1823-Pos
Effects of Human Cardiac Troponin T Mutations Associated with Cardiomyopathy
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Mutations in troponin, an important muscle protein complex, can result in cardiomyopathy by interfering with the normal muscle activity of the heart. Troponin T (TnT) is the largest subunit of troponin and is involved in binding the troponin complex to the thin filament. Investigation of two mutations associated with cardiomyopathy in TnT, I90M and R173Q, showed different physiological characteristics. The TnT I90M mutation was identified as the causative agent of familial hypertrophic cardiomyopathy (FHC) in a large multi-generational Chinese family and at least two family members with this mutation died of sudden cardiac death. Another mutation in TnT, R173Q, was identified as the underlying cause of dilated cardiomyopathy (DCM). Patients with the TnT R173Q mutation experienced prenatal onset DCM and supraventricular tachycardia at a young age. Functional troponin complexes containing wild-type or mutant TnT's demonstrated similar maximal actomyosin ATPase activity. The inhibitory ability of the troponin complexes containing the I90M mutation was significantly reduced relative to wild-type TnT. Most RCM mutations investigated to date showed a reduced ability to inhibit actomyosin ATPase activity but the RCM mutation, R173Q, did not affect the inhibitory ability of troponin. The mutations showed increased (I90M) and decreased (R173Q) calcium sensitivity of actomyosin ATPase activity consistent with what has been observed for most FHC and DCM mutations. The mutations reduced the rate of degradation of these proteins by calpain relative to wild-type TnT. Overall, these results suggest that although calcium sensitivity may be an indicator of the type of cardiomyopathy no clear trends in maximal or minimal ATPase activity exist that can be used to characterize DCM and FHC mutations.

1824-Pos
Functional Consequences of a Novel Cardiac Troponin T Mutation Linked to Infantile Restrictive Cardiomyopathy
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A novel double deletion in cardiac troponin T (cTnT) of two highly conserved amino acids (N100 and E101) was identified in the cardiac cDNA of a pediatric transplant recipient. The patient previously presented with restrictive and hypertrophic cardiomyopathy. Family work-up was negative, and she was found to harbor a *de novo* mutation. Electron microscopy revealed the presence of myofibrillar disarray and fibrosis. To further define this cTnT mutation as a cause of the disease, functional studies were performed. Functional effects of the single and double cTnT mutants (ΔN100, ΔE101 and ΔN100/ΔE101) were analyzed in porcine skinned papillary muscle. Fibers were displaced with exogenous cTnT mutants or WT, Ca^{2+} unregulated force was measured and then reconstituted with binary cTnI.cTnC complex. The ΔN100 and ΔE101 mutations showed opposing changes in the Ca^{2+} sensitivity of force development compared to WT. The ΔN100 mutation increased this by 0.29 pCa units and the ΔE101 mutation, in contrast, decreased it by 0.28 pCa units. Interestingly, the ΔN100/ΔE101 mutation shifted the Ca^{2+} sensitivity to the left (+ 0.19 pCa units). This finding is compatible with the preserved systolic function in this patient. ΔE101 was the only mutation that decreased the maximal force recovery compared to WT. In contrast, ΔN100 and ΔN100/ΔE101 did not show significant changes in this parameter. Both ΔN100 and ΔN100/ΔE101 exhibited decreased cooperativity of force development, suggesting altered intra-filament protein-protein interactions. These data show that residue N100 has a predominant effect over E101 and its absence is much more deleterious for cTnT function. In addition, the strength of the functional data validates this novel cTnT deletion mutant as the cause of this cardiomyopathy. Supported by NIH HL-42325(JDP), AHA 0825368E (JRP), AHA 09POST2300030 (MSP) and CIHR GMHD 79045 (GA).

1825-Pos
Biophysical and Biochemical Studies of Human Slow Skeletal Troponin T Isoforms in Slow Skeletal Muscle
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A paucity of information exists concerning the functional roles of the human slow skeletal troponin T isoforms (HSSTnT isoforms) in different muscle types. Three HSSTnT isoforms have been found in slow skeletal muscle: HSSTnT1 (+ Exons 5 and 12), HSSTnT2 (+5, −12), HSSTnT3 (−5, −12) and HSSTnT4 (−5, +12, only found at the mRNA level). Soleus rabbit skinned fibers were displaced with HSSTnT1, 2, 3 or 4 and reconstituted with human SSTnI-C/STnC complex. The extent of Tn displacement was analyzed by measuring the Ca^{2+} unregulated force (UF) at pCa 8.0 after SSTnT treatment. The UF ranged from 63 to 73%. The Ca^{2+} sensitivity increased between SSTnT isoforms: isoform 1 (pCa_{50} = 5.73) < isoform 2 (pCa_{50} = 5.80) < isoform 3 (pCa_{50} = 5.84). HSSTnT4 yielded a pCa_{50} = 5.78. Using a reconstituted fast skeletal muscle system, the actomyosin ATPase activity containing different HSSTnT isoforms was determined. The HSSTnT isoforms did not alter ATPase activation or inhibition in the presence or absence of Ca^{2+}. Potential interactions between human cardiac troponin C (HcTnC), rabbit skeletal tropomyosin (RsTm) and human cardiac troponin I (HcTnI) with SSTnT were mapped. Dot blot analysis using HRP conjugated proteins revealed new interactions between SSTnT peptides and HcTnC, RsTm and HcTnI. These results may help identify the functional differences that occur between SSTnT isoforms due to their alternative splicing. Supported by NIH HL-042325(JDP) and AR-050199 (JDP) and AHA 0825368E (JRP).

1826-Pos
Does the DCM Functional Phenotype Predominate over that of HCM and RCM?
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In vitro investigations into Hypertrophic Cardiomyopathy (HCM) and Restrictive Cardiomyopathy (RCM) show that mutations in cardiac Troponin T (cTnT) produce a pathogenic state via increase in myofilament Ca^{2+} sensitivity, whereas mutations in cTnT that cause Dilated Cardiomyopathy (DCM) decrease Ca^{2+} sensitivity and maximal force. Our aim was to determine whether combinatory mutations of an HCM, RCM and a DCM in cTnT yield an intermediate or dominant functional phenotype that could be correlated with the clinical condition seen in patients. Standard laboratory methods were used for cloning, expression and purification of the WT and mutants: ΔK210 (DCM), I79N (HCM), ΔE96 (RCM), ΔK210/I79N, ΔK210/ΔE96 and ΔE96/I79N. Porcine papillary skinned fibers were displaced with cTnT WT or mutant and reconstituted with HCTnI-TnC. The Ca^{2+} sensitivity of force development, maximal force and basal force were evaluated. The extent of TnT displacement was analyzed by measuring the unregulated tension at pCa 8.0 after cTnT treatment and none of the mutants showed an inability to displace the native cTn complex. Both double mutants (ΔK210/I79N and ΔK210/ΔE96) containing the DCM mutant showed a rightward shift in the Ca^{2+} sensitivity with a decrease in maximal force. In addition, the ΔK210 mutation rescued the impaired relaxation produced by the RCM mutation (ΔE96). From the skinned fiber data, ΔK210 has a dominant effect over I79N and DE96 mutations in cTnT. Circular dichroism measurements demonstrated that all three double mutants had lower alpha helical content than WT. In contrast, single mutants I79N (significantly) and DK210 (showed a tendency) to increase alpha helical content. These results suggest that cTnT can exist in multiple conformations that may be responsible for these distinct functional phenotypes. NIH HL-67415 and HL-42325 (JDP), AHA 0825368E (JRP).

1827-Pos
Cardiomyopathy-Causing Deletion K210 in Cardiac Troponin T Alters Phosphorylation Propensity of Sarcomeric Proteins
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Ca^{2+} desensitization of myofilaments is indicated as a primary mechanism for the pathogenesis of familial dilated cardiomyopathy (DCM) associated with the deletion of lysine 210 (ΔK210) in cardiac troponin T (cTnT). ΔK210 knock-in mice closely recapitulate the clinical phenotypes documented in patients with this mutation. Considerable evidence supports the proposition that phosphorylation of cardiac sarcomeric proteins is a key modulator of function and may exacerbate the effect of the deletion. In this study we investigate the impact of K^{210} deletion on phosphorylation propensity of sarcomeric proteins. Quantitative analysis of cardiac myofibrils isolated from ΔK210 hearts identified a decrease in basal phosphorylation of cTnI (46%), cTnT (29%) and MyBP-C (31%) compared with wild type controls. Interestingly, immunoblot analyses with phospho-specific antibodies show augmented phosphorylation of cTnT-Thr^{203} (28%) and decreased phosphorylation of cTnI-$Ser^{23/24}$ (41%) in mutant myocardium. *In vitro* kinase assays indicate that ΔK210 increases phosphorylation propensity of cTnT-Thr^{203} three fold without changing cTnI-$Ser^{23/24}$