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Structural Investigation of the Acidosis Resistant Cardiac Troponin I Mutant A162H
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Cardiac muscle contraction is regulated by troponin in a Ca^{2+}-dependent manner. After Ca^{2+} binds to the N-domain of troponin C (cNTnC), the switch region of troponin I (cTnI) associates with cNTnC stabilizing an open conformation that favors contraction. During myocardial ischemia, heart muscle becomes acidic (pH ~6.2) reducing calcium sensitivity and ATPase activity. The acidosis-resistant neonatal TnI isoform contains a histidine residue (H130) that is replaced by an alanine in adult TnI; substitution of this alanine with histidine restores calcium sensitivity and ATPase activity *in vitro* and enhances myocardial contractility *in vivo* (Dargis (2002) JBC; Day (2006) Nature Medicine). However, the effect of this substitution on the switch peptide affinity and mode of interaction with cNTnC is unknown. We used NMR spectroscopy to investigate the binding of an extended switch region of cTnI (residues 144-170), containing the A162H substitution (analog of H130 in skeletal), to cNTnC to assess binding under normal and acidic conditions. We found that the histidine enhances the switch peptide affinity for cNTnC by a factor of ~5 at pH 7 and by more than 40 fold at pH 6. We also investigated whether electrostatics are responsible for the apparent enhancement in binding by determining the pK_a of this histidine and glutamate residues on cNTnC, since deviation from normal values indicates an electrostatic interaction. We found that glutamate residues E15 and E19 in cNTnC show a biphasic titration curve when A162H cTnI is bound, with the first pK_a corresponding to glutamate ionization and the second is associated with H162. In conclusion, cTnI A162H improves myocardial performance during acidosis by making electrostatic interactions with E15 and E19 on cNTnC, increasing the binding affinity of the switch peptide and promoting the open state of cNTnC.

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Transgenic Mice with a Low Expression Rate of the FHC Linked Mutation cTnI-ΔK184 Exhibit Predominantly Signs of Diastolic Dysfunction
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Point mutations in genes coding for cardiac troponin-I (cTnI) were linked to familial hypertrophic cardiomyopathy (FHC). We have shown that cardiac myofibrils isolated from a transgenic mouse model carrying the FHC-linked mutation cTnI-ΔK184 in which >90% of cTnI (h-TG) is mutated have an increase in Ca^{2+}-sensitivity and in passive force, and a slower rate of relaxation (Iorga et al., 2008). In patients the mutated protein amounts to typically <50%. Therefore, we investigated how myofibrillar function is affected in a mouse model in which only ~10% of cTnI (l-TG) carries the mutation. In skinned fibers from the l-TG mice, Ca^{2+}-sensitivity was similar in WT and TG mice at 110% of slack length. After pre-stretching the fibers to 125% of slack length, Ca^{2+}-sensitivity in TG fibers was higher compared to WT (pCa_{50} 5.32 in WT and 5.49 in l-TG, $p<0.01$). In contrast, the increase in passive force and the slow down of the rate of relaxation was present not only in myofibrils from h-TG but also from l-TG mice. In line with these findings, tip catheter measurements showed an increase in end diastolic pressure in both h-TG and l-TG mice *in vivo*. Taken together our results demonstrate that even with a very low expression rate of the FHC-linked mutation, cTnI-ΔK184 signs of diastolic dysfunction both at the myofibrillar and organ level can be detected. The effects of the mutation on the systolic function in contrast appear to depend on the expression level of the mutant protein. This dose dependency may thus underlie at least in part the variable gene-phenotype relation.

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Cardiac Troponin I C-Terminal Mutations (K178E and K179E) Cause Severe Heart Failure and Early Mortality in Transgenic Mice
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The C-terminal region of cardiac troponin I ($cTnI_{164-210}$) plays an important role in cardiac muscle contraction. Most genetic cardiomyopathies in human are associated with the mutations in cTnI C-terminus. Among them, K178E mutation was reported to cause restrictive cardiomyopathy (RCM) in human. Deletion of K177 is related to hypertrophic cardiomyopathy (HCM). Since the amino acids numbered 173-181 bind to actin, which increases the inhibitory effect of TnI, we hypothesize that K177 and K178 are both critical for normal cardiac function. We modeled the RCM mutation of lysine 178→glutamate (K178E) in human cTnI by cardiac specific expression of the mutated protein ($cTnI^{179Glu}$ in mouse sequence) in transgenic mice. In addition, we created $cTnI^{178Glu}$ transgenic mice expressing cTnI K178E as a comparison. The RCM $cTnI^{179Glu}$ transgenic mice showed a dramatic phenotype of a high rate of early death. Some survived TG mice were infertile. Interestingly, the mutation of cTnI K178E (cTnI K177E in human sequence) had a similar phenotype as shown in RCM $cTnI^{179Glu}$ mice. The replacement of the mutant cTnI K178E in $cTnI^{178Glu}$ mouse lines was about 20-40%. Bi-atrial enlargement was a dramatic sign observed in most of the $cTnI^{178Glu}$ TG mice, and was developed early at age of 2 weeks. Severe cardiac dysfunction and early death were observed in these TG mice as well. The data from transgenic mouse studies indicate that like RCM cTnI K179E mutation, the cTnI K178E mutation in mouse hearts can also cause severe cardiomyopathy with heart failure and early death, suggesting that cTnI C-terminus is critical in maintaining normal cardiac function.
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Rescue of a Dilated Cardiomyopathy Mouse Model Caused by a Mutation in Tropomyosin (E54K) by Expression of Slow Skeletal Troponin I
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Dilated cardiomoypathy (DCM) is a disease characterized by decreased contractility and enlargement of cardiac chambers. We have previously shown that E54K mutation in α-tropomyosin (TM54) reduces myofilament Ca^{+2} sensitivity and causes DCM. Thus, we hypothesized that sensitization of the myofilament to Ca^{+2} in early phase of DCM would rescue the phenotype of the disease. Four groups were generated: non-transgenic (NTG), TM54,slow skeletal troponin I (ssTnI) and TM54/ssTnI. To sensitize TM54 myofilament we crossbred TM54 mice with ssTnI expressing mice, which increased Ca^{+2} sensitivity based on force pCa measurements. The systolic function was significantly reduced in the TM54 mice compared to NTG, but restored in TM54/ssTnI mice. TM54 mice also showed increased diastolic LV dimension and HW/BW ratios when compared to NTG, which were improved in the TM54/ssTnI group. Phosphorylation levels of ERK 1/2 trended toward a decrease in TM54 compared to ssTnI control and was restored in the TM54/ssTnI group. β-myosin heavy chain expression was increased in the TM54 animals compared to NTG. When the TM54 animals were crossed with ssTnI, the expression of β-myosin heavy chain significantly decreased thereby partially restoring normal isoform distribution. Analysis by 2D-DIGE indicated a significant decrease in two phosphorylated spots of troponin I in the TM54/ssTnI animals compared to NTG and TM54. The decrease in troponin I phosphorylation contributes to the increased Ca^{+2} sensitivity in the ssTnI transgenic crossed with TM54 transgenic animals. Analysis by 2D-DIGE also indicated no significant changes in troponin T, regulatory light chain, myosin binding protein C and tropomyosin phosphorylation. Our data indicate that myofilament sensitization to Ca^{+2} may be a useful preventative therapeutic strategy in sarcomere-linked DCM associated with decreased sensitivity.

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Molecular Mechanisms of A164H cTnI
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Troponin I (TnI) has a central, isoform-dependent role in ischemic contractile failure. The fetal heart expresses the slow skeletal TnI (ssTnI) isoform which confers protection from ischemia-mediated contractile failure relative to the adult expressed cardiac TnI (cTnI) isoform. A single codon substitution in cTnI, A164H, reverts significantly to an ssTnI phenotype, in specifics, conferring protection from ischemia-mediated contractile failure. Importantly, unlike ssTnI, cTnI A164H does not alter contractile performance under baseline conditions. The molecular mechanisms for the marked enhancement in myocyte function under pathophysiological conditions with no detected effects under baseline conditions have yet to be determined. Isoform specific residues in helix 4 of TnI were investigated through structure/function analysis to gain insight into this mechanism. Molecular dynamics simulation for cTnI, Q157R/A164H/E166V/H173N cTnI (QAEH), A164H cTnI, and ssTnI in complex with cTnC, showed that substitution of cTnI with the ssTnI residues alters the intermolecular interactions between TnI and cTnC. These findings suggest that these residues are important for the conformation of helix 4 in regards to cTnC. To investigate if these substitutions alter function, adult cardiac myocytes were transduced with adenovirus expressing QAEH cTnI and sarcomere dynamics were analyzed. QAEH cTnI showed a similar phenotype to ssTnI, increasing