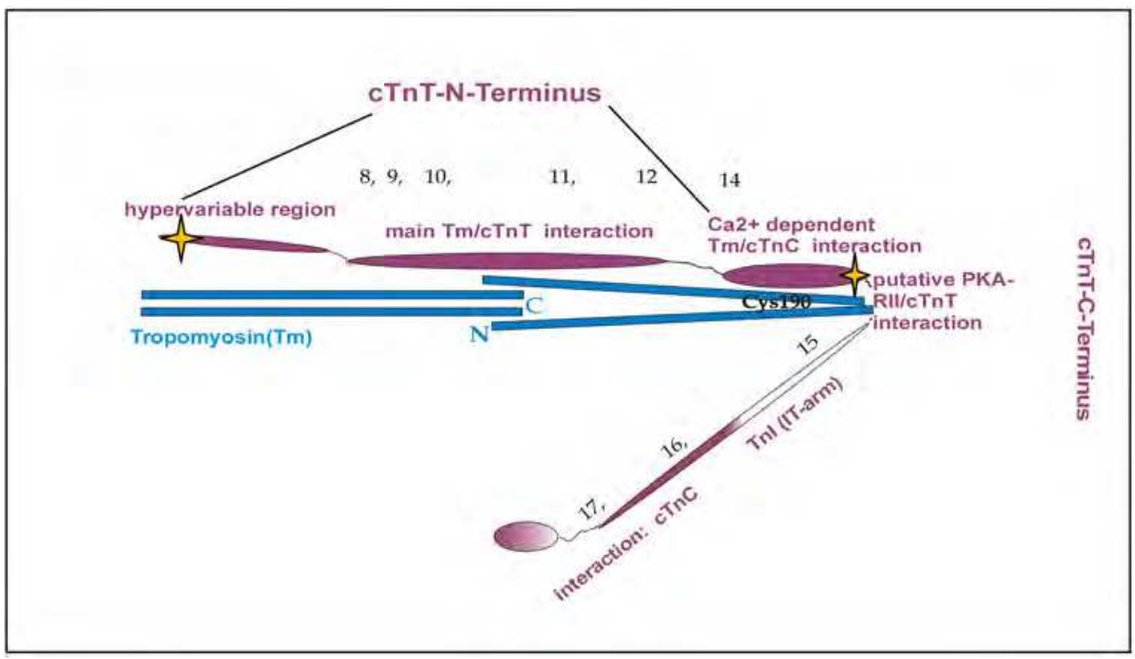


Fig. 1. Organisation , interaction and phosphorylation sites of cardiac troponin T. Exons known to contain cardiomyopathy mutations are indicated by numbers

1.2 Cardiac troponin C (cTnC)

cTnC is the calcium binding subunit of the cardiac troponin complex. Structurally the protein belongs to the EF-hand calcium binding protein super family together with parvalbumin, the first described protein of this family, Calmodulin, skeletal muscle troponin C etc. cTnC is composed of two lobes connected by a flexible linker (Sia et al., 1997) (Fig.2).

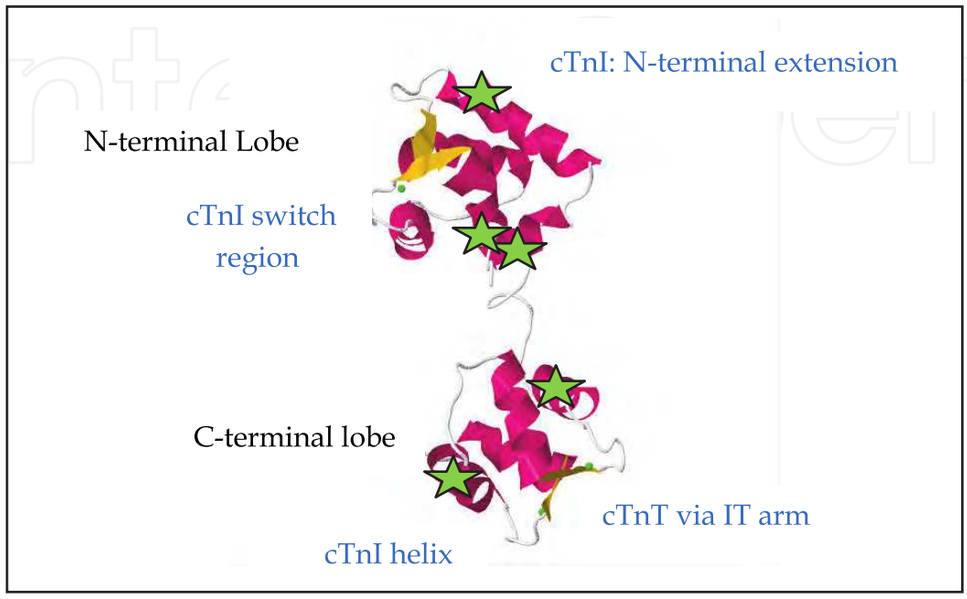


Fig. 2. Interaction sites and cardiomyopathy inducing single amino acid exchanges in cTnC

The structure was taken from PDP 1AJ4 based on the work of (Sia et al., 1997). The 3 D structure of cTnC in calcium (green points) saturated from is shown. Helices are given as magenta ribbons. The position of cardiomyopathy mutations is indicated by stars. Interacting proteins are given in blue.

Each lobe contains two parallel EF hands, each of which forms the helix-loop-helix divalent metal binding domain. Helices in a protein have been labeled by capital letters, with A assigned to the most N-terminally located helix. Thus E and F are the loop flanking helices in parvalbumin forming the metal binding motif. The name EF-hand for the helix loop helix metal binding motif is based on this nomenclature. The short helices of about 10-12 residues are arranged perpendicular. The loop is composed of 12 residues essential for calcium coordination in a pentagonal bipyramidal configuration. The residues 1, 3, 5, 7, 9 and 12 (X, Y, Z, -Y, -X, -Z) are involved in Ca^{2+}-coordination. In position 12 there is a conserved glutamate or aspartate residue providing two oxygens for calcium binding (Structure Reference: PDB: 2PMY). The calcium binding residues preferentially have an acidic side chain, but also the protein backbone is involved. In cTnC the two EF hands (III, IV) in the C-terminal lobe are the high affinity Ca^{2+}-, Mg^{2+}-binding sites, which contain metal ions also at low (relaxing) intracellular Ca^{2+}-concentration. This C-terminal domain of cTnC provides the platform for binding of cTnI and of cTnT and therefore is pivotal for the integrity of the troponin complex. Helices of the metal bound C-terminal domain exhibit a hydrophobic pocket, where cTnI is bound (Gasmi-Seabrook et al., 1999). cTnT binds to Calcium sites III and IV at the end of the rigid coiled coil. Besides the structural role of the C-terminal lobe, there is now strong evidence that it plays an active part in thin filament activation (Fuchs &

Grabarek, 2011). Alteration in Ca^{2+}-/Mg^{2+}-binding to sites III and IV might alter the interaction with cTnT and the coiled coil structure. Also the identification of cardiomyopathy causing alterations in this part of cTnC points to the involvement in regulation.

The N-terminal EF-hand I is not able to bind Ca^{2+} due to an insertion of Val and the replacement of two Asp residues involved in Ca^{2+}-coordination by Ala and Leu residues. Therefore there is only one functional active Ca^{2+}-binding site (site II) in the N-terminal domain of cTnC, which is a high affinity Ca^{2+}-specific binding site. Binding and release kinetics of Ca^{2+} to cTnC within the thin filament are such that Ca^{2+}-binding and release occurs within one contraction cycle (Davis & Tikunova, 2008). Saturation of cTnC with Ca^{2+} is obtained upon increase in intracellular Ca^{2+}-concentration after influx from sarcoplasmatic Ca^{2+}-store upon membrane depolarization. Therefore site II is named the regulatory Ca^{2+}-binding site. Due to the non functional site I there is no conformational switch from closed to open solely upon Ca^{2+} -binding as is observed for skeletal muscle TnC and the C-terminal lobe of cTnC (Sia et al., 1997). For the stabilization of the open conformation of the N-terminal lobe cTnI binding is required (Dong et al., 1999; Li et al., 1999). There exist multiple interaction sites for cTnI throughout the complete cTnC molecule. The amphiphilic part of helix 1 in cTnI (residues 43-65) binds via several polar and van der Waals interaction to the C-terminal cTnC lobe and around residue 10 of the N-terminal helix of cTnC. Furthermore residues 93-161 in the C-terminal lobe of cTnC interact with the IT-arm (Takeda et al., 2003), The N-terminal lobe of cTnC forms the Ca^{2+}-dependent binding site for the cTnI switch region (Takeda et al., 2003) and a phosphorylation dependent binding site near the nonfunctional Ca^{2+}-binding loop with the flexible heart specific N-terminal cTnI extension (see below) (Schmidtmann et al., 2005).

1.3 Cardiac troponin I (cTnI)

cTnI, the inhibitory subunit of the troponin complex is a very flexible molecule consisting of helices and random coils. In solution cTnI exhibits no tertiary structure. A specific spatial orientation is only obtained within the ternary troponin complex. cTnI is build up in a modular fashion (Fig. 3).

1.3.1 The N-terminal extension

The N-terminal extension of about 31 amino acids (length is dependent on species) is heart specific and resembles the hypervaraible region of cTnT. It contains conserved amino acid stretches; thus at the very N-terminus there is a acidic region (Sadayappan et al., 2008) followed by a proline rich sequence, which forms a polyprolin helix and functions as a rigid spacer to keep the N-terminus extended (Howarth et al., 2007). Then the phosphorylation region follows, which contains two adjacent located serine residues at position 22 and 23 (numbered without starter methionine). Both residues are substrates for PKA (Swiderek et al., 1990; Mittmann et al., 1990). In its dephosphorylated state the N-terminal cTnI arm interacts between residues 10-30 with cTnC around amino acid residue 29 (Fig. 2) (Finley et al., 1999; Gaponenko et al., 1999; Abbott et al., 2000; Ward et al.,2003, 2004; Schmidtmann et al., 2002, 2005). This interaction also seems to stabilize the open conformation of the cTnC-N-terminal lobe (Abbott et al., 2000, 2001; Ward et al., 2004). Bisphosphorylation of the two serine residues 22 and 23 by PKA upon ß- adrenergic stimulation releases the interaction with cTnC. According to Howarth et al, (2007) the bisphosphorylated arm contains a helix

comprising amino acids 21-30 being stabilized by salt bridges between phosphate and preceeding arginine residues (Jaquet et al. 1998). The release of the extension from cTnC takes place due to the insertion of negative charges followed by conformational changes. This allows a different interaction, which is directed by the acidic part of the N-terminal cTnI arm and therefore needs a positively charged partner (Sadayappan et al., 2008). Clusters of basic amino acid residues are provided by the regulatory C-terminal domain of cTnI itself, but also an additional interaction with actin cannot be excluded. The release of the N-terminal arm from cTnC leads to a reduction of the Ca^{2+} -affinity of cTnC, in myofilament Ca^{2+}-sensitivity (Zhang et al 1995; Reiffert et al., 1996) and to enhanced cross bridge cycling (Kentish et al.,2001; Turnbull et al., 2002). There is evidence that the main action occurs via interaction with cTnI, which stabilizes cTnI binding to the thin filament (Sakthivel et al., 2005).

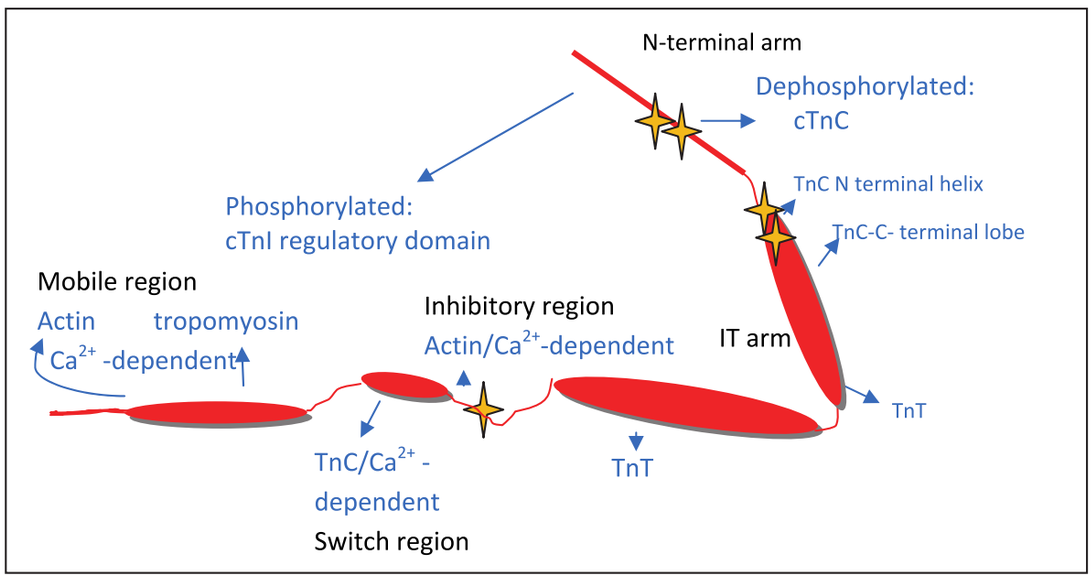


Fig. 3. Organization of cTnI, its interactions (blue) and phosphorylation sites (indicated by ✦)

1.3.2 Constitutive cTnC and cTnT binding sites

The cTnC binding site is located subsequent to the N-terminal extension. It forms a helix (helix 1 according to the nomenclature of Takeda et al., 2003) which contacts the N-terminal cTnC helix and reaches to the C-terminal lobe of cTnC. It strongly binds to the hydrophobic pocket of the C-terminal cTnC lobe. The C-terminal part of helix 1 also interacts with cTnT via several hydrogen bonds and hydrophobic interactions. This helix is followed by another helical binding site for cTnT (helix 2). Helix 2 forms a coiled coil with a helix located in the C-terminal half of cTnT. The two helices are part of the IT arm (Takeda et al., 2003). Both these binding sites in cTnI are independent on reversible Ca^{2+} -binding to the regulatory Ca^{2+} binding loop in cTnC. In helix 1 there are two serine residues (Ser43/45) which are phosphorylated by PKC upon alpha adrenergic stimulation. Ser43/45 are positioned near residue 10 of the N-terminal helix in cTnC-N-terminal lobe as well as near the C-terminal lobe. Thus, phosphorylation might alter these interactions. In mice phosphorylation of these sites by PKC upon α-adrenergic stimulation is responsible for the negative inotropic effect and might be influenced by the cTnT phosphorylation state. The physiological effect of PKA

dependent phosphorylation at Ser22/23 seems not to be impaired by Ser43/45 phosphorylation (Montgomery et al., 2002).

1.3.3 Regulatory C-terminal region

The C-terminal region of cTnI can functionally be subdivided into the inhibitory region comprising amino acids 137-148, the helical switch region (amino acids 150-159) and the C-terminal mobile region. The inhibitory region binds to actin/tropomyosin in the relaxed state, i.e. when the regulatory Ca^{2+} binding loop of cTnC contains no Ca^{2+}. Within this region there is another heart specific PKC phosphorylation site at Thr 144. The physiological role of this phosphorylation site is not quite clear (Solaro & Kobayashi, 2011), though investigations by Tachampa et al., (2007) imply that Thr144 is involved in length dependent activation of tension development in thin filament bundles. Phosphorylation might modulate this function probably by loosening the interaction of cTnI inhibitory region with actin.

Upon Ca^{2+}- saturation of cTnC the inhibitory region is released from actin and the switch region binds to the cTnC-N-terminal lobe inducing the formation of the hydrophic pocket. Strength of interaction is sensible to small conformational changes in the cTnC-N-lobe also affecting Ca^{2+}-binding affinity. The C-terminal mobile region following the switch region is a very important cTnI region for regulation of muscle contraction, though not much is known about this region. It provides a second actin binding site and a tropomyosin binding site. Under relaxing conditions, this mobile region is fixed to tropomyosin (Pirani et al., 2005; Galinska et al., et al., 2008) and actin, and is released from actin/tropomyosin upon Ca^{2+}-saturation of cTnC. Thus the C-Terminus stabilizes the blocked state of the thin filament (Galinska et al., 2010). Probably this effect is intensified by the interaction of cTnT binding to tropomyosin actin. Thus cTnT- N-terminus and cTnI-C-terminus both support the inhibitory region of cTnI in keeping tropomyosin in the blocking position. Hereby troponin complexes on opposite sites cooperate, one complex providing cTnT/tropomyosin interaction the other cTnI/tropomyosin/actin interaction (Paul et al., 2009; Solaro & Kobayashi, 2011). But cardiac TnI is not only involved in regulation of inhibition, but also of activation. Evidence came from investigations of Galinska et al., (2010) using truncated cTnI and normal length cTnI. They showed that the C-terminus is also involved in stabilization of tropomyosin in the active state (Ca^{2+} - saturated cTnC). Truncation may occur *in vivo* due to proteolysis in myocardial stunning (reversible ischemia/reperfusion injury) (Foster et al., 2003) and due to cardiomyopathy mutations.

2. Cardiomyopathy inducing troponin mutations

A large number of mutations have been detected in genes encoding for the three cTn subunits in patients suffering of cardiomyopathies (tables 1-6). Resulting phenotypes are highly variable, even within a family carrying the same mutation (see below), indicating that modifiers, environment or polymorphisms are involved in disease development. Wang et al., (2005) was the first group who detected a polymorphism in the MYBPC3 (cardiac myosin binding protein C gene) that might be able to modify the expression of hypertrophy. Also combinations of more than one mutation determine the disease development. Therefore it is still impossible to correlate phenotype and genotype, though an immense progress has been made in the understanding of molecular pathogenesis. The goal to understand phenotype development remains. Since the disease is primarily caused by

mutations, it is crucial to improve knowledge on the dysfunctions on molecular level in detail of as many mutants as possible. Thus one might be able to detect common features and mechanisms which might allow detection of a link to phenotype development. Though the molecular effects of only a couple of mutations have been thoroughly investigated a first common rule, namely an enhancement in Ca^{2+}- sensitivity of the myofilament for HCM/RCM-mutants and a decrease in Ca^{2+}- sensitivity for DCM mutants has been stated by Robinson et al., (2007). However it seems to be too simple and does not explain the development of HCM or RCM or the often very low degree of hypertrophy despite large Ca2+ sensitivity changes and susceptibility to malignant arrhythmia. Furthermore, the rule does not apply to all mutations investigated. One problem is that analysis of Ca^{2+} -sensitivity resulted in many opposing statements. Thus for example Nakaura et al., 1999 did not observe enhanced Ca^{2+} -sensitivity of force in skinned fibres with cTnT-F110I, whereas Hernandez et al., 2005 described enhanced Ca^{2+} -sensitivity of force development as well as of actomyosin ATPase activity. One main reason lies in the complexity of the systems used, as reconstituted proteins, skinned fibers, myofibrils, isolated cardiomyocytes (adult or neonatal), transgenic animals. In general results obtained with higher organised system sseem to be more reliable. But additionally species differences might account for differing results. For example according to Rust et al., (1999) overexpression of cTnT-I79N in rat cardiomyocytes resulted in suppressed contractile performance, whereas others using mice myofibrils suggested hypercontractility.

2.1 Mutations in TNNT2, the gene encoding cTnT

Most mutations in TNNT2 detected in patients lead to familial hypertrophic cardiomyopathy (HCM), only a few to restricted cardiomyopathy (RCM) or dilated hypertrophic cardiomyopathy (DCM) (Table 1). Families with DCM mutations in TNNT2 mostly exhibit a severe disease progression with poor prognosis.

2.1.1 HCM inducing mutations

HCM-mutations in TNNT2 are found in about 10% of the HCM cases, and thus TNNT2 belongs to the more abundant troponin disease genes. There is no known HCM mutation which is located within the hypervariable N-terminal region of cTnT. All mutations identified in patients suffering from HCM are located either near or within the N-terminal main interaction site for tropomyosin (amino acids 79-182) or within the C-terminal half (amino acids 203-288) which contains multiple interaction and putative phosphorylation sites (Table 1; Fig. 1). This distribution of mutations implies that they affect the interaction either with tropomyosin (Tm) and/ or the other troponin subunits and might influence phosphorylation dependent effects. The majority of gene loci, where mutations have been identified in patients encode the N-terminal region of cTnT interacting with the overlap of Tm (Table 1; Fig. 1). Mutations in the Tm binding region of cTnT (amino acids 92-183) destabilize Tm binding to actin filaments (Palm et al., 2001). Mutations within the main Tm binding site further weaken the end-to-end Tm interaction, which is responsible for the cooperativity (Palm et al., 2001). Also a lowered affinity of troponin to actin/Tm could be expected. Indeed cTnT-F110I, located at the C-terminal end of the main Tm binding region, impairs binding of troponin to actin/Tm by altering the dynamic properties of the tail region (Hinkle & Tobacman, 2002). It reduces its flexibility. Flexibility of the cTnT-tail is an important feature for its interaction with Tm overlap region (Hinkle & Tobacman, 2002).

Mutation	Disease	Exon/Intron	Reference
Phe70Leu (F70L)	HCM	8	Richard et al., (2003) Circ. 107: 2227-32
Pro77Leu (P77L)	HCM	8	Varnava et al., (2001) Circ. 104: 1380-4
Ile79Asn (I79N)	HCM , RCM, DCM	8	Thierfelder et al., (1994) Cell 77:701-12; Watkins et al., (1995) NEJM. 332: 1058-64; Rust et al., (1999) JCI. 104: 1459-67; Yanaga et al., JBC. (1999) 74:8806-12; Varnava et al., (2001) Circ. 104: 1380-4 ; Palm et al., (2001) Biophys J. 81: 827-37 ; Westermann et al., (2006) Eur J Heart Fail. 8:115-21 ; Menon et al., (2008) Clin Genet, 74(5): 445-54 ; Baudenbacher et al.,(2008) JCI. 118 :3893-903 ; Midde et al., (2011) JMCC [Epub ahead of print]
Glu83Lys (E83K)	HCM	8	Mogensen et al., (2003) J Med Genet 40: e59
Val85Leu (V85L)	HCM	8	Konno et al., (2005) J Intern Med. 258: 216-24.
Asp86Ala (D86A)	HCM	8	Van Driest et al., (2003) Circ. 108: 445-51
Arg92Trp (R92W)	HCM	9	Moolman et al., (1997) JACC. 29: 549-55; Moolman-Smook et al., (1999) Am J Hum Genet. 65:1308-20; Fujino et al., (2001) Clin Cardiol. 24: 397-402; Varnava et al., (2001) Circ. 104:1380-4; Palm et al., (2001) Biophys J. 81:2827-37; Waldmuller et al., Hum Mutat (2002) 19:560-9; Ackerman et al., (2002) JACC. 39: 2042-8; Van Driest et al., (2003) Circ. 108: 445-51; Shimizu et al., (2003) Clin Cardiol 26: 536-9; Konno et al., (2005) J Intern Med. 258:216-24
Arg92Leu (R92L)	HCM	9	Forissier et al., (1996) Circ. 94: 3069-73 ; Varnava et al., (2001) Circ. 104: 1380-4 ; Palm et al., (2001) Biophys J. 81:2827-37 ; Richard et al., (2003) Circ. 107: 2227-32
Arg92Gln (R92Q)	HCM	9	Thierfelder et al., (1994) Cell 77:701-12; Watkins et al., (1995) NEJM. 332:1058-64; Yanaga et al., (1999) JBC. 274: 8806-12; Palm et al., (2001) Biophys J. 81:2827-37 ; Cuda et al., (2002) Hum Mutat 19:309-10; Robinson et al., (2002) JBC. 277: 40710-6; Hinkle & Tobacman (2003)JBC. 278: 506-13; Javadpour et al., (2003) JCI. 112: 768-75; Torricelli et al., (2003) Am J Cardiol 92:1358-62; Van Driest et al., (2004) JACC. 44: 1903-10
Arg94Leu (R94L)	HCM	9	Varnava et al., (1999) Heart 82: 621-4; Varnava et al., (2001) Circ. 104:1380-4 ; Palm et al., (2001) Biophys J. 81:2827-37
Arg94Cys (R94C)	HCM	9	Mogensen et al., (2003) J Med Genet. 40: e59
Lys97Asn (K97N)	HCM	9	Barr, Seidman et al., (2001) originally posted on URL: http://www.cardiogenomics.org
Ala104Val (A104V)	HCM	9	Nakajima-Taniguchi et al., (1997) JMCC. 29: 839-43; Palm et al., (2001) Biophys J. 81: 2827-37 ; Hinkle & Tobacman (2003) JBC. 278: 506-13.

Mutation	Disease	Exon/Intron	Reference
Phe110Ile (F110I)	HCM	9	Watkins et al., (1995) NEJM. 332: 1058-64; Anan et al., (1998) Circ. 98: 391-7; Yanaga et al., (1999) JBC. 274: 8806-12; Lin et al., (2000) Cardiol. 93:155-62 ; Palm et al., (2001) Biophys J. 81: 2827-37 ; Hinkle & Tobacman (2003) JBC. 278: 506-13; Konno et al., (2005) J Intern Med. 258: 216-24; Hernandez et al., (2005) JBC. 280: 37183-94
Phe110Leu (F110L)	HCM	9	Torricelli et al., (2003) Am J Cardiol. 92: 1358-62
Phe110Val (F110V)	HCM	9	Richard et al., (2003) Circ. 107: 2227-32 ; Torricelli F et al., (2003) Am J Cardiol. 92: 1358-62
Lys124Asn (K124N)	HCM	9	An et al., (2004) Zhonghua Xue Za Zhi 84:1 340-3
Arg130Cys (R130C)	HCM	10	Torricelli et al., (2003) Am J Cardiol. 92: 1358-62 ; Song et al., (2005) Clin Chim Acta 351: 209-16
Glu163Lys (E163K)	HCM	11	Watkins et al., (1995) NEJM. 332: 1058-64; Palm et al., (2001) Biophys J. 81: 2827-37
Glu160del (ΔE160)	HCM	11	Watkins et al., (1995) NEJM. 332: 1058-64; Palm et al., (2001)Biophys J. 81: 2827-37 ; Richard et al., (2003) Circ. 107: 2227-32 ; Mogensen et al., (2003) J Med Genet. 40:e59; Torricelli et al., (2003) Am J Cardiol. 92:1358-62; Capek & Skvor (2006) Meth Inf Med. 45: 169-72
Ser179Phe (S179F)	HCM	11	Ho et al., (2000) Circ. 102:1950-5
Glu244Asp (E244D)	HCM	14	Watkins et al., (1995) NEJM. 332: 1058-64; Yanaga et al., (1999) JBC. 274: 8806-12; Moore, Seidman et al., (2004) URL: http://www.cardiogenomics.org
Lys247Arg (K247R)	HCM	14	Garcia-Castro et al., (2003) Clin Chem. 49: 1279-85
Asn271Ile (N271I)	HCM	15	Richard (2003) Circ. 107:2227-32
Lys273Glu (K273E)	HCM	15	Fujino et al., (2002) Am J Cardiol. 89:29-33; Venkatraman et al., (2003) JBC 278: 41670-6; Konno et al., (2005) J Intern Med. 258: 216-24
IVS15+1G>A	HCM	15	Thierfelder et al., (1994) Cell 77: 701-12; Watkins et al., (1995) NEJM. 332: 1058-64; Watkins et al., (1996) JCI. 98: 2456-61. ; Mukherjea et al., (1999) Biochem. 38: 13296-301; Redwood et al., (2000) Circ Res. 86: 1146-52; Varnava et al., (2001) Circ. 104: 1380-4

Mutation	Disease	Exon/Intron	Reference
Arg278Cys (R278C)	HCM	16	Watkins et al., (1995) NEJM. 332:1058-64; Yanaga et al., (1999) JBC. 274:806-12; Elliott et al., (1999) NEJM. 341: 1855-6; Barr, Seidman et al., 2002 and Moore, Seidman et al., (2003, 2004) URL:http://www. cardiogenomics.org; Van Driest et al., (2003) Circ. 108: 445-51; Garcia-Castro et al., (2003) Clin Chem 49: 1279-85; Garcia-Castro et al., (2003) Rev Esp Cardiol. 56: 1022-5; Torricelli (2003) Am J Cardiol 92: 1358-62; Theopistou et al., (2004) Am J Cardiol 94: 246-9; Miliou et al., (2005) Heart 91: 966-7; Hernandez et al., (2005) JBC. 280:371 83-94; Ingles et al., . (2005) J Med Genet. 42:e59, Sirenko et al., (2006) J Physiol. 5755.1: 201-13
Arg278Pro (R278P)	HCM	16	Erdmann et al., 1998. (on-line); Van Driest et al., (2003) Circ. 108: 445-51; Miliou et al., (2005) Heart 91: 966-7
Arg286Cys (R286C)	HCM	16	Richard et al., (2003) Circ. 107: 2227-32 ; Miliou et al., (2005) Heart 91: 966-7
Arg286His (R286H)	HCM	16	Van Driest et al., (2003) Circ. 108: 445-51 ; Van Driest et al., (2004) JACC. 44: 1903-10
Trp287ter (W287ter)	HCM	16	Richard et al., (2003) Circ. 107: 2227-32.
Arg113Trp (R113W)	DCM	9	Mogensen et al., (2004) JACC. 44: 2033-40 ; Mirza et al., (2005) JBC. 280 :28498-506
Arg141Trp (R141W)	DCM	10	Li et al., (2001) Circ. 104:2188-93 ; Venkatraman et al., (2003) JBC. 278: 41670-6 ; Villard et al., (2005) EHJ. 26: 794-803 ; Mirza et al., (2005) JBC. 280 :28498-506
Ala172Ser (A172S)	DCM	11	Stefanelli et al., (2004) Mol Genet Metab. 83: 188-96
Arg205Leu (R205L)	DCM	13	Mogensen et al., (2004) JACC 44: 2033-40; Mirza et al., (2005) JBC. 280 :28498-506
Lys210del (ΔK210)	DCM	13	Kamisago et al., (2000) NEJM. 343: 1688-96; Hanson et al., (2002) J Card Fail. 8 : 28-32 ; Venkatraman et al., (2003) JBC 278: 41670-6 ; Mogensen et al., (2004) JACC 44: 2033-40.
Asp270Asn (D270N)	DCM	15	Mirza et al., (2005) JBC. 280 :28498-506
Glu96del (ΔE96)	RCM	9	Peddy et al., (2006) Pediatrics 117:1830-3; Pinto et al., (2008) JBC. 283:2156-66
Asn100del/Glu101 Del (ΔN100/ΔE101)	RCM	9	Pinto et al., (2011)JBC 286:20901-12 (double mutation)
Glu136Lys (E136K)	RCM	10	Kaski et al., (2008) Heart 94:1478-84

Updated from: Genomics of Cardiovascular Development, Adaptation, and Remodeling. NHLBI Program for Genomic Applications, Harvard Medical School. [*june, 2011 accessed*] and OMIM database. ter designates termination of sequence resulting in a truncated protein, del and Δ are synonyms for a deleted amino acid. The one letter code is given in brackets.

Table 1. Mutations in TNNT2

Since the cTnT- N-terminus contributes to the inhibition of the actin/myosin interaction (Tobacman, 1988), one might assume that also inhibition is affected by mutations. Indeed inhibition of is reduced due to cTnT-F110I (Knollmann & Potter, 2001; Gomes et al., 2004). A similar decrease in inhibition has been described for I79N (Yanaga et al., 1999), though this amino acid exchange is positioned N-terminally of the main Tm interaction site. Nevertheless I79N and F110I exhibit several similarities. Thus, Midde et al., 2011 showed that rigor crossbridges in I79N or F110I containing filaments were disordered, indicating that disruption in thin filament structure may lead to severe contractile dysfunction. I79N, as most investigated HCM mutants, enhances the Ca^{2+}- sensitivity of force development and actomyosin ATPase activity, which might contribute to enhanced contractility and higher energy consumption (Lin et al., 1996; Sweeney et al., 1998; Chandra et al., 2005). Furthermore, mouse hearts with I79N or R92Q could not increase cardiac performance upon ß-adrenergic stimulation (Knollmann et al., 2001; Javadpour et al., 2003), performance of I79N transgenic even worsened upon isoproterenol treatment (Sirenko et al., 2006). Such an effect as well as Ca^{2+} –sensitivity increase has not been observed with a R278C, a mutation located in the cTnT-C-terminus. These findings suggest that the region around amino acids 79 and 92 is important for Ca^{2+} -signal transmission and affects Ca^{2+}-regulation. Furthermore dysfunction is especially prominent under ß-adrenergic stimulation, which might explain the high risc for cardiac sudden death of these mutations. Causality between enhanced Ca^{2+}-sensitivity and the potential for the development of malignant arrhythmias has been shown by Baudenbacher et al., (2008) for I79N. They described altered action potential duration due to the mutation.

Mutations in TNNT2 gene encoding the C-terminal half of cTnT might affect the interaction with tropomyosin at the second Ca^{2+} -dependent binding site, as well as binding to cTnI and cTnC. Most mutations in this part lead either to single amino acid exchanges, deletions of single amino acids or to C-terminally truncated cTnT molecules due to splice site mutations. IVS15+1G>A is a splicing donor mutant which might result in two truncated proteins. In one mutant exon 16 is skipped encoding the C-terminal 14 amino acids, in the other mutants seven amino acids replace the C-terminal 28 amino acids encoded by exon 15 and 16 (Thierfelder et al., 1994). Both mutants are able to form a heterotrimeric troponin complex, however their affinity towards cTnI is drastically reduced (Mukherjea et al., 1999). The impaired interaction with cTnI may be the cause for reduced inhibitory capacity of troponin at low Ca^{2+}-concentrations described by Redwood et al., (2000) and accelerated cross bridge kinetics (Stelzer et al., 2004). Furthermore a reduced binding of troponin to the thin filament has been reported dependent on its regulatory states (Knollmann & Potter, 2001; Burhop et al., 2001), indicating that the C-terminal part of cTnT stabilizes binding of cTn to the thin filament and affects Ca^{2+} -regulation. Increased Ca^{2+}-sensitivity and cooperativity (Nakaura et al., 1999)) and impaired switching off myosin cycling at low Ca^{2+}-concentrations is observed (Burhop et al., 2001). Up to date nearly nothing is known on how cardiomyopathy inducing mutations in TNNT2 influence phosphorylation dependent effects of PKC dependent cTnT or PKA dependent cTnI phosphorylation. According to a study of Nakaura et al., (1999) effects of truncated cTnT were independent on PKA dependent cTnI phosphorylation.

2.1.2 RCM inducing mutations

There are only few RCM mutations detected in TNNT2 (Table 1). Most RCM mutations in genes encoding for cardiac troponin subunits are found in TNNI3 (see below). However,

interestingly I79N, originally listed as HCM mutation, might also cause RCM or DCM even in members of the same family. This indicates that other factors besides the mutation, as for example polymorphisms etc. (see below) are determinant for the development of the specific disease. Also in mice RCM might be evolved by I79N (Table 1). The other two RCM mutations (Table 1), a deletion of an acidic residue (E96del) and replacement of a glutamic acid residue by a basic lysine (E136K) have been detected in children developing RCM with very poor prognosis. E96del poorly inhibited actomyosin activity at low Ca^{2+} -concentrations (Pinto et al., 2008) confirming that the cTnT N-terminus is important for the inhibitory capacity of the cardiac troponin complex. A first double deletion (Table 1) leads to the deletion of two amino acids, located adjacently within the main Tm binding site. In filaments with adult, not fetal cTnT an increase in Ca^{2+}-sensitivity and decrease in cooperativity has been observed so far (Pinto et al., 2011).

2.1.3 DCM inducing mutations

Patients with DCM mutations in cTNNT2 exhibit a malignant prognosis as do also most of the FHC mutations in cTNNT2. Investigations on molecular level are largely missing to date. All patients showed decreased cardiac function (Kamisago et al., 2000) in contrast to enhanced contractility often observed in patients and transgenic animals carrying HCM mutations. In accordance *in vitro* analysis of DCM-mutations revealed a decreased Ca^{2+}-sensitivity (Mirza et al., 2005). Venkatraman et al., 2005 showed that ΔK210, the most prominent example for DCM causing cTnT-variation, decreases Ca^{2+}-sensitivity of force and actomyosin ATPase activity as well as maximal force and ATPase activity not only with adult, but also with fetal cTnT. The deletion of K210 occurs in the second Ca^{2+} -sensitive tropomyosin binding region near the PKC phosphorylation site and possibly near the binding site for PKA regulatory subunit II. This implies that Ca^{2+} -regulation as well as PKC dependent phosphorylation of cTnT and/or PKA dependent phosphorylation of other sarcomeric proteins might be affected. Detailed information is missing.

2.2 Mutations in TNNC1, the gene encoding cTnC

Mutations in the TNNC1 gene occur seldom. Far less than 1% of the genetic disorders leading to familial cardiomyopathies in patients are due to mutations in TNNC1. Up to date only six HCM and one DCM inducing mutations, all single amino acid exchanges, have been identified (Fig.2; Table 2). A8V, L29Q and C84Y are located in the N-terminal and N122fs, E134D, D145E and G159D in the C-terminal domain of cTnC (Fig. 2). L29Q was the first mutation detected in the cTnC gene (Hoffmann et al., 2001). Since there was only one family showing this mutation, it is not clear if this mutation causes HCM. However, according to Schmidtmann et al., 2005 and Liang et al., 2008 this amino acid replacement has the potential to cause a cardiomyopathy. Replacement of leucine by glutamine destabilizes the interaction of the N-terminal cTnI arm with cTnC (Schmidtmann et al., 2005; Baryshnikova et al., 2008). The release of the N-terminal cTnI arm occurs also upon bisphosphorylation of cTnI after ß-adrenergic stimulation and contributes to a reduction in Ca^{2+}-sensitivity of the actomyosin ATPase activity (see below). Consistent with this effect for L29Q a small reduction of Ca^{2+}- sensitivity has been described (Schmidtmann et al., 2005). However, in the phosphorylated state of cTnI, an enhanced Ca^{2+}-sensitivity of the actomyosin ATPase activity was observed, indicating an altered ß-adrenergic responsiveness. Divergent results might be obtained in systems differing in complexity

mutation	disease	exon	reference
Leu29Gln (L29Q)	HCM	3	Hoffmann et al., (2001). Hum. Mutat. 17: 524; Dweck et al., (2008). J Biol Chem. 283: 33119-28; Liang et al., (2008). Physiol. Gen. 33:257-66; Schmidtmann et al., (2005). *FEBS J.* 272(23):6087-97.
Ala8Val (A8V)	HCM	1	Landstrom et al., (2008). JMCC 45: 281-288 ; Pinto et al., (2009) JBC. 284(28):19090-100; Pinto et al., (2011).JBC. 286(2):1005-13.
Cys84Tyr (C84Y)	HCM	4	Landstrom et al., (2008). JMCC 45: 281-288. Pinto et al., (2009) JBC. 284(28):19090-100
Gln122Alafsx30 (Q122fs)	HCM	4	Chung et al., (2011) Cardiol. Young [Ehead of print]
Glu134Asp (E134D)	HCM		Pinto et al., (2009) JBC. 284(28):19090-100
Asp145Glu (D145E)	HCM	5	Landstrom et al., (2008). JMCC 45: 281-288. Pinto et al., (2009) JBC. 284(28):19090-100; Pinto et al., (2011).JBC. 286(2):1005-13.
Gly159Asp (G159D)	DCM	6	Mogensen et al., (2004). JACC 44: 2033-2040; Mirza et al., (2005). JBC 280: 28498- 506.

Modified from: OMIM database (http://omim.org); the one letter code is given in brackets; fs designates frameshift.

Table 2. Mutations in TNNC1

(Dweck et al., 2008). Thus, in a higher organized system, namely mouse cardiomyocytes, Liang et al. (2008) described an enhanced Ca^{2+}-sensitivity of force generation dependent on sarcomere length. They showed that also Ca^{2+}- binding affinity to site II was higher in cTnC-L29Q than in cTnC wild type, whereas Ca^{2+}-dissociation rate was not affected. This is in agreement with former findings of Gillis et al., 2005, that residues 2, 28-30 affect Ca^{2+}-binding properties of the regulatory Ca^{2+}-binding site. Replacement of these residues might increase cardiomyocyte contractility. Also the other FHC mutation in TNNC1, leading to A8V, C84Y and D145E replacements in cTnC, enhance Ca^{2+} -sensitivity of force development (Landstrom et al., 2008). For E134D no influence on Ca^{2+}-sensitivity could be observed. It remains unclear how this mutation leads to contractile dysfunction. Since E134 is located near contact sites of cTnI around Ser43/45 effects of PKC dependent phosphorylation might be influenced. Also A8V in the N-terminal helix of cTnC- N-lobe is located near a contact site with cTnI- Ser43 in H1 of cTnI (Li et al., 2004, Takeda et al., 2003). No data on the impact of these mutations on PKC phosphorylation are available up to date.

The proximity of A8 and E134 to the same cTnI region underlines findings of Smth et al. (1999) that the cTnC-N-terminal helix is spatially near the C-lobe. Latest kinetical investigations by Pinto et al, (2011a) showed that Ca^{2+} off rates are delayed for A8V and D145E, both stabilizing cross bridges. Thus structural disturbances at the cTnC-N-terminus not only alter structure of the N- but also of the C-terminal lobe and vice versa and may impair cTnC-cTnI interaction. C84Y is located at the end of the EF hand helix flanking regulatory Ca^{2+}- binding loop at the transition to the central linker region. This residue probably is involved in forming the binding platform for the cTnI switch region and thus might destabilize binding of cTnI in Ca^{2+} -saturated state of cTnC (Fig. 2). D145E is located in Ca^{2+} -binding loop IV, in +Z position (see above) indicating that divalent cation binding might be affected (Pinto et al., 2009). Another amino acid exchange in the C-terminal lobe, G159D, is linked to DCM. Consistent with all other investigated DCM causing mutations G159D reduces Ca^{2+}-sensitivity of the actin-myosin interaction and decreases contractility (Mirza et al., 2005). The hydrophobic residues at position 156,157 and 160 make contact to cTnI (Gasmi-Seabrook et al., 1999). Thus the G156D exchange may considerably disturb the hydrophobic interaction with cTnI and also might affect interaction with cTnT. In the troponin complex G159D reduced the opening (Ca2+ binding) and closing rates (Ca2+ dissociation) of the N-terminal domain of cTnC. Alteration in opening rate was also observed for L29Q, indicating that both mutants alter structural transition kinetics (Dong et al., 2008). PKA dependent phosphorylation of cTnI also affects kinetics in that it enhances the closing rate. This effect was abolished by L29Q and G159D implying that phosphorylation signal transduction is impaired as was earlier proposed for L29Q by Schmidtmann et al. (2005).

2.3 Mutations in TNNI3, the gene encoding cTnI

Most mutations in the gene encoding cTnI, are located in exon 7 and 8, which encode the regulatory C-terminal region of cTnI a few are located in the N-terminal heart specific extension. In patients they mostly induce either HCM or RCM. There are only three mutations identified up to date in TNNI3, which are linked to DCM.

2.3.1 Mutations in TNNI3 linked to HCM

Only one mutation has been identified in exon 3 encoding part of the heart specific N-terminal cTnI arm (Table 3). This mutation results in an R20C (numbering without starter methionine) amino acid exchange, which is located within the consensus sequence for PKA (Fig. 3). The consensus sequence is present in cTnI in a dublicated form (Mittmann et al., 1992) enabling phosphorylation of two adjacent located serine residues (Ser22, 23) by PKA (Fig. 4). The exchange of the middle arginine in a series of three arginine residues (position 20 or 21 dependent on the inclusion of the starter methionine) impairs phosphorylation of the two serine residues and reduces the phosphorylation effect *in vitro* as shown by Gomes et al. (2005). Since both phosphorylation sites are affected, susceptibility towards proteolysis is enhanced. Phosphorylation protects to a certain extent towards proteolysis (Barta et al., 2003). But also the amino acid exchange itself might enhance susceptibility towards protein degradationas proposed by Gomes et al. (2005). Thus probably impairment of PKA dependent phosphorylation as well as enhanced protein digestion might be the main effect of this mutant protein for disease development.

*Designates phosphorylation sites, arg 20 is indicated

Fig. 4. Consensus sequence in cTnI for PKA.

The first 6 mutations in TNNI3 causing HCM were described by Kimura et al. (1997) (Table 3). R145G, for example, is located within the inhibitor region of cTnI, which binds to actin/tropomyosin at low intracellular Ca^{2+} -concentrations (relaxed state) and blocks actin/myosin interaction (Fig. 3). Mutations located in the inhibitory region most probably affect inhibitory capacity of cTnI by altering actin/cTnI interaction in the relaxed state. Indeed R145G in fibers resulting from transgenic mice reduce inhibition (James et al., 2000; Wen et al., 2008) and binding affinity towards actin. Ca^{2+} -sensitivity of force development in skinned papillary muscles from these mice was enhanced with maximal force being decreased (Krüger et al., 2005; Wen et al ., 2008). These findings are in accordance with enhanced Ca^{2+}-sensitivity and reduced maximal actomyosin ATPase activity described by Deng et al. (2001). Also energy consumption in transgenic mice was increased as shown by Wen et al., 2008, indicating hypercontractility. In isolated rat cardiomyocytes as in myofibrils from transgenic mice contractile parameters were reduced (James et al., 2000; Kruger et al., 2005; Reis et al., 2008) and Ca^{2+} -regulation and ß -adrenergic response was impaired (Lang et al., 2002; Reis et al., 2008). The dynamic properties of contraction were severely suppressed upon ß- adrenergic stimulation (Reis et al., 2008). This again supports the idea that impairment of ß- adrenergic signaling might be important for disease development. Furthermore, R145G might also affect PKC dependent phosphorylation at Thr144, which has not been investigated thoroughly yet. Kobayashi et al. (2004) showed that effects of exchange of all PKC sites Thr144/ Ser43/45 by a glutamic acid residue, which is thought to mimic phosphorylation, are reduced due to the mutation. Pseudophosphorylated PKC sites reduce the Ca^{2+} -dependent opening of the N-terminal lobe of cTnC (Kobayashi et al., 2004). However, a more differentiated analysis is needed.

Mutations in the switch region (Table 3, Fig. 3), which binds to the N-terminal lobe of cTnC upon Ca^{2+}-saturation, are thought to impair binding to N-terminal lobe of cTnC under Ca^{2+}-saturating conditions. Thus, they probably affect Ca^{2+}- dissociation from cTnC and the conformational switch of cardiac troponin I needed for transmission of the Ca^{2+}- signal. An example for such a mutation is Ala157Val.

Mutations located in the mobile C terminal cTnI region may alter cTnI Tm/actin interaction. This may affect inhibition as well as activation. The mobile C-terminus together with N-terminal part of cTnT of the opposing troponin complex stabilizes the tropomyosin position in the blocked state. Indeed for C-terminally truncated cTnI impaired relaxation kinetics, enhanced Ca^{2+} sensitivity and disturbed cooperativity has been observed (Narolska et al., 2006, Tachampa et al., 2009). A small decrease in inhibitory capacity has been described for R162W and K185del (Redwood et al., 1998). Mutations located at the very C-terminus of cTnI as G203S or K206Q do not exhibit an effect on inhibition (Deng et al., 2003, Köhler et al, 2003). Again there are conflicting results concerning Ca^{2+}-sensitivity alterations. Transgenic mice with cTnI-G203S exhibit altered Ca^{2+} -regulation and show prominent altered expression of cytosketetal, contractile proteins and of proteins involved in energy production (Tsoutsman et al., 2006; Lam et al., 2007). Not much is known about other

mutations at the same position, G203R (replacement of glycin by the positively charged larger and less flexible amino acid arginine) and a frameshift (fs) mutation (table 3). One would expect that dysfunction due to these sequence alterations are more prominent than for G203S. Also K206Q is not well characterized. It enhances maximal actomyosin ATPase activity and alters dynamics of the actin myosin interaction (Deng et al., 2003). According to our own latest investigations (abstract, Saes et al., DGK, Mannheim April 2011) this part of cTnI interacts with actin; the replacement of lysine in position 206 by glutamine abolishes cTnI-C-terminus/actin interaction indicating a stabilization of the activated state. Furthermore, K206Q as well as G203S impair transduction of the PKA dependent phosphorylation signal *in vitro* (Deng et al., 2003). This implies that PKA dependent phosphorylation at the cTnI-N-terminus modulates not onlyfunction of inhibitory and switch region, but also of the mobile domain.

2.3.2 Mutations in TNNI3 linked to RCM

Mutations linked to RCM occur mostly in the regulatory domain as do HCM inducing mutations and may share even the same locus (Table 3). Thus for example R145G induces FHC and R145W, RCM. Indeed RCM and HCM have some clinical as well as molecular characteristics in common. Both diseases show diastolic dysfunction and all RCM mutations investigated lead to enhanced Ca^{2+}- sensitivity as do most of the HCM inducing mutations. Many of them reduce maximal tension a well as maximal actomyosin ATPase activity and impair inhibition (Gomes et al., 2005; Davis et al., 2008; Kobayashi & Solaro, 2006; for review see Parvatayar et al., 2010). Thus it is unclear how the RCM phenotype develops. But it indicates that the type of exchange (for example R145G HCM and R145W RCM) might be important for the disease development. A glycine exhibits a much smaller van der Waals volume, higher flexibility and higher hydrophilicity than a tryptophan at the same position and thus may alter interactions and dynamic properties very differently. Ca^{2+}-regulation is accompanied by allosteric transitions which are dependent on dynamic properties of the proteins involved. Thus alteration in dynamic properties affects Ca^{2+} regulation and might determine the type of dysfunction (Lassalle, 2010).

2.3.3 Mutations in TNNI3 linked to DCM

The first mutation identified (autosomal recessive) is located at the very N-terminal end of the heart specific N-terminal arm of cTnI and leads to a conservative replacement of the amino acid alanine by valine, which both are hydrophobic (Table 3). However, in contrast to alanine, valine is branched, takes double of the van der Waals volume and has a higher hydropathy index. These altered physicochemical properties might produce local structure disturbances and therefore modify interactions. According to Murphy et al., 2004 this amino acid exchange affects cTnI/cTnT interaction, though no direct interaction of the N-terminal arm with cTnT has been described so far. There are several contacts of the non phosphorylated N-terminus with cTnC- N-terminal lobe and of the phosphorylated N-terminus with the regulatory C-terminal region of cTnI. The mechanism how this mutation may alter cTnI/cTnI interaction is not clear. Further investigations are needed. Lately Carballo et al. (2009) described two new DCM mutations in cTNNI3 with an autosomal dominant trait leading to a severe onset of the disease. K36Q is located near constitutive cTnC interaction site and in the putative hinge region important for phosphorylation dependent movement of the cTnI-N-terminal arm. Therefor this mutation possibly might

affect structural integrity of the troponin complex and ß-adrenergic responsiveness. N185K is located within the mobile cTnI C-terminal region. *In vitro* both amino acid replacements reduce Ca^{2+} -sensitivity of the actomyosin ATPase and decreased maximal ATPase activity (Carballo et al., 2009) considered as typical for DCM.

mutation	disease	exon	reference
Arg21Cys (R21C /R20C)	HCM	3	Barr, Seidman et al. (2001) first posted on URL: http: //www. cardiogenomics.org; Arad et al., (2005) Circ. 112:2805-1 ; Gomes et al.,(2005) JMCC 39 :754-65
Arg141Gln (R141Q)	HCM	7	Richard et al., (2003) Circ. 107:2227-32 ; Van Driest et al., (2003) Circ. 108 : 445-51
Leu144Pro (L144P)	HCM	7	Merk, Seidman et al., (2005) first posted on URL:http://www. cardiogenomics.org
Arg145Gly (R145G/R146G)	HCM	7	Kimura et al., (1997) Nat Genet. 16:379-82. Takahashi-Yanaga, et al., (2000) J Biochem (Tokyo) 127:355-7, Elliott et al. (2000) JBC 275:22069-74 ; Deng et al., (2001) Biochem. 40:14593-602; Takahashi-Yanaga et al., (2001)JMCC 33:2095-107 ; Lang et al., (2002) JBC 277(4):11670-8 ; Burton et al., (2002) Biochem J 362:443-51 ; Lindhout et al., (2002) Biochem 41:7267-74 and (2005) Biochem 44:14750-9; Kruger et al., (2005) J Physiol 564: 347-57, Wen et al.,(2008) JBC 283: 20484-94; Reis et al., (2008) *Pflugers Arch*. 457:17-24
Arg145Gln (R145Q)	HCM	7	Kimura et al., (1997) Nat Genet 16:379-82 ; Taka-hashi-Yanaga et al., (2001) JMCC 33: 2095-107; Mogensen et al., (2004) JACC 44: 2315-25.
Ala157Val (A157V)	HCM	7	Richard et al., (2003) Circ. 107: 2227-32 ; Mogensen et al., (2004) JACC 44:2315-25; Brito & Madeira (2005)Rev Port Cardiol. 24:1137-46 ; Meder et al., (2009) J Cardiol 15: 274-8
Arg162Trp (R162W)	HCM	7	Kimura et al., (1997) Nat Genet 16: 379-82; Elliott et al., (2000) JBC 275: 22069-74; Takahashi-Yanaga et al., (2001) JMCC 33: 2095-107
Arg162Gln (R162Q)	HCM	7	Van Driest et al., (2003) Circ.108: 445-51; Mogensen et al., (2004) JACC 44: 2315-25 ; Doolan et al., (2005) JMCC 2005 38: 387-93; Cheng et al., (2005) JACC 46:180-1; Ingles et al., (2005) J Med Genet. 42 :e59.
Arg162Pro (R162P)	HCM	7	Richard et al., (2003)Circ. 2003 107: 2227-32; Doolan et al., (2005) JMCC 38: 387-93; Ingles et al., (2005) J Med Genet. 42: e59
Ser166Phe (S166F)	HCM	7	Van Driest et al., (2003) Circ. 108: 445-51; Van Driest et al., (2004) JACC 44: 1903-10; Mogensen et al., (2004) JACC 44: 2315-25
Lys178del (ΔK178)	HCM	7	Richard et al., (2003) Circ. 107: 2227-32
Lys183Glu (K183E)	HCM	7	Mogensen et al., (2004) JACC 44: 2315-25

mutation	disease	exon	reference
Lys183del (ΔK183)	HCM	7	Kimura et al., (1997) Nat Genet. 16: 379-82; Kokado et al., (2000) Circ. 102: 663-9; Takahashi-Yanaga et al., (2001) JMCC 33: 2095-107; Kohler et al., (2003) Physiol Gen. 14: 117-28 ; Konno et al., (2005) J Int Med. 258: 216-24.
Arg186Gln (R186Q)	HCM	8	Richard et al., (2003) Circ. 107:2227-32; Mogensen et al., (2004) JACC 44: 2315-25
Ile195Met (I195M)	HCM	8	Barr, Seidman et al., (2001) first posted on URL: http://www. cardiogenomics.org
Asp196Asn (D196N)	HCM	8	Richard et al., (2003) Circ. 107: 2227-32; Mogensen et al., (2004) JACC 44: 2315-25; Nimura et al., (2002) Circ. 105 : 446-51
Leu198Val (L198V)	HCM	8	Merk, Seidman et al., (2005) first posted on URL:http://www. cardiogenomics.org
Leu198Pro (L198P)	HCM	8	Doolan et al., (2005) JMCC 38: 387-93
Ser199Gly (S199G)	HCM	8	Mogensen et al., (2004) JACC 44: 2315-25
Ser199Asn (S199N)	HCM	8	Mogensen et al., (2004) JACC 44: 2315-25; Brito & Madeira (2005) Rev Port Cardiol. 24: 1137-46
Glu202Gly (E202G)	HCM	8	Mogensen et al., (2004) JACC 44: 2315-25
Gly203Arg (G203R)	HCM	8	Mogensen et al., (2004) JACC 44: 2315-25
Gly203Ser (G203S)	HCM/ Wolff Parkinson syndrom	8	Kimura et al., (1997) Nat Genet. 16:379-82; Kokado et al., (2000) Circ. 102: 663-9 ; Takahashi-Yanaga et al., (2001) JMCC 33: 2095-107 ; Burton D et al., (2002) Biochem J 362: 443-51; Kohler et al., (2003) Physiol Gen. 14: 117-28, Deng et al., (2003) JMCC 35: 1365-74; Tsoutsman et al., (2006) JMCC 41: 623-32; Nguyen et al., (2007) Int J Cardiol. 119: 245-8; Lam et al., (2010) JMCC 48: 1014-22
Gly203fs (G203fs)	HCM	8	Morner et al., (2000) JMCC 32: 521-5 and (2003) 35: 841-9; Richard et al., (2003) Circ. 107: 2227-32
Arg204Cys (R204C)	HCM	8	Barr, Seidman et al., (2002) first posted on URL: http://www. cardiogenomics.org
Arg204His (R204H)	HCM	8	Doolan et al., (2005) JMCC 38: 387-93; Ingles et al., (2005) J Med Genet. 42: e59
Lys206Gln (K206Q)	HCM	8	Kimura et al., (1997) Nat Genet. 16:379-82; Taka-hashi-Yanaga et al., (2001) JMCC 33: 2095-107; Kohler et al., (2003) Physiol Gen. 14: 117-28; Deng et al., (2003) JMCC 35: 1365-74
Ala2Val (A2V)	DCM, recessive	1	Murphy et al., (2004) Lancet 363: 371-2
Lys36Gln (K36Q)	DCM, dominant	3	Carballo et al., (2009) Circ. 105 : 375-82
Asn135Lys (N135K)	DCM dominant	7	Carballo et al., (2009) Circ. 105 : 375-82
Leu144Gln (L144Q)	RCM	7	Mogensen et al., (2003) JCI 111:209-16; Gomes et al., (2005) JBC 280: 30909-15
Arg145Trp (R145W)	RCM	7	Mogensen et al., (2003) JCI 111: 209-16; Mogensen et al., (2004) JACC 44: 2315-25; Gomes et al., (2005) JBC 280: 30909-15; Cheng (2005) J Am Coll Cardiol 46: 180-1

mutation	disease	exon	reference
Ala171Thr (A171T)	R CM	7	Mogensen et al., (2003) JCI 111: 209-16; Gomes et al., (2005) JBC 280: 30909-15
Lys178Glu (K178E)	RCM	7	Mogensen et al., (2003) JCI 111: 209-16; Gomes et al., (2005) JBC 280: 30909-15
Asp190His (R190H)	RCM	8	Mogensen et al., (2003) JCI 111: 209-16; Gomes et al., (2005) JBC 280: 30909-15
Asp190Gly (D190G)	RCM	8	Davis et al., (2008) JMCC 44: 891-904
Arg192His (R192H)	RCM	8	Mogensen et al., (2003) JCI 111: 209-16; Gomes et al., (2005) JBC 280: 30909-15

In brackets the one letter code and positions due to species specifities are given; fs designates frameshift.

Table 3. Mutations in TNNI3

In summary, there seem to be three major factors on the molecular level which help to understand phenotype development and might be directive for future investigations. 1) Mutations might severely affect affinity to other thin filament proteins and/or enhance susceptibility to proteolysis. In both cases structural integrity of the thin filament would be disturbed, which would in turn affect contractile function. It might even affect sarcomeric structure. 2) Mutations alter dynamical properties of the protein. Changes in dynamics might affect inter- and intramolecular interactions, Ca^{2+}-regulation, ß –adrenergic responsiveness and PKC mediated phosphorylation and thereby may induce contractile dysfunction in various degrees. 3) Combinations of mutations might lead to additive or compensatory effects.

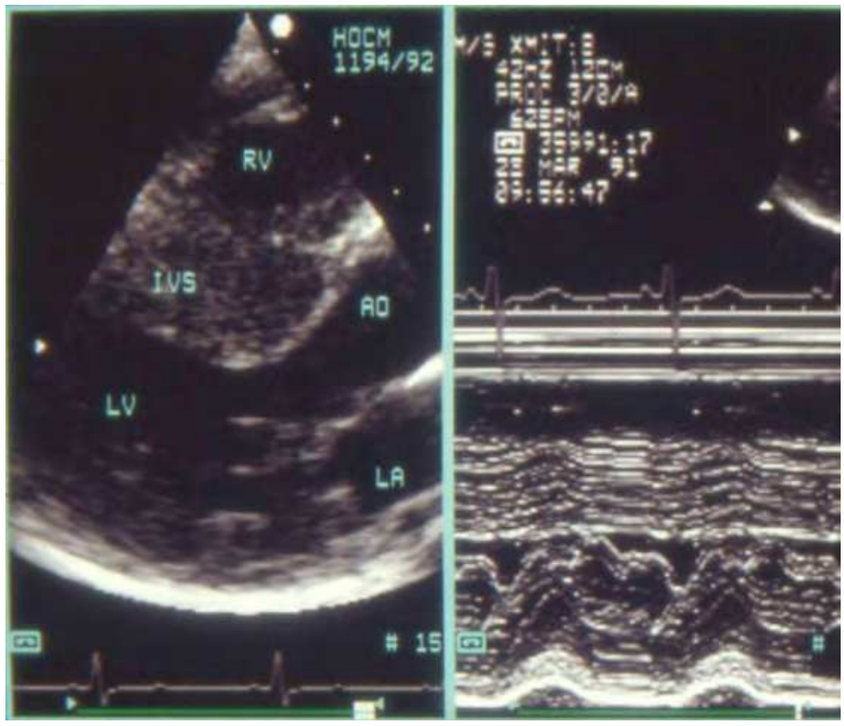
3. Clinical presentation

3.1 Diagnosis of HCM

In 1989 (Jarcho et al., 1989) and 1990 (Geisterfer-Lowrance et al., 1990), the first "disease genes" have been identified in family members with inherited hypertrophic cardiomyopathy (HCM). This identification of disease genes has raised many expectations, among others in the better understanding of the molecular mechanisms of disease development, in a more reliable identification of patients at risk, and in new concepts of treatment (Keren et al.; 2008, Lippi et al., 2009; Marian, 2010; Ho, 2010a; Watkins et al, 1995; 2011). The following paragraphs will focus on the clinical presentation of patients with mutations in the genes encoding troponin C, T and I (TNNC1, TNNT2, and TNNI3) in the context of HCM. For clinical presentation of HCM patients in general the reader is referred to an excellent book chapter from Fatkin et al., (2007).

HCM is clinically suggested in patients by the presence of unexplained left ventricular hypertrophy (LVH, usually defined as ventricular wall thickness ≥ 15 mm or ≥ 13 mm in relatives of a HCM patient; Elliott et al., 2008) and a non-dilated left ventricle with preserved or even enhanced global systolic function (Fig.5). Diagnosis relies on the electrographic and echocardiographic demonstration of hypertrophy. LVH may be diffuse or more segmentally distributed (proximal and/or midportion of the interventricular septum, apex, anterior or lateral wall), but no single morphologic expression appears to be specific (Klues et al., 1995). In fact, differentiation of LVH secondary to HCM may be difficult from other diseases affecting the ventricles, e.g. hypertrophy secondary to infiltrative diseases (e.g. amyloidosis),

Fabry's disease (Monserrat et al., 2007), glycogen storage disorders (Arad et al., 2005a), or systemic arterial hypertension. These diagnostic difficulties may rise with advanced age.



(IVS=septum, LV/RV=left/right ventricles, Ao=aorta ascendens, LA=left atrium)

Fig. 5. 2D and M-mode echocardiogram demonstrating severe hypertrophy (> 25 mm) of the septum

Besides LVH, left ventricular outflow obstruction is one of the most suspicious features of this disease. Braunwald & Ebert (1962) noted first the dynamic component of this obstruction. Later on the systolic anterior motion ("SAM") of the anterior leaflet of the mitral valve was recognized as the major contributor of left ventricular outflow obstruction and the more or less significant accompanying mitral regurgitation (Marian 2010). In a series of 320 consecutive HCM patients, this obstructive pathology at resting conditions (defined as a gradient ≥ 50 mmHg at rest) was found in 37% of patients (Maron et al., 2006). In the remaining patients, 52% developed dynamic outflow gradients during exercise or maneuvers which decrease afterload or increase contractility. These high numbers, however, should be cautiously extrapolated for the general HCM population because of referral bias and patients selection criteria.

Abnormal diastolic function (prolonged LV relaxation and increased LV chamber stiffness) is an almost universal feature of HCM. It appears that diastolic dysfunction is a very early manifestation of HCM, even before morphological evidence of hypertrophy occurs (Nagueh et al., 2001; Ho et al., 2009). Today, diastolic dysfunction is suggested if the ratio between early diastolic peak filling velocity (the E wave in transmitral Doppler) and early diastolic peak velocity of the mitral annulus (the E′ derived from tissue Doppler) exceeds the value 15 in the presence of normal systolic function (Fig. 6; Ommen et al., 2000; Paulus et al., 2007).

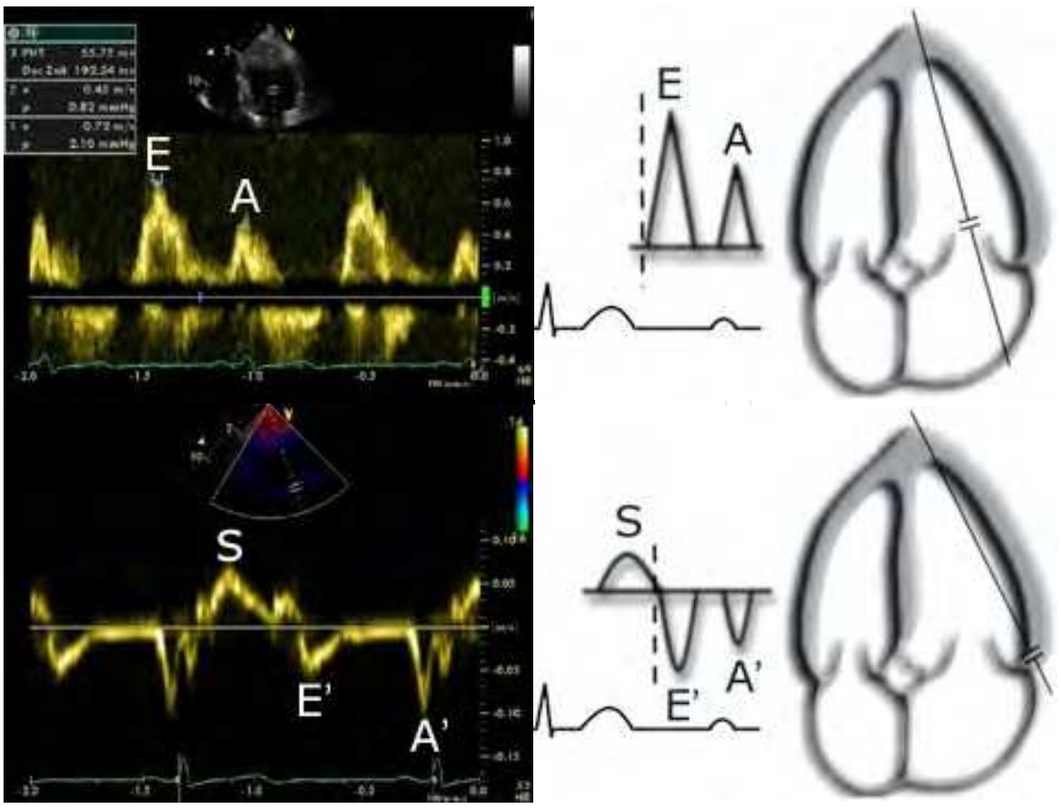


Fig. 6. Transmitral Doppler (top) and tissue Doppler (bottom) allowing quantification of the early diastolic peak filling velocity (E) and early diastolic peak velocity of the mitral annulus (E'). The ratio E/E' is used to determine diastolic dysfunction.

The clinical presentation of HCM patients shows a remarkable diversity: some individuals experience none or minor symptoms, others may develop dyspnoe at exercise or at rest, angina pectoris, palpitations, atrial fibrillation, dizziness, presyncope and syncope, fatigue or finally end stage heart failure requiring cardiac transplantation (Ho, 2010a).

The changes on ECG are very variable and include left axis deviation, occurrence of Q waves, a positive Sokolow index for hypertrophy, conduction abnormalities, ST-T depression or other abnormalities, negative T waves and giant T waves (particularly observed in Japanese patients with apical type of HCM (Sakamoto et al., 1976). The ECG abnormalities may not parallel hypertrophy in all cases. In fact, ECG abnormalities are more frequently found in HCM patients as echocardiographic abnormalities. Konno et al. (2005) observed ECG abnormalities (in particular ST-T abnormalities) in about 54% of genetically affected, but nonhypertrophic patients at echocardiography. However, almost all of ECG abnormalities (perhaps except giant T waves) are unspecific, and do also occur in patients with advanced age for various other reasons.

The underlying histopathology is characterized by gross cardiac hypertrophy, myocyte hypertrophy, disarray of cardiac cells, interstitial fibrosis, hyperplasia of the media of coronary arteries. The cardiac myocyte disarray (Fig. 7) appears to be a hallmark of HCM, not infrequently involving up to 20% of the ventricles (Maron & Roberts, 1979; Elliot & McKenna, 2004).

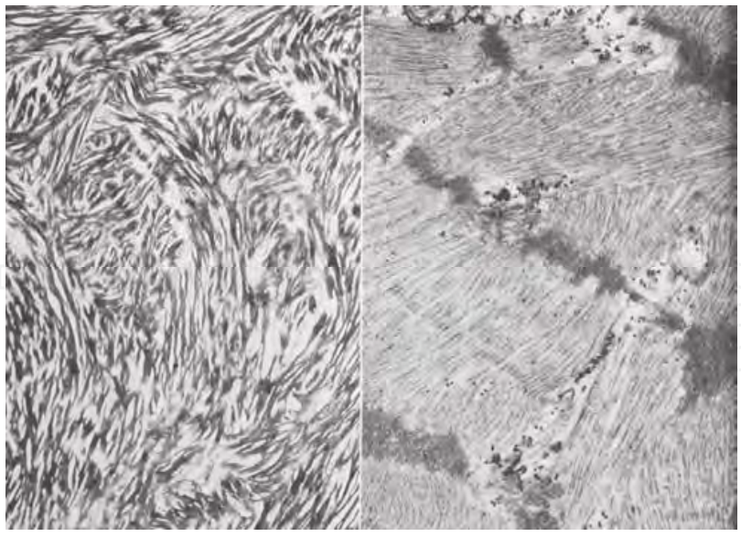


Fig. 7. Histology of the heart of a patient with HCM demonstrating typical disarray of the myocytes and myofibrils.

3.2 Complications and general prognosis in HCM

A major concern in the management of patients with HCM is prognosis. Sudden cardiac death (which may account for 50% of the disease-related death), atrial fibrillation with the risk of stroke, and congestive heart failure (CHF) are the leading contributors to the morbidity and mortality associated with HCM (Keren et al., 2008; Marian 2010). Overall, HCM is a "benign" disease with an annual mortality rate of 0.5-1% in unselected HCM-affected subjects (Cannan et al. 1995). However, sudden death may the first clinical manifestation of this disease, particularly in young otherwise healthy appearing subjects (Maron et al., 1996). In a meta-analysis by Liberthson (1996), HCM was the most frequent single cause of sudden death in children and young adults. This was supported by a large series of Maron (2003) who analyzed 387 young athletes who died suddenly, and found that HCM was the cause in 26.4%. The overall risk of sudden death appears to be similar in males and females (Olivotto et al., 2005), by contrast, 90% of 134 athletes with sudden cardiac death were males (Maron et al., 1996).

The risk for sudden cardiac death can be effectively reduced by the prophylactic implantation of an internal automated defibrillator (ICD), the main problem is, however, to identify those who will profit from this device. So far, no single risk factor (except surviving cardiac arrest) has been identified which may clearly justify prophylactic ICD implantation. Nowadays, a more comprehensive approach is used combining informations/findings (family history of sudden death, severe cardiac hypertrophy, history of presynope or syncope, non-sustained or sustained ventricular tachycardia) (Kofflard et al., 2003; Marian 2003; Frenneaux 2004; Marian 2010). It is a new challenge to integrate genotype testing in this risk assessment algorithm, although the power of genetic testing appears to be up to now more the identification of non-carriers in HCM families obviating the need for clinical screening and follow-up examinations (Keren et al., 2008; Pinto et al., 2011). The ethical, legal and societal implications of genetic testing for cardiac diseases in clinical practice has been discussed elsewhere (Tester & Ackerman, 2011). Furthermore, the pro and contra of genotyping in predicting prognosis in HCM has been recently discussed in Circulation (Ho, 2010b; Landstrom & Ackerman, 2010).

The true penetrance of all clinical presentations is not known and may be underestimated because the clinical diagnosis of HCM is not robust. Furthermore, clinical findings may either vary over time or the disease-related abnormalities may have a variable onset during life. For example, progressive increase in LV wall thickness has been observed in adolescents and young adults with HCM, whereas wall thickness remains to be more stable in the elderly (Maron et al., 1986; Semsarian et al., 1997). This may, however, not be true for all mutations. Revera et al. (2007) re-evaluated 22 carriers with an Arg92Trp (TNNT2 gene) mutation after an average of 11 years. With age, left ventricular hypertrophy increased (≥ 5 mm wall thickness, as assessed by echocardiography) in 50% of individuals. These later points are of particular importance since most clinical studies have a cross-sectional design, and prospective longitudinal studies are scarce.

All groups are in agreement that the phenotypic heterogeneity in HCM patients/families cannot be explained by the genetic defect alone. Other factors must be involved including sex, additional disease such as arterial hypertension, and environmental factors. This heterogeneity is particularly striking in families with one genetic defect, but demonstrating phenotypes of different cardiomyopathies. For example, Menon et al. (2008) identified a large family with a mutation in the TNNT2 gene (Ile79Asn). This mutation affected 9 family members. Mutation carries showed clinically a restrictive cardiomyopathy in 2, a non-obstructive HCM in 3, dilated cardiomyopathy in 2, a mixed cardiomyopathy in 1, and mild concentric hypertrophy in 1 family member. Genetic factors others than the causal sarcomere mutation may affect the penetrance and severity of cardiac abnormalities, referred as the modifier genes. In this regard, variants of the angiotensin-1 converting enzyme (ACE-1) gene are discussed as potential modifiers, increasing the risk for sudden cardiac death (Marian et al., 1993) and the severity of LVH (Lechin et al., 1995). Beside modifier genes, others factors must also determine the phenotype, as pressure or volume load, since manifestations of HCM are predominantly restricted to the LV, although the mutant sarcomeric protein is expressed in both ventricles. Finally, homozygous mutations (as it has been described for a child with a Ser179Phe mutation in the TNNT2 gene who died suddenly at the age of 17 (Ho et al., 2000)), or multiple mutations within the sarcomere protein encoding genes may cause a more severe type of HCM. For example, Girolami et al. (2010) identified in a cohort of 488 unrelated index HCM patients 4 patients (0.8%) who harbored triple mutations of the sarcomere proteins. The triple sarcomere defects were associated with an adverse outcome.

Mutations in the troponin genes (TNNC1, TNNT2, TNNI3) are accounting for less than 10% of patients with HCM. In a large series of 197 index patients living in France, Richard et al. (2003) detected disease-casing mutations in 63%, whereby mutations within the TNNT2 and TNNI3 gene accounted for 4% each.

3.3 TNNT2

In MEDLINE, reports on approx. 194 HCM patients from various living areas with a mutation of the TNNT2 gene have been published so far (Thierfelder et al., 1994; Watkins et al., 1995; Forissier et al., 1996; Moolman et al., 1997; Anan et al., 1998; Elliott et al., 1999; Ho et al., 2000; Varnava et al., 2001; Richard et al., 2003; Van Driest et al, 2003; Garcia-Castro et al., 2003; Torricelli et al., 2003; An et al., 2004; Theopistou et al., 2004; Miliou et al., 2005; Konno et al., 2005; Capek & Skvor, 2006; Menon et al., 2008; Xu et al., 2008; Gimeno et al., 2009). Table 4 summarizes those reports (86 families, 188 genetically affected subjects) which

provided at least prognostic informations and/or some data on the phenotype. The median age (at the time of examination) is 40 years, 56.1% are females. Maximal left ventricular wall thickness was less than 15 mm in 49.5% of genetically affected subjects, only in 9.7% of patients, the wall thickness exceeded 25 mm. The ECG was abnormal in the majority of affected subjects (78.8%). Congestive heart failure (CHF, NYHA class ≥ II) was present in 42.9% of subjects. Prognostic data were available for 30 affected families, 19 lamented sudden cardiac death within their families. In additional 31 index patients, sudden cardiac death, heart transplantation or an ICD implantation was reported on 11 patients. Watkins et al. (1995) overlooked a series of 67 subjects over the age of 16 years who had TnT mutations. He noted that 24% of these individuals did not fulfill the clinical diagnostic criteria of HCM. The risk for disease-related death, however, was high (sudden cardiac death, death related to CHF). Varnava et al. (2001) investigated histologically 75 hearts with HCM. Blood samples from relatives and/or affected patients (before death) were available in 50 cases, allowing genotyping. Mutations in the TNNT2 gene were found in 9/50 patients, 8 of the 9 patients died suddenly. The heart weight was less, but the degree of disarray was significantly more in those patients with a mutation of the TNNT2 gene as compared to mutations of other genes. Other authors confirmed this constellation "minor LVH and high risk for sudden cardiac death" particularly for the following mutations: Phe87Leu (Gimeno et al., 2009), Arg92Trp (Moolman et al., 1997), Ala104Val (Nakajima-Taniguchiet et al., 1997), and Lys273Glu (Fujino et al., 2002).

3.4 TNNI3

Reports on approx. 99 patients have been published so far (Kimura et al., 1997; Kokado et al., 2000; Mörner et al., 2000; Niimura et al., 2002; Richard et al., 2003; Van Driest et al., 2003; Mogensen et al., 2004; Arad et al., 2005b; Brito & Madeira, 2005; Doolan et al., 2005; Sheng et al., 2008). Table 5 summarizes the reports (41 families, 87 genetically affected subjects) which provided prognostic informations or some data on phenotype. The median age (at the time of examination) is 46 years, 55.1% are females. Maximal left ventricular wall thickness was less than 15 mm in 53.2% of genetically affected subjects, in none the wall thickness exceeded 25 mm. An abnormal ECG was observed in 90.5% of genetically affected patients. Congestive heart failure was present in 36.4% subjects. In 24 of 37 families, sudden cardiac death or cardiac arrest occurred.

3.5 TNNC1

Only six cases with mutations of the TNNC1 gene have been published so far (Hoffmann et al., 2001; Landstrom et al., 2008; Chung et al., 2011) (Table 6). The first missense mutation within the TNNC1 gene (Leu29Gln) was described by Hoffmann et al. (2001) in a 60 year-old man with a moderate LV hypertrophy, ECG abnormalities and heart failure. The number of cases is too low to characterize a typical phenotype. Three of the 6 cases underwent myectomie, in a 19 year-old man, sudden cardiac death occurred.

3.6 Prognosis in patients with mutations of the genes encoding cTnT and cTnI

Overall, patients with mutations within the gene encoding cardiac troponin T and I are characterized by minor/moderate left ventricular hypertrophy, the maximal wall thickness of the LV does exceed 25 mm in a small percentage only. Despite the presence of minor/moderate LV hypertrophy, the rate of abnormal ECGs is high (> 75%). Furthermore,

congestive heart failure is not an infrequent complication (35-40% of the genetically affected patients). The rate of sudden cardiac death/arrest appears to be very high (> 30% of index patients or in related family members). Whereas some mutations are associated with sudden cardiac death at any age (e.g. the Lys183 deletion mutation in he TNNI3 gene; Kokado et al., 2000), others are associated with disease-related death predominantly in older carriers (e.g. the Phe87Leu mutation within the TNNT2 gene; Gimeno et al., 2009), or at younger age (e.g. the Arg92Trp mutation within troponin T; Moolman et al., 1997). Of note, the degree of disarray of the myocytes may not parallel the magnitude of hypertrophy: Gambrin et al. (2008) described a 22 year old women who underwent cardiac transplantation for restrictive filling pattern and CHF. Histology revealed severe disarray in the absence of hypertrophy. Genetic analysis disclosed an Arg204His mutation in the TNNI3 gene.

The mechanism of sudden cardiac death in patients with HCM is still under debate (Ho, 2010 a,b). For many years, the ischemia hypothesis has been proposed, that is increased oxygen demand due to increased LV mass and wall stress, combined with reduced oxygen supply due to reduced capillary density and abnormal narrowed intramural coronary arteries. This balance may further deteriorate if myocardial bridging with systolic compression of epicardial coronary artery is present (Yetman et al., 1998). The ischemia hypothesis is supported by a study of Spirito et al. (2000). These authors investigated the relation between the magnitude of hypertrophy and mortality in 480 consecutive patients with HCM. Over a follow-up period of 6.5 years, 65 patients died (23 sudden death, 15 CHF-related, 27 noncardiac cause or stroke). The risk of sudden death increased progressively with the wall thickness (0 per 1000 person-years for a wall thickness ≤ 15 mm, up to 18.2 per 1000 person-years for those with a wall thickness ≥ 30 mm).

3.7 What we can learn from patients with troponin mutations

What can we learn from the patients with troponin mutations? One important message to the clinicians is that the risk of sudden cardiac death does not go along with the severity of left ventricular hypertrophy/wall thickness. Furthermore, sudden cardiac death may occur in all age groups with troponin mutations. These observations imply that other mechanisms than ischemia-triggered rhythm disturbances may account for the excessive risk of sudden cardiac death in this subgroup (and other subgroups?) of patients with HCM. In transgenic mice expressing the TnT-I79N (Ile79Asn) mutation, no ventricular hypertrophy or fibrosis was detected, but ventricular ectopy and the rate of stress-induced ventricular tachycardia were significantly increased (Knollmann et al, 2003). Baudenbacher et al. (2008) showed in this mouse model that the risk of developing ventricular tachycardia appears to be directly proportional to the degree of Ca^{2+} sensitization caused by different troponin T mutations (TnT-I79N, TnT-F110I, and TnT-R278C). They gave first evidence that reduction of Ca^{2+} -sensitivity (by blebbistatin) in myofilaments acts "antiarrhythmic". This work by Baudenbacher and coworkers clearly demonstrates that changes in the intracellular Ca^{2+} -sensor cardiac troponin are associated with arrhythmias, and histological/anatomical changes which do often later develop in the course of hypertrophic cardiomyopathy, are not a prerequisite for these life-threatening arrhythmias. In our laboratory, we studied the effects of the cTnI-R145G mutation on adrenergic signalling in isolated rat ventricular cardiomyocytes (Reis et al., 2008). This mutation hinders the transduction of the phosphorylation signal from troponin to the thin filament. Upon adrenergic stimulation of the cardiomyocytes, rates of shortening and relengthening were significantly suppressed.

This suppression was evident in response to ß$_2$- but not ß$_1$-adrenergic stimulation. These data demonstrate that adrenoceptor-mediated signalling may be altered by troponin mutations. Since sudden cardiac death in patients with HCM often occurs during exercise or physical activity, thus conditions with increased sympathetic activity, altered adrenergic signalling may be a potential player in the pathogenesis of life-threatening arrhythmias.
Many other mechanism are currently under investigation, including the role of the myocyte enhancer factor 2, transforming growth factor, connective tissue factor, and periostin in the development of hypertrophy, diastolic dysfunction, and myocardial scarring (Seidman & Seidman, 2011); also altered intracellular calcium handling and abnormalities in myocardial energetics are under discussion to participate in this complex phenotype (Ho, 2010a,b). Despite obvious progress, the precise link between the molecular defect and the complex phenotype HCM is still not understood.

Patients/ Family	Age/Sex	Clinical Presentation						References
		Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
Ser69Phe								
n=1 (9 unrelated SCD) Area: U.K.	29 M	n.a.	n.a.	n.a.	n.a.	n.a.	SCD	Varnava et al., 2001
Pro77Leu								
n=1 (9 unrelated SCD) Area: U.K.	37 M	n.a.	n.a.	n.a.	n.a.	n.a.	SCD	Varnava et al., 2001
Ile79Asn								
n=9 (1 family + unrelated p.) Area: multi-ethnic/racial	n.a.	13.4±4 (n=4)	n.a.	n.a.	n.a.	n.a.	4 SCD in family	Watkins et al., 1995
n=1 (9 unrelated SCD) Area: U.K.	16 M	n.a.	n.a.	n.a.	n.a.	n.a.	SCD	Varnava et al., 2001
n=9 (1 family) Area: U.S.A.	50 F[1]	n.a.	n.a.	n.a.	n.a.	+	DCM, death at age 64	Menon et al., 2008
	68 F	10	9	normal	+	+	DCM, death at age 73	
	66 F[1]	18	10	n.a.	+	+	-	
	46 M[1]	16	12	n.a.	+	+	mixed DCM/HCM	
	58 M[1]	12	10	normal	+	+	RCM type	
	53 F[1]	14	11	n.a.	+	+	RCM type	
	49 F[1]	16	9	n.a.	+	+	-	
	40 F[1]	24	8	n.a.	+	+	-	
	48 F	14	10	n.a.	-	-	-	

Patients/ Family	Clinical Presentation							References
	Age/Sex	Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
Asp86Ala								
n=1(389 unrelated p.) Area: U.S.A.	39 M	n.a.	30	n.a.	n.a.	+	-	Van Driest et al., 2003
Phe87Leu								
n=7 (1 family) Area: Spain	52 F	14	n.a.	n.a.	+	+	SCD in family	Gimeno et al., 2009
	39 M	13	n.a.	n.a.	+	+	"	
	40 F	14	n.a.	n.a.	+	+	", ICD	
	30 F	18	n.a.	n.a.	+	+	", ICD	
	30 M	27	n.a.	n.a.	+	-	"	
	29 F	25	n.a.	n.a.	+	+	"	
	9 M	12	n.a.	n.a.	+	-	"	
Arg92Gln								
n=1 (150 unrelated p.) Area: Toscana	23 M	29	n.a.	III	+	-	-	Thierfelder et al., 1994; Torricelli et al., 2003
n=32 (3 families + unrelated p.) Area : multi-ethnic/racial	n.a.	n.a.	15.0±6 (n=21)	n.a.	n.a.	n.a.	SCD in 11 p	Watkins et al., 1995
Arg92Trp								
n=18 (2 families) Area: South Africa	56 F	14	12	n.a.	+	n.a.	SCD in family	Moolman et al., 1997
	57 F	13	12	n.a.	+	n.a.	"	
	41 F	12	13	n.a.	+	n.a.	"	
	28 F	24	11	n.a.	+	n.a.	"	
	35 F	13.8	7.5	n.a.	-	n.a.	"	
	23 F	6	6	-	+	n.a.	"	
	21 M	normal	normal	-	+	n.a.	"	
	38 M	9	9	-	+	n.a.	"	
	35 F	8	8	-	+	n.a.	"	
	28 M	7	8	-	+	n.a.	"	
	47 F	7	7	-	-	n.a.	"	
	27 F	6	6	-	-	n.a.	"	
	9 F	4	4	-	-	n.a.	"	
	28 M	8.5	8.5	-	-	n.a.	"	
	15 F	5	5	-	-	n.a.	"	
	11 F	7	7	-	-	n.a.	"	
	34 F	18-20	11	n.a.	+	n.a.	"	
	56 F	8	7	-	+	n.a.	"	
n=1 (389 unrelated p.) Area : U.S.A.	27 F	n.a.	32	n.a.	n.a.	-	-	Van Driest et al., 2003
n=2(9 unrelated SCD) Area.U.K.	22 M	n.a.	n.a..	n.a.	n.a.	n.a.	SCD	Varnava et al., 2001
	6 F	n.a.	n.a.	n.a.	n.a.	+	transplant	

Patients/ Family	Clinical Presentation							References
	Age/Sex	Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
Arg92Leu								
n=1 (9 unrelated SCD) Area: U.K.	26 M	n.a.	n.a.	n.a.	n.a.	n.a.	SCD	Varnava et al., 2001
n=4 (1 family) Area: France	43 F	n.a.	19	n.a.	-	+	CHF at 44 y	Forissier et al., 1996
	23 M	n.a.	35	n.a.	+	+	n.a	
	20 F	n.a.	n.a.	apical	+	+	n.a.	
	45 F	n.a.	10	n.a.	+	-	n.a.	
Arg94Leu								
n=2 (9 unrelated SCD) Area: U.K.	17 M	n.a.	n.a.	n.a.	n.a.	n.a.	SCD	Varnava et al., 2001
	21 F	n.a.	n.a.	n.a.	n.a.	n.a.	SCD	
Ala104Val								
n=4 (1 family) Area: Japan	50 F	15	8	n.a.	+	+	SCD at age 50	Nakajima-Taniguchi et al., 1997
	36 F	17	6	n.a.	+	+	SCD at age 36	
	54 F	17	8	n.a.	+	+	ventricular tachycardia	
	33 F	20	12	n.a.	+	-	-	
Phe110Ile								
n=2 (1 family) Area : multi-ethnic/racial	n.a.	n.a.	17 (n=2)	n.a.	n.a.	n.a.	-	Watkins et al., 1995
n=16 (6 families) Area : Japan	38 F	27	17	III	+	-	-	Anan et al., 1998
	69 F	22	14	III	+	-	SCD in family	
	47 F	20	13	III	+	-	SCD in family	
	87 F	9	9	IV	+	-	-	
	48 M	10	10	IV	+	-	-	
	42 F	11	11	normal	-	-	-	
	64 M	23	10	II	+	-	-	
	70 M	20	11	II	+	-	-	
	47 F	13	13	III	+	-	-	
	45 M	12	10	normal	+	-	-	
	39 F	13	13	III	+	-	-	
	24 F	11	11	IV	+	-	-	
	56 F	19	11	II	+	-	SCD in family	
	53 M	15	13	III	+	-	"	
	31 F	21	12	II	+	-	"	
	28 F	10	10	normal	+	-	"	
Phe110Val								
n=3 (150 unrelated p.) Area: Italy	28 F	32	n.a.	III	+	-	-	Torricelli et al., 2003
	48 F	21	n.a.	III	+	n.a.	-	
	82 M	15	n.a.	II	+	n.a.	-	

Patients/ Family	Clinical Presentation							References
	Age/Sex	Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
Lys124Asn n=1 (71 unrelated p.) Area : China	41 F	n.a.	n.a.	LVH	n.a.	n.a.	-	An et al., 2004
Arg130Cys n=2 (150 unrelated p.) Area: Italy	55 M	23	n.a.	III	+	-	-	Torricelli et al., 2003
	58 F	13	n.a.	n.a.	+	-	-	
Glu160 n=32 (2 families +unrelated p.) Area : multi-ethnic/racial	n.a.	n.a.	17.5±5 (n=14)	n.a.	n.a.	n.a.	SCD in 14	Watkins et al., 1995
n=2 (150 unrelated p.) Area: Italy	50 F	20	n.a.	III	+	-	-	Torricelli et al., 2203
	55 M	22	n.a.	III	+	+	-	
Glu163Lys n=5(1 family + unrelated p.) Area. multi-ethnic/racial	n.a.	n.a.	19.8±8 (n=5)	n.a.	n.a.	n.a.	SCD in 0	Watkins et al., 1995
Ser179Phe n=1 (1 family) Area: Kuwait	17 M	25	11	n.a	+	-	SCD	Ho et al., 2000
Glu244Asp n=1 (1 family) Area: multi-ethnic/racial	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	SCD in 0	Watkins et al., 1995
Lys247Arg n=1 (30 unrelated p.) Area: Spain	60 F	23	n.a.	n.a.	n.a.	n.a.	-	Garcia-Castro et al., 2003
Intron 15 G1→A n=28 (1 family +unrelated p.) Area: multi-ethnic/racial	n.a.	n.a.	17.5±5 (n=17)	n.a.	n.a.	n.a.	SCD in 9	Watkins et al., 1995
n=1 (9 unrelated SCD) Area. U.K.	15 M	n.a.	n.a.	n.a.	n.a.	n.a.	SCD	Varnava et al., 2001
Asn271Ile 3 (1 family) Area: Spain	62 M	22	n.a.	n.a.	+	-	-	Gimeno et al., 2009
	36 M	14	n.a.	n.a.	+	-	-	
	31 F	9	n.a.	n.a.	-	-	-	

Patients/ Family	Clinical Presentation							References
	Age/Sex	Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
Lys273Glu								
n=8 (2 families) Area: Japan	58 F	7	8	n.a.	n.a.	n.a.	DCM features	Fujino et al., 2002
	32 F	23	7	asym.	n.a.	n.a	-	
	29 F	23	10	asym.	n.a.	n.a.	-	
	75 F	15	13	n.a.	n.a.	n.a.	SCD in family	
	78 F	15	11	asym.	n.a.	n.a.	"	
	75 F	16	9	asym.	n.a.	n.a.	"	
	49 F	20	9	asym	n.a.	n.a.	"	
	46 M	21	10	asym	n.a.	n.a.	"	
Arg278Cys								
n=3 (1 family + unrelated p.) Area: multi-ethnic/racial	n.a.	n.a.	16.3±6 (n=3)	n.a.	n.a.	n.a.	SCD in 1	Watkins et al., 1995
n=3(389 unrelated p.) Area: U.S.A.	57 M	n.a.	20	n.a.	n.a.	-	-	Van Driest et al.,, 2003
	66 M	n.a.	15	n.a.	n.a.	+	pacemaker	
	74 M	n.a.	23	n.a.	n.a.	+	TASH	
n=1 Area: U.K.	57 M	12	n.a.	n.a.	+	+	late onset HCM	Elliott et al., 1999
n=1 (30 unrelated p.) Area: Spain	60 F	22	n.a.	n.a.	n.a.	n.a.	-	Garcia-Castro et al., 2003
n=8 (2 families) Area: Spain	55 F	22	n.a.	n.a.	+	+	-	Gimeno et al., 2009
	59 M	22	n.a.	n.a.	+	+	-	
	27 M	12	n.a.	n.a.	-	-	-	
	30 M	10	n.a.	n.a.	-	-	-	
	29 M	10	n.a.	n.a.	-	-	-	
	21 M	40	n.a.	n.a.	+	+	ICD-Impl.	
	64 M	26	n.a.	n.a.	+	-	-	
	33 M	11	n.a.	n.a.	+	-	-	
n=6 (2 families) Area: Greek	40 M	20	11	asym	+	+	SCD in family	Theopistou et al., 2004
	71 F	13	12	conc.	+	+	"	
	41 M	10	8	normal	-	n.a.	"	
	38 M	10	10	normal	-	n.a.	"	
	14 F	7	8	normal	-	n.a.	"	
	13 M	22	11	asym.	+	-	SCD age 15	
n=1 (150 unrelated p.) Area: Italy	66 M	24	n.a.	III	+	+	-	Torricelli et al., 2003

Patients/ Family	Age/Sex	Clinical Presentation						References
		Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
Arg278Pro								
n=1 (389 unrelated p.) Area: U.S.A.	47 M	n.a.	19	n.a.	n.a.	+	ICD	Van Driest et al., 2003
n=1 (143 unrelated p.) Area : Greek	18 M	n.a.	n.a.	LVH	n.a.	n.a.	-	Miliou et al., 2005
Arg286Cys								
n=1 (143 unrelated p.) Area : Greek	26 F	n.a.	n.a.	LVH	n.a.	n.a.	-	Miliou et al., 2005
Arg286His								
n=2 (389 unrelated p.) Area: U.S.A.	49 M	n.a.	20	n.a.	n.a.	-	-	Van Driest et al., 2003
	39 M	n.a.	25	n.a.	n.a.	+	myectomy	

Table 4. Mutations in the TNNT2 Gene Associated with HCM

Number of affected patients is given (out of a group of unrelated HCM patients or families with HCM). Age=age at investigation if not otherwise noted; [1]age at diagnosis; n.a.=data not available; SCD=sudden cardiac death; DCM=dilated cardiomyopathy like-type; RCM=restrictive cardiomyopathy like-type; CHF=congestive heart failure; TASH=transcoronary septal ablation; ICD=internal automated defibrillator; area=living area of study patients. +Left ventricular hypertrophy (LVH) is classified according to according to Maron et al. (1981) type I=confined to the anterior segment of the ventricular septum, type II=involved anterior and posterior septum, type III=involvement of both the septum and the free wall of the left ventricle, and type IV=regions other than the basal and anterior septum (e.g. apical area).

Patients/Family	Age/Sex	Clinical Presentation						Reference
		Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
Arg21Cys								
n=1 (15 unrelated p.) Area: Europa	40 F	n.a.	n.a.	apical	n.a.	n.a.	SCD in family	Arad et al., 2005
Pro82Ser								
1 pt. with late-onset HCM Area: n.a.	> 40	n.a.	n.a.	LVH	+	+	n.a.	Niimura et al., 2002
Arg141Gln								
n=1 (389 unrelated p.) Area: U.S.A	41 M	n.a.	25	n.a.	n.a.	-	-	Van Driest et al., 2003
Arg145Gln								
n=1 (1 family) Area : U.K.	n.a.	21	n.a	apical	+	n.a.	n.a	Mogensen et al., 2004

	Clinical Presentation							
Patients/Family	**Age/Sex**	**Septum (mm)**	**Wall (mm)**	**LVH type+**	**ECG**	**CHF**	**Prognosis**	**Reference**
Ala157Val								
n=5 (3 families) Area : U.K.	n.a.	n.a.	n.a.	n.a.	+	n.a.	SCD in family	Mogensen et al., 2004
n=1 (1 family) Area: Portugal	24 M	n.a.	n.a.	LVH	*	*	SCD at age 44	Brito & Madeira, 2005
Arg162Gln								
n=7 (3 families) Area: U.K.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a	SCD in family	Mogensen et al., 2004
n=2 (389 unrelated p.) Area: U.S.A.	76 F	n.a.	17	n.a.	n.a.	+	myectomy	Van Driest et al., 2003
	33 M	n.a.	19	n.a.	n.a.	+	SCD in family	
n=3 (1 family) Area: Australia	70 M	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Doolan et al., 2005
	44 M	n.a.	10	normal	-	-	-	
	40 M	n.a.	10	normal	-	-	-	
Arg162Pro								
n=2 (1 family) Area: Australia	52 F	n.a.	9	normal.	-	-	-	Doolan et al., 2005
	25 F	n.a.	14	n.a.	+	-	cardiac arrest at age 21	
Ser166Phe								
n=3 (389 unrelated p.) Area: U.S.A.	48 F	n.a.	22	n.a.	n.a.	+	myectomy	Van Driest et al., 2003
	79 F	n.a.	14	n.a.	n.a.	+	myectomy	
	21 F	n.a.	17	n.a.	n.a.	+	myectomy	
n=1 (1 family) Area: U.K.	n.a.	19	n.a.	n.a.	+	n.a	-	Mogensen et al., 2004
Lys183Glu								
n=3 (1 family) Area: U.K.	n.a.	n.a.	n.a.	n.a.	+	n.a	-	Mogensen et al., 2004
Lys183del								
n=25 (7 families) Area: Japan	65 F	13	7	I/II	+	n.a.	SCD in family	Kokado et al., 2000
	61 F	17	12	I/II	+	n.a.	"	
	51 F	9	10	normal	+	n.a.	"	
	46 F	20	10	I/II	+	n.a.	"	
	36 F	16	8	I/II	+	n.a.	"	
	48 F	7	7	normal	+	n.a.	"	
	36 M	18	13	III	+	n.a.	"	
	33 F	21	9	I/II	+	n.a.	"	
	47 F	10	8	normal	+	n.a.	"	
	27 F	13	9	III	+	n.a.	"	
	8 M	5	5	normal	-	n.a.	"	
	85 M	14	10	I/II	+	n.a.	"	
	56 F	23	11	IV	+	n.a.	"	
	48 F	13	14	IV	+	n.a.	"	
	71 F	11	10	normal	+	n.a.	"	

Patients/Family	Clinical Presentation							Reference
	Age/Sex	Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
	49 M	5	9	normal	+	n.a.	"	
	24 F	20	11	I/II	+	n.a.	"	
	23 F	9	8	normal	+	n.a.	"	
	66 F	11	11	normal	+	n.a.	"	
	62 M	13	12	normal	+	n.a.	"	
	35 M	12	11	normal	-	n.a.	"	
	48 M	10	13	LVH	+	n.a.	"	
	24 M	13	10	LVH	+	n.a.	"	
	68 F	19	11	I/II	+	n.a.	"	
	78 F	22	13	I/II	+	n.a.	"	
Arg186Gln n=5 (2 families) Area: U.K.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a	SCD in family	Mogensen et al., 2004
Asp196Asn n=4 (2 families) Area: U.K.	n.a.	n.a.	n.a.	n.a.	+	n.a	-	Mogensen et al., 2004
n=1 with late-onset HCM Area: n.a.	>40	n.a.	LVH	+	+	n.a.	n.a.	Niimura et al., 2002
Leu198Pro n=1 (1 family) Area: Australia	15 M	n.a.	22	n.a.	+	-	SCD at age 15	Doolan et al., 2005
Ser199Gly n=1 (1 family) Area: U.K.	n.a.	n.a.	n.a.	apical	+	n.a.	-	Mogensen et al., 2004
Ser199Asn n=8 (2 families) Area: U.K.	n.a.	n.a.	n.a.	n.a.	+	n.a.	SCD in family	Mogensen et al., 2004
n=1 (1 family) Area: Portugal	52 M	n.a.	n.a.	LVH	+	-	cardiac arrest at age 61	Brito & Madeira, 2005
Glu202Gly n=1 (1 family) Area: U.K.	n.a.	19	n.a.	n.a.	+	n.a.	-	Mogensen et al., 2004
Gly203Arg n=2 (1 family) Area: U.K.	n.a.	n.a.	n.a.	n.a.	+	n.a.	-	Mogensen et al., 2004
Gly203 frameshift n=4 (1 family) Area: Sweden	71 F	9	9	normal	+	+	-	Mörner et al., 2000
	61 M	15	8	LVH	+	-	-	
	64 M	16	14	LVH	+	-	-	
	27 F	8	7	normal	-	-	-	
Arg204His n=3 (1 family) Area: Australia	37 M	n.a.	17	n.a.	+	-	cardiac arrest at age 17	Doolan et al., 2005
	9 M	n.a.	7	normal	+	-	-	
	5 M	n.a.	5	normal	+	-	-	

Table 5. Mutations in the TNNI3 Gene Associated with HCM

Patients/ Family	Clinical Presentation							Reference
	Age/Sex	Septum (mm)	Wall (mm)	LVH type+	ECG	CHF	Prognosis	
Ala8Val n=1 (1025 unrelated p.) Area: U.S.A./ Caucasian	37M	18	n..a	n.a.	n.a.	+	myectomy	Landstrom et al., 2008
Leu29Gln n=1 Area: Germany	60 M	15	15	n.a.	+	+	n.a.	Hoffmann et al. ,2001
Cys84Tyr n=1 (1025 unrelated p.) Area: U.S.A./ Caucasian	17 M	19	n.a.	n.a.	n.a.	-	n.a.	Landstrom et al., 2008
Glu134Asp n=1 (1015 unrelated p.) Area: U.S.A./ Caucasian	22 F	26	n.a.	n.a.	n.a.	+	myectomy	Landstrom et al., 2008
Asp145Glu n=1 (1025 unrelated p.) Area: U.S.A./ Caucasian	58 M	22	n..a	n.a.	n.a.	+	myectomy	Landstrom et al., 2008
c.363dupG frameshift n=1(1 family) Area: U.S.A.	19 M	n.a.	n.a.	n.a.	n.a.	n.a.	SCD	Chung et al., 2011

Table 6. Mutations in the TNNC1 Gene Associated with HCM

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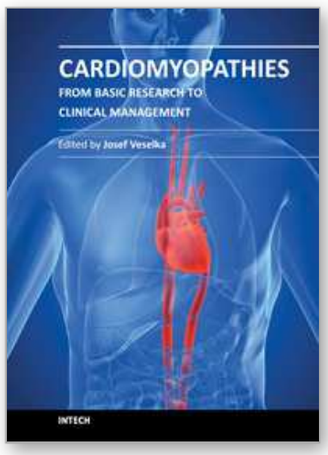

Cardiomyopathies - From Basic Research to Clinical Management
Edited by Prof. Josef Veselka

ISBN 978-953-307-834-2
Hard cover, 800 pages
Publisher InTech
Published online 15, February, 2012
Published in print edition February, 2012

Cardiomyopathy means "heart (cardio) muscle (myo) disease (pathy)". Currently, cardiomyopathies are defined as myocardial disorders in which the heart muscle is structurally and/or functionally abnormal in the absence of a coronary artery disease, hypertension, valvular heart disease or congenital heart disease sufficient to cause the observed myocardial abnormalities. This book provides a comprehensive, state-of-the-art review of the current knowledge of cardiomyopathies. Instead of following the classic interdisciplinary division, the entire cardiovascular system is presented as a functional unity, and the contributors explore pathophysiological mechanisms from different perspectives, including genetics, molecular biology, electrophysiology, invasive and non-invasive cardiology, imaging methods and surgery. In order to provide a balanced medical view, this book was edited by a clinical cardiologist.

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Kornelia Jaquet and Andreas Mügge (2012). Consequences of Mutations in Genes Encoding Cardiac Troponin C, T and I – Molecular Insights, Cardiomyopathies - From Basic Research to Clinical Management, Prof. Josef Veselka (Ed.), ISBN: 978-953-307-834-2, InTech, Available from: http://www.intechopen.com/books/cardiomyopathies-from-basic-research-to-clinical-management/consequences-of-mutations-in-genes-encoding-troponin-c-t-and-i-molecular-insights

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