DCM gene. Two of the identified mutations are missense located in the BAG domain of BAG3 known to interact with the chaperone Hsp70 (Heat Shock Protein 70). BAG3 is a cytoprotective co-chaperone involved in proteostasis, and plays a role in autophagic clearance of misfolded or degraded proteins.
The aim of the study is to confirm that BAG3 mutations are responsible for DCM and to identify underlying molecular mechanisms. We studied biochemical and cellular effects of these mutations in rat neonatal cardiomyocytes (RNC) and HeLa cells, with transient overexpression of GFP-fused BAG3 proteins. Fusion protein location was observed by immunofluorescent microscopy. BAG3-wt/mut overexpression in RNC lead to normal expression at the Z-disc. However 48 hours post-transfection, RNC overexpressing BAG3-mut displayed cellular atrophy and disorganized sarcomeres. Potential perturbation of BAG3 partner interaction was investigated using GST pull-down, which demonstrated a loss of BAG3-mut/Hsp70 interaction. The effect of BAG3 mutations on autophagy was examined via western-blot monitoring LC3-II and on proteostasis using a luciferase based reporter assay. These experiments demonstrated that BAG3-mut overexpression, under stress conditions, seems to alter proteostasis regulation.
These results suggest an effect of BAG3 mutants on cardiomyocyte survival, with a faulty proteostasis response to proteotoxic stress, which could be linked to a loss of chaperone interaction. It reveals a new putative pathological mechanism leading to DCM.

P4205 | BENCH
BigIcan is beneficial in angiotensin II induced heart failure by preventing cardiac inflammation and remodeling improving LV function and mortality by preventing transdifferentiation of myofibroblasts

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Biglycan is a small leucine rich proteoglycan and is important for the structural integrity of the extracellular matrix, but also serves as a danger signal and triggers sterile inflammation. Whether biglycan is beneficial or delirious in angiotensin induced heart failure, a model were remodeling as well as inflammation plays a crucial role, is unkown. The present study investigated whether gene deletion of biglycan influences cardiac inflammatory stress response, adverse remodeling as well as apoptosis leading to cardiac dysfunction and mortality after 3 weeks of angiotensin induced heart failure in vivo.
Methods and results: Biglycan knockout mice (BGN-/-) and their controls (WT) were subjected to receive angiotensin II (ANGII) or saline for 3 weeks via osmotic mini pump and 21 days later LV function was analyzed invasively. ANGII induced significant cardiac inflammation (increased CD3 (+3.5 fold) and CD11b+ (+5 fold) cells) as well as cardiac dysfunction in WT-ANGII animals. Interestingly, deletion of BGN impaired cardiac function (significantly impaired stroke work, cardiac output and diastolic function) when BGN-/- ANGII were compared to WT-ANGII. This was associated with increased inflammation (CD3, CD11, cytokine expression TNF-alpha) as well as increased collagen accumulation as detrimental LV remolding in BGN-/- ANGII compared to WT-ANGII. This all increased mortality in BGN-/- ANGII (45% vs 0%) in ANGII induced heart failure. Interestingly, we documented an increased number of myofibroblasts in BGN-/- ANGII, which might explain the accumulation of matrix as well as increased inflammatory response compared to WT-ANGII
Conclusions: Loss of biglycan increased the inflammatory response, which impaired cardiac remodeling and function during ANGII induced heart failure leading to high mortality in vivo. Therefore, biglycan in ANGII induced heart failure seems to be beneficial by preserving the structural integrity of the matrix and preventing increased transdifferentiation of fibroblasts to myofibroblasts.

P4206 | BENCH
Endonuclease G modulates myocardial energy metabolism and function at advanced age

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Background: We have recently shown that endonuclease G (Endo G) is localized in heart mitochondria and necessary for mitochondrial DNA homeostasis and respiration, and modulates cardiomyocyte size. In the present study we investigated the impact of EndoG deficiency on myocardial function and its relation with age.
Methods and results: Studies were performed in EndoG-KO mice and wildtype littermates (WT) of two ages: adults (6-12 months) and old (>24 months). In adult animals, blood pressure monitoring (telemetry) demonstrated no differences between EndoG-KO and WT (mean blood pressure 118.2±4.2 vs 112,2±6.9 mmHg, p = ns), and P NMR spectroscopy of isolated, perfused hearts showed no differences in ATP/creatine phosphate (CrP) ratio (1.17±0.09 vs 1.29±0.2, p=ns). Echocardiography failed to demonstrate any difference in left ventricle end-diastolic diameter, mass or ejection fraction (EF) (p = ns). By contrast, in old mice Endo G deficiency was associated with a reduction in myocardial ATP/CrP ratio in (0.97 vs 1.31, $p < 0.05$), higher left ventricular end-diastolic diameter (4.95 vs 4.65mm,p = 0.05) and left ventricular mass (119.5 vs 100.9 mg, $p < 0.05$) and reduced left ventricular EF (60.7 vs 66.85%, $p < 0.05$), despite no differences in blood pressure (127,1±3.3 Endo G - KO vs 110.3±5.6 WT, p = ns). Analysis of mitochondrial respiration demonstrated a significant reduction in ADP-stimulated O2 consumption with either Complex I (38.9 vs 31 nmolO/minxCS, p = 0.003) or Complex II substrates (69.3 vs 60.1 nmolO/minxCS, p = 0.02).
Conclusion: Endo G deficiency impairs energy metabolism and heart function, but these effect are only observable in old animals. These results suggests that the role of EndoG in heart function becomes more important at advanced age.

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The immune protein MD-2 is a novel independent predictor for long term mortality in dilated cardiomyopathy and non-inferior to nt-pro BNP

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Background: Myeloid Differentiation protein 2 (MD-2) is strongly involved in activation of the innate immune receptor Toll-Like Receptor 4 (TLR4). Its role in cardiac diseases is unknown. Since cardiac inflammation is often associated with heart failure, we investigated in the present study the impact of MD-2 level at first hospital admission in patients with Dilated Cardiomyopathy (DCM) on long term mortality.
Methods and results: We included 174 patients between 2005 and 2011 with biopsy-proven DCM in our study. MD-2 protein expression in serum from patients was quantified by ELISA (USCN Life Science, USA) and nt-pro brain natriuretic peptide (nt-pro BNP) level were quantified during routine diagnostics at first hospital admission. The primary end point of this study was all-cause mortality. Information on vital status of patients was obtained from official resident data files. For descriptive statistics the study population was divided into two groups according to the patients' MD-2 measurement ($\geq$ and $<$ median of all patients). Survival analysis was done calculating Kaplan–Meier curves and log-rank test was used for curve comparison. Multivariable Cox regression models were applied to assess the association between MD-2 level and all-cause mortality, with adjustment for age and cardiovascular risk factors. A value of $p < 0.05$ was considered statistically significant.
All-cause mortality in DCM patients with MD-2 level $\geq$ overall median (302.44 ng/ml) was strongly associated with increased mortality when compared to patients with MD-2 level $<$ overall median (31.0% versus 8.1%, $p<0.001$). Multivariable Cox regression analyses demonstrate, that this effect was independent from age, LV ejection fraction and other cardiovascular risk factors including diabetes, BMI, arterial systolic and diastolic blood pressure and smoking with a hazard ratio of 1.004 ($p<0.001$) for MD-2 as continuous variable. ROC analyses showed no statistically difference between prediction of mortality regarding MD-2 vs. nt-pro BNP (AUC: 0.79 versus 0.74, p=0.40).
Conclusion: MD-2, quantified at first hospital admission, is an independent predictor for mortality in DCM and is non-inferior to nt-pro BNP suggesting a relevant role of this protein in the pathophysiology of DCM.

P4208 | BEDSIDE
The association of cardiovascular reactivity to mental stress with mortality in patients with chronic heart failure

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Objective: Psychological factors have been related to poor outcome in patients with heart failure. Pathophysiological mechanisms explaining this link may include the cardiovascular response to acute mental stress. The current study examined whether heart rate and blood pressure responses to acute mental stress predicted mortality in patients with chronic heart failure.
Methods: Patients with HF (N=100, 26% female, mean age 65±12 years) underwent a public speech task, during which heart rate (HR) and blood pressure (BP) were recorded. Their all cause mortality status was assessed 4.8 years thereafter. Heart rate reactivity was recoded into high, low, and negative responsiveness based on the 25th (bpm $\leq$0) and 75th (>6 bpm) percentile. Blood pressure reactivity was recoded in high (>12.1 mmHg), medium (2.3-12.1 mmHg) and low (<2.3 mmHg) responsiveness based on the 25th and 75th percentile. The following covariates were added to the Cox proportional hazards regression: gender, left ventricular ejection fraction, use of beta blocking agents (HR), use of ACEi/ARB medication (BP), and presence of implanted devices. A p value of <.10 was considered of interest, due to the limited sample size.
Results: At follow-up, 31 patients had died (31%), of whom 15 from cardiac causes. Results from the Cox proportional hazards regression showed that blunted HR reactivity (between 0.5-6 bpm; HR=2.3, 95% CI=0.9-6.2, p=.09) and low diastolic BP reactivity (HR=3.1, 95% CI=1.3-7.9, p=.02) were significantly associated with an increased risk of mortality in HF independent of included covariates (all ns, except ACEi/ARB use (HR=0.3, 95% CI=0.1-0.8, p=.02)). SBP reactivity was not significantly related to future mortality risk.
Conclusion: In this preliminary study, low heart rate and diastolic blood pressure reactivity to acute mental stress were independent associates of all cause mortality in patients with chronic heart failure. The observed blunted autonomic and hemodynamic response to mental stress is in accordance with physical stress