Chronic Heart Failure – Pathophysiology and Mechanisms

Analysis of the pathophysiology of heart failure with reduced and preserved ejection fraction: a non-invasive hemodynamic evaluation

Prior studies have demonstrated key differences in heart failure patients with reduced (HFrEF) vs preserved ejection fraction (HFpEF), but have been confounded by baseline differences in age, body composition, and functional disability. We used combined cardiopulmonary-exercise stress echocardiography (CPET-ESE) to analyse the differences in the HF phenotypes after matching for these baseline factors.

Methods: Subjects with HFrEF (n = 70) and HFpEF (n = 70) were matched 1:1 for age, sex, body mass index, peak oxygen consumption, and minute ventilation/carbon dioxide production slope. All the patients performed a symptom-limited graded ramp bicycle CPET-ESE.

Results: During a median follow-up of 23 months, there were 10 deaths and 67 HF hospitalisations. The distribution of events was not different between HFpEF and HFrEF (HR 1.97; 95% CI 1.01–3.84; Log-Rank P = 0.045). The differences in the pathophysiological characteristics were analysed and the following parameters were compared: peak tricuspid annular plane systolic excursion/systolic pulmonary artery pressure 0.42 ± 0.2 vs 0.55 ± 0.2 mmHg/mL (HFpEF; P < 0.01), as well as left atrium-left ventricle (LA-LV) interaction (LA reservoir strain/LV global longitudinal strain) 1.5 ± 0.8 vs 2.2 ± 1.1 in HFpEF; P < 0.01. When compared to HFrEF, subjects with HFpEF displayed a higher prevalence of metabolic syndrome and higher values of high-sensitivity C-reactive protein (3.68, interquartile range IQR 2.12–6.66 vs. 2.19, IQR 1.37–4.97 mg/L; P < 0.01).

Conclusions: Despite similar baseline characteristics and disease severity, HFrEF and HFpEF reflect different pathophysiological mechanisms. RV-PALA-LV uncoupling, low-grade systemic inflammation and metabolic syndrome are more common in patients with HFpEF than HFrEF.

Plasma P-Selectin: a prognostic biomarker in heart failure with preserved ejection fraction

Purpose: To assess the association of P-Selectin with mortality in HFpEF.

Methods: Prospective, observational study of 130 HFpEF patients (mean age 72 ± 10, males 50%) who underwent extensive phenotyping with blood sampling, six-minute walk testing, echocardiography, cardiovascular magnetic resonance imaging (CMR), calculation of the Meta-Analysis Global Group in Chronic Heart Failure Risk (MAGGIC) scores and blinded plasma P-Selectin measurement.

Results: Overall, HFpEF was characterised by a high burden of co-morbidity: hypertension 91%; obesity 63%; diabetes 50% and metrics of diastolic dysfunction: B-type natriuretic peptide (BNP) 140 [66–258] ng/L; left ventricular mass indexed (LVMI) 53 ± 15 g/m², maximal left atrial volume indexed (LAVImax) 54 ± 26 ml/m²; 

Conclusions: Above median P-Selectin levels were associated with greater risk of all-cause mortality (HR 1.97; CI 1.016–3.837; Log-Rank P < 0.045 – see Figure). Excluding components of the MAGGIC score, there were 12 parameters demonstrating univariate association with the endpoint of P < 0.1 including: diastolic blood pressure, previous HF hospitalization, loop diuretic use, haemoglobin, BNP, echocardiographic E/E′, LVMI, indexed right ventricular end-diastolic and end-systolic volumes, LAVImax, myocardial infarction (MI) detected by CMR and plasma P-Selectin. Following multivariable Cox proportional hazards regression analysis and when added to MAGGIC scores, only P-Selectin (adjusted hazard ratio [HR] 1.707; 95% confidence interval [CI] 1.099–2.560; P = 0.017) and MI detected by CMR (HR 2.377; CI 1.114–5.075;
Nicotinamide treats cardiac hallmarks of experimental heart failure with preserved ejection fraction

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Introduction: Coincidence of congestive heart failure (CHF) and chronic kidney disease (CKD) results in poor survival rate, therefore new therapeutic approaches are needed.

Objective: The aim of the study was to examine if renal denervation (RDN) would improve the survival rate in CHF induced by creation of aorto-caval fistula (ACF).

Methods: This was studied in fawn-hooded hypertensive rats (FHH), a genetic model showing CHF development. Fawn-hooded low-pressure rats (FHL), without CKD, served as controls. RDN was performed four weeks after creation of ACF and the follow-up period was 10 weeks.

Results: We found that intact (non-denervated) ACF FHH exhibited survival rate of 58.8% (5 of 34 rats), significantly lower than in intact ACF FHL (81.3%, 26/32 rats). In intact ACF FHL rats albuminuria remained stable throughout the study whereas in ACF FHH it increased significantly, up to a level 40-fold higher than the basal values. RDN did not improve the survival rate in either ACF FHL or ACF FHH and did not alter the course of albuminuria in ACF FHL. In contrast, RDN attenuated the rise in albuminuria in ACF FHH and at the end of the study it was about 40% lower than in intact ACF FHH.

Conclusions: Our present results support the notion that even modest CKD increases CHF-related mortality. While RDN did not attenuate CHF-dependent mortality in ACF FHH, it delayed the progressive rise in albuminuria, suggesting that renal denervation could be a novel therapeutic measure for the treatment of CHF, particularly the form associated with CKD.

Funding Acknowledgements: Ministry of Health of the Czech Republic grant no. nu 20-02-00052 awarded to LG and VM.

Effect of epoxyeicosatrienoic acid analogue alone or combined with angiotensin-converting enzyme inhibitor on the course of congestive heart failure induced by aorto-caval fistula in hypertensive rats

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Introduction: The long-term prognosis of patients with congestive heart failure (CHF) remains poor, thus new therapeutic strategies for the treatment are needed. The current knowledge suggest that targeting the eicosanoid system could be a promising approach. Absolute or relative lower plasma and organ levels of epoxyeicosatrienoic acids (EET) might contribute to progression of CHF.

Purpose: To examine if chronic treatment by epoxyeicosatrienoic acid analogue (EET-A) would attenuate the course of CHF induced by volume overload achieved by creation of the aorto-caval fistula (ACF).

Methods: In Ren-2 transgenic rats (TGR), a model of angiotensin II-dependent hypertension, chronic treatment by EET-A was achieved by 14,15-EET analogue (100 mg.L−1 in drinking water) and combined therapy by EET-A + ACEI (14,15-EET analogue, 100μg.L−1 and trandolapril, 2 mg.L−1 in drinking water). ACF was created at the animal age of 8 weeks, 2 weeks later the animals were randomly divided into 4 groups and the therapy began (EET-A + ACEI n = 32 or placebo n = 30). Sham-operated TGR represented a control group (n = 8). The follow-up period was 20 weeks. GraphPad Prism 7 was used for statistical analysis with log-rank (Mantel-Cox) test for analysis of survival data.

Results: All sham-operated rats survived till the end of study. Untreated CHF rats began to die at the week 3 after creation of ACF and all the animals died by the
week 12. The treatment with the EET-A or with ACEi similarly improved the survival rate, and the final rate was 45% in EET-A group (14 of 31 rats; vs. placebo $P < 0.01$) and 62% in ACEi group (20 of 32 rats; vs. placebo $P < 0.01$), with non-significant intergroup difference – ACEi vs. EET-A, $P = 0.09$. Combined therapy by EET-A + ACEi improved survival similarly with the final rate 72% (23 of 32, vs. placebo and 62% in ACEi group (20 of 32 rats; vs. placebo).

Conclusion: Our results suggest that in ACF TGR the treatment with EET analogue markedly attenuates CHF-dependent mortality and reduces albuminuria, both to the same extent as with ACEi treatment, however combination treatment by EET-A and ACEi did not show significant additional improvement in survival over ACEi alone.

Statin is associated with lower cancer risk and cancer related mortality in patients with heart failure: a territory-wide study

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Funding Acknowledgements: None

Background: Patients with heart failure (HF) is associated with a high risk of cancer. The effect of statin use among patients with HF on cancer risk and cancer-related mortality is nonetheless unknown.

Methods: Using a territory-wide clinical information registry, statin use was ascertained among all eligible patients with HF (N = 87102) from 2003 to 2015. Inverse probability of treatment weighting was used to balance baseline covariates between statin users (5926 patients) with statin nonusers (36176 patients). Competing risk regression with Cox proportional-hazard models was performed to estimate the risk of cancer and cancer-related mortality associated with statin use.

Results: Of all eligible subjects, 11052 (12.7%) were diagnosed with cancer. Statin use among patients with HF was associated with primary endpoint hazard ratio (HR) 1.41; 95% confidence interval (CI): 1.06;1.90, $P = 0.02$). This result seemed to be mainly driven by history of HF (HR 1.61; 95%CI: 1.13;2.27, $P = 0.01$) versus HFpEF (HR 1.19; 95%CI: 0.79;1.81, $P = 0.41$).

Conclusions: History of HF in COVID-19 patients was associated with a higher risk of in-hospital death or orotracheal intubation, suggesting that they are at increased risk for clinical deterioration.

Multivariate analysis

<table>
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<th>Hazard Ratio</th>
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<tr>
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<tr>
<td>Age</td>
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</tr>
<tr>
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<td>1.28;1.95</td>
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<tr>
<td>Diabetes</td>
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<td>Coronary artery disease</td>
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<tr>
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<td>Chronic kidney disease</td>
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<td>Global HF</td>
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<tr>
<td>qSOFA score</td>
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<td>1.68;2.64</td>
</tr>
</tbody>
</table>

HF denotes Heart Failure; qSOFA, Quick Sepsis-related Organ failure Assessment; RAAS, renin-angiotensin-aldosterone system inhibitors

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On behalf of: Critical COVID-19 France Investigators

Funding Acknowledgements: NCT04344327

Background: Data concerning COVID-19 patients with pre-existing heart failure are scarce.

Aims: To investigate the incidence, characteristics and outcomes of COVID-19 patients with a history of heart failure (HF) with preserved (HFpEF) or reduced (HFrEF) ejection fraction.

Methods: All patients hospitalized for COVID-19 across 24 centres in France were included from February 26 to April 20, 2020. Primary endpoint was a composite of in-hospital death or orotracheal intubation.

Results: Of 2809 patients (66.4 ± 16.9 years), 317 patients (11.2%) had a history of HF. Among them, 49.2% had HFpEF and 50.8% had HFrEF. COVID-19 severity at admission, defined by a quick sequential organ failure(qSOFA) score>1, was similar in patients with versus without a history of HF. Before and after adjustment for age, sex, male, cardiovascular comorbidities and qSOFA score, history of HF was associated with the primary endpoint hazard ratio (HR) 1.41; 95% confidence interval (CI): 1.06;1.90, $P = 0.02$). This result seemed to be mainly driven by history of HF (HR 1.61; 95%CI: 1.13;2.27, $P = 0.01$) versus HFrEF (HR 1.19; 95%CI: 0.79;1.81, $P = 0.41$).

Conclusions: History of HF in COVID-19 patients was associated with a higher risk of in-hospital death or orotracheal intubation, suggesting that they are at increased risk for clinical deterioration.
Ketone bodies improve myocardial function in heart failure

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Funding Acknowledgements: None

Background: Beta hydroxybutyrate (BHB) is the main ketone body produced during fasting or carbohydrate deprivation as an alternative fuel. Mounting evidence suggests that after myocardial infarction (MI), mitochondrial impairment and metabolic failure coincide with increased levels and utilization of BHB. However, whether the observed increase in BHB is adaptive or maladaptive in damaged myocardium, has never been evaluated.

Aim: Our main scope was to explore the effects of BHB on cardiac function after ischemia both in vivo and in vitro.

Methods and Results: In cultured cardiomyoblasts, the administration of BHB (3 mMol) reduces the activation of caspase-3 in response to ischemia, as well as the number of tunnel positive nuclei. Specifically, the mitochondrial apoptotic pathway seems affected, as BHB reduces cytochrome-C release induced by ischemia. Mitochondrial structure and interconnections, soundly affected by ischemia, were significantly retained in presence of BHB, as well as mitochondrial membrane potential (assessed via TMRE). The preserved mitochondrial health was further supported by higher levels of PGCG1-alpha detected in cells exposed to ischemia plus BHB as compared to ischemia alone. To explore the in vivo effects of BHB on ischemia damaged- myocardium, we administered carbohydrate-null diet (ketogenic diet, KD) or standard diet-supplemented with BHB, to post-MI mice. Both groups treated with KD and BHB supplemented diet displayed preserved left ventricular ejection fraction respect to untreated infarcted mice. The protective effects of BHB on cardiac phenotype were mirrored by increased levels of PGCG1-alpha in the myocardium of treated mice, in terms of protein and transcription levels. Interestingly, in the hearts of mice fed KD and BHB supplemented diet, we observed a marked difference in histone acetylation pattern.

Conclusions: BHB protects cardiac cells from apoptotic and mitochondrial damage induced by ischemia. Through its ability to regulate epigenetic modifications, BHB could activate a gene expression program able to support mitochondrial function, thereby representing a powerful therapeutic strategy.

Myocardial Disease – Pathophysiology and Mechanisms

M1/M2 ratio predicts cardiac systolic function after myocardial infarction with ST elevation (STEMI)

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Funding Acknowledgements: None

Background: In response to myocardial infarction (MI), cardiac monocytes and macrophages regulate inflammation and scar formation playing a crucial role in the response to ischemia. In this study, we sought to examine the dynamic changes of the 2 monocyte subsets in a contemporary population with anterior STEMI and correlate these changes with the short-term cardiovascular outcome.

Methods We enrolled prospectively 64 patients with a diagnosis of anterior STEMI, with total ischemic time inferior to 4h. Levels of classical and nonclassical peripheral monocytes were evaluated by FACS analysis at hospital admission (time 0), 48 and 96h post-admission. After 96 h from admission all patients underwent a cardiac echography for the evaluation of cardiac volumes, left ventricular ejection fraction (LVEF) and left ventricular global longitudinal strain; patients were divided into two groups according to the LVEF (LVEF <45% and LVEF ≥45%).

Results Baseline characteristics, pharmacological treatment, total ischemia time and post-PCI TIMI flow did not show significant differences with the exceptions of diuretics use that was prevalent in the LVEF ≤45% population (20% n=4 in LVEF ≤45%; n=0 in LVEF >45%; P=0.001), cardiac arrest (20% n=4 in LVEF ≤45%; n=0 in LVEF >45%; P=0.001), and ventricular fibrillation (20% n=4 in LVEF ≤45%; n=0 in LVEF >45%; P=0.001). Total peripheral monocyte counts were not different between the two populations at the three time-point (P=0.486 at T0; P=0.213 a T48; P=0.456 a T96). However, M1 monocytes were significantly increased at T0, T48 and T96 in the LVEF ≤45% (T0: 63.8%, T48: 60%, T96: 55.8%) compared to ischemia alone. Also M2 monocytes were significantly increased at T0, T48 and T96 in the LVEF ≥45% (T0: 85%, T48: 98%, T96: 91%) than the LVEF ≤45% (T0: 67%, T48: 44.9%, T96: 36.9%; P= 0.005 for T0, P=0.002 for T48, P=0.000 for the T96). The T48 and T96 M1/M2 ratio was reduced in the LVEF ≤45% group. Moreover, the M1/M2 ratio at 48 and 96 h presented a significant positive linear correlation with the LVEF (R=0.063 at T0, P=0.272; R=0.128 at T48, P=0.016; R=0.316 at T96, P=0.001) confirmed by the logistic regression (OR=1.646 at T0, P=0.019; OR=4.339 at T48, P=0.005; OR=4.635 at T96, P=0.014).

Conclusions: In summary, elevation of M1 monocytes and a lower M1/M2 ratio are related to worse LVEF post-STEMI, possibly indicating a more pronounced pro-fibrotic state. Our data demonstrate that M1 and M2 monocytes expression can predict the outcome of the systolic cardiac function in a short time after STEMI.

Targeting GRK2 to prevent aortic valve calcification

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Funding Acknowledgements: None

Background: Calcific aortic valve stenosis (CAVS) is a clinically relevant issue due to the lack of drugs for prevention or treatment. CAVS is driven by endothelial dysfunction and inflammation. A novel therapeutic strategy should target specific molecules involved in the regulation of both endothelial function and immune responses. G protein-coupled receptor kinase 2 (GRK2) regulates desensitization and downregulation of G protein-coupled receptors, but more recently, it has been shown to interact with an extensive repertoire of proteins. We previously demonstrated that the lack of this protein in the endothelium promotes vascular inflammation and atherosclerosis in mice due to increased mitochondrial reactive oxygen species (ROS).

Purpose: This study aimed to evaluate the role of GRK2 in Aortic Valve Calcification (AVC) by in vivo and in vitro studies.

Methods: To reach our purpose we evaluated GRK2 expression in mitochondria isolated from the valve of patients with calcified (CAVS) aortic valve leaflets vs. control ECs. We also performed histological analysis by using 12 months old mice with selective endothelial knock-out of GRK2 (Tie2Cre;GRK2fl/fl) in EC compared to control (GRK2fl/fl) to evaluate the presence of microcalcification in the aortic valve. Finally, we cloned a small sequences of the PH domain of JARKt into the pcDNA3 to induce GRK2 localization into mitochondria.

Results: In vitro, we observed a significant downregulation of GRK2 expression into the mitochondria of CAVS VECs than control VECs, which associates to an increased ROS production. Histological analysis revealed that GRK2fl/fl mice of 12 months-old display presence of microcalcification, as expected. However, this phenotype is significantly more pronounced in the Tie2Cre;GRK2fl/fl, demonstrating that the lack of GRK2 in the EC accelerates the calcific degeneration of the aortic valve in mice. A previous report demonstrated that JARKt transfection in macrophage increases mitochondrial biogenesis and reduces ROS production. Here, we cloned several small sequences of the PH domain of JARKt into the pcDNA3.1 plasmid, named as PH1-4. We found that the transfection into HEK293 cells of the PH4 potently increased GRK2 localization into the mitochondria as compared to JARKt, PH4, and pcDNA3 as control. PH3 also determined increased biogenesis and reduced ROS production after AngII stimulation. These data support the concept that a smaller portion of the PH domain of JARKt can reproduce its biological effect.

Conclusion: In conclusion, our data suggest a direct involvement of GRK2 expression/localization in the pathogenesis of CAVS. Intracellular re-localization of GRK2 could be a novel strategy to prevent AVC in a pathophysiological condition such as ageing.

Microarray analysis to identify potential biomarkers in adverse cardiac remodeling in patients with myocardial infarction: pilot study

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Funding Acknowledgements: None

Introduction: The mechanism of adverse cardiac remodeling following myocardial infarction (MI) remains unclear. It consists of variety of changes at different biological levels. Transcriptome is one of the most intriguing levels. Nowadays we have series of transcriptome databases with experimental data on post-infarction cardiac remodeling. Obviously, not all experimental data can be extrapolated to the clinical studies. The response to ischemic injury in the infarct zone and remote myocardium
has a definite spatio-temporal sequence and underlies the mechanism of cardiac remodeling. However, potential differences in gene expression between the infarct zone and remote myocardium in patients with MI are not investigated yet.

**Purpose:** The purpose of the research was to identify the differentially expressed genes (DEGs) between the infarct zone and remote myocardium in patients with MI.

**Methods:** The study included 4 patients with fatal MI type 1. All patients died within 48 h of MI. The post-mortem examination was performed according to country policy. In each case, we obtained myocardial samples from the infarct zone and remote myocardium. Genome-wide gene-level expression was assessed by microarray analysis. We used transcriptome analysis software to analyze and visualize global expression patterns of genes. DEGs were defined as gene-level fold change < -2 or >2 and adjusted P-value of < 0.05.

**Results:** We studied expression of 21448 genes. A total of 71 DEGs, including 41 up-regulated genes and 31 down-regulated genes, were identified (Figure 1). Up-regulated genes included FGR, DTA, BMS, PFGFR1, TRAF1, PLCB1, PRDM1, SELE, PLP1, ANKRD13A, MTA3, ATP6V0C, MMS22L, ATXN1, OR10G8, NAA16, MYH9, PRELID1, NPM3, GGT5, HAGH, VAPM1, CUECD2, AP1AR, Cbfbfs5, PECR, TSPYL2, SORBS1, CCG1L, FRMD6, GRK4, CYTIP, TCTC3B, DOCK2, FGFR4, KLR3, SLTRK6, DNAJC5G, CADM2, SNIP1, down-regulated genes included FAM44B, PL51, CAMK1, SW5, CYP2S1, CRB3, ULBP1, RBPM52, GTF2F1, MCS1R, MP3C, CDDC121, CARM1, PHLP2, OR10G8, NAA16, MYH9, PRELID1, NPM3, GGT5, HAGH, UP54, SUSD6, HYOU1, MRI4658, BCL7C, TCT7B, ADSS1, HYOU1, RCAN1, COBL. Our analysis revealed that up-regulated DEGs were mainly enriched in the platelet activation and aggregation pathways, in cytochrome C pathway, in chemokine signaling pathway, in negative regulation of mononuclear cell migration, in neurons necrosis caused by energy deficiency, in positive regulation of extracellular matrix disassembly, in positive regulation of mast cell degranulation.

**Conclusion:** The results of our pilot study have demonstrated differences in gene expression between the infarct zone and remote myocardium in clinical settings. Following steps of our research can include bioinformatics analysis and validation process.

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**Basic Science – Cardiac Biology and Physiology**

**Mechano-energetic uncoupling underlies lack of inotropic reserve in Barth syndrome cardiomyopathy**

**Mechanistically, TissueFactor (TF) was identified as a direct target of miR-145, while miR-885 targets the von Willebrand Factor (vWF). Equally important, miR-424 has been associated with hyper-coagulability whereas low levels of miR-103a have been observed in deep vein thrombosis, although precise mechanisms have not been defined for these miRNAs. Using a twostage multiple regression analysis, exosomal miR-424 was an independent predictor of thromboembolic events (i.e. pulmonary embolism and acute myocardial infarction) in COVID-19 patients (Wald: 4.180; P < 0.005), whereas miR-103a independently regulated D-dimer levels (P < 0.001).

**Conclusions:** To our knowledge, this is the first study showing a significant contribution of exosomal non-coding RNAs in COVID19. Limitations of our study include the relatively small patient population and the fact that we did not determine the exact source of exosomal non-coding RNAs. Nevertheless, we speculate that a main source could be represented by endothelial cells and/or platelets, which express these miRNAs in normal conditions.

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**Cardiovascular Disease**

**MicroRNAs drive endothelial dysfunction and thromboembolism in COVID-19**

**Background:** Endothelial dysfunction and thromboembolism are frequently observed in COVID-19 patients, indicating the potential role of microRNAs in these processes. Previous studies have identified microRNAs that are differentially expressed in COVID-19 patients compared to healthy controls. These microRNAs are known to regulate key pathways associated with endothelial dysfunction and thromboembolism.

**MicroRNAs drive endothelial dysfunction and thromboembolism in COVID-19**

**J Gambardella†1; M Morelli‡1; C Sardu; p Maggi§1; A Matarrese†1; R Marrella†1; V Messina§; S Jankauska§; G Padiloso‡; G Gaetano Santulli†1; University Hospital Federico II, Naples, Italy; † Albert Einstein College of Medicine, The Bronx, USA; ‡ Varelli University, Naples, Italy; § AO dei Colli-Monaldi Hospital, Naples, Italy; † Funding Acknowledgements: None**

**Background:** Thromboembolic complications play a crucial role in the clinical outcome of COVID-19. Emerging evidence has shown that exosomal microRNAs (miRNAs) are functionally involved in several pathologic processes. In March 2020, we were the first group to functionally link COVID-19 and endothelial dysfunction.

**Methods:** To test the hypothesis that exosomal miRNAs are a key determinant of thrombosis in COVID-19, we enrolled 26 COVID-19 patients. The study was approved by the local Ethical Committees. Circulating exosomes were isolated as described above. We assessed the expression of selected exosomal miRNAs in patients with COVID-19 compared to healthy controls.

**Results:** We observed significant dysregulation of multiple exosomal miRNAs in COVID-19 patients, including miR-126, miR-146a, and miR-155. These miRNAs are known to target various genes involved in endothelial function and thrombosis.

**Conclusion:** Our findings suggest that exosomal miRNAs play a crucial role in the development of endothelial dysfunction and thromboembolism in COVID-19, highlighting the potential therapeutic opportunities for targeting these miRNAs.
major clinical problems in BTHS, i.e., the inability of the heart to increase contractility during exercise and the increased risk of arrhythmias.

**TMEM43 mutation p.S358L results in changes in cardiac morphology and performance in transgenic zebrafish**

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**Funding Acknowledgements:** Grant number 01E01504, Federal Ministry of Edu-
cation and Research, Germany (BMBF)

**Background:** Arrhythmogenic cardiomyopathy (ACM) is an inherited heart muscle disorder, predisposing to arrhythmias, heart failure and sudden cardiac death. A fully penetrant heterozygous missense mutation c.1073C>T (p.S358L) within the highly conserved gene of transmembrane protein 43 (TMEM43) has been genetically identified to cause a severe subtype of ACM. A second variant (c.332C>T; p.F111L) within TMEM43 has not been functionally validated yet. TMEM43 is an integral protein of the inner nuclear membrane and is involved in maintaining the structural integrity of the nuclear envelope.

**Methods and Results:** We generated cardiomyocyte-restricted transgenic zebrafish lines that overexpress eGFP-linked full-length human wildtype TMEM43 and the respective mutations using the Tol2-system. Mutant transgenic zebrafish are viable and survive until adulthood. At 72 h post fertilisation cardiac phenotyping of the F3 incross generation revealed an elevated percentage of cardiac development defects in TMEM43 mutant zebrafish larvae. Furthermore, both TMEM43 mutant zebrafish lines showed a decreased end-diastolic and end-systolic ventricular area. However, the overall ventricular contractility was enhanced, indicated by increased fractional shortening and fractional area change values. Moreover, the contractions appeared to be much more heterogeneous with hypercontractility localised at certain areas of the ventricle. To identify the reason for the smaller ventricular size, whole-mount immunofluorescence (IF) staining unveiled a significantly reduced cross-sectional area of the ventricular cardiomyocytes in the p.S358L-mutant zebrafish. As the mean nuclear area was also smaller, the nuclear-cytoplasmic ratio remained unchanged, as well as the number of overall nuclei. This leads to the hypothesis that pathways involved in growth and proliferation are potentially affected. To analyse whether the mutation in TMEM43 has an impact on its localisation, we performed IF staining on fixed larvae and paraffin sections of adult hearts. In TMEM43-WT and -p.F111L, the TMEM43-eGFP fusion protein localised properly at the nuclear envelope and the endoplasmic reticulum, whereas TMEM43-p.S358L displayed strikingly a delocalisation in the cytoplasm and a reduced overall expression. Assessment of the heart morphology at 5 months of age showed that TMEM43-p.S358L ventricles of dissected hearts remain significantly smaller compared to controls as indicated by a reduced ventricular surface area/body length ratio. On ultrastructural level TMEM43-p.S358L ventricular tissue displayed an elevated proportion of nuclei that exhibit expanded perinuclear space and bulging of the outer nuclear membrane.

**Conclusion:** Our results demonstrate that intact TMEM43 seems essential for nuclear morphology and proper cardiac development in transgenic zebrafish. Further examinations will focus on pathways involved in cardiomyocyte growth and proliferation.

**A 3D model for myocardial infarction in living myocardial slices**

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**Funding Acknowledgements:** The Institute of Molecular and Translational Therapeutic Strategies (IMTTS)

**Introduction:** Cryoinjury has been recently used as an alternative approach to mimic myocardial infarction in various in vivo studies. This model, often performed in rodents or zebrafish, was shown to be representative for human infarcts encountered in clinical practice. Here, we performed cryoinjury on rat living myocardial slices, which are ultrathin sections of cardiac tissue that maintain the native multicellularity, architecture, and structure of the heart. This in vitro 3D model preserves tissue viability in the absence of coronary perfusion due to free diffusion of oxygen and nutrients into its innermost cells, preventing ischemic damage and allowing for chronic culture.

**Purpose:** The aim of this study is to establish in vitro model of myocardial infarction in living myocardial slices using cryoinjury.

**Methods:** Living myocardial slices were generated from rat left ventricular tissue and were subjected to cryoinjury to damage 30% of their length. Cryoinjured slices, along with control slices, were cultured while electromechanically stimulated and mechanically stretched to achieve a sarcomere length of 2.1 μm. Force measurements of freshly prepared slices and cultured slices for 24 and 48 h were performed. Histological studies were done to assess tissue viability, fibrosis markers and cardiomyocyte cross-sectional area. Eventually, slices were cut into two parts: peri-injury (including the cryoinjury with 1 mm margins of neighbouring tissue) and remote regions. Both regions were then analyzed for cardiac remodeling marker genes applying real-time PCR.

**Results:** Cell viability assessment using live/dead cell staining demonstrated complete death of cells in the cryoinjured area of myocardial slices. Measurement of contractile force of cryoinjured slices after 24 h in culture revealed reduced maximal contractility and slower kinetics, including time to peak, time to relax and decay rate compared to control slices, indicating that contractile function of myocardial slices deteriorated significantly following cryoinjury. Gene expression analysis highlighted an increase in fibrotic gene activation in the remote region, as well as an increased expression of inflammatory markers in the peri-injury region of myocardial slices compared to control. Quantification of cardiomyocyte cross-sectional area demonstrated hypertrophy of cardiomyocytes in the remote region of cryoinjured slices compared to both the peri-injury area and the control slices.

**Conclusions:** Based on contractile, structural and selective transcriptional analysis, we here report a reproducible model of myocardial infarction in living myocardial slices. This 3D model could be utilized for investigating various aspects of cardiac biology, such as electrophysiology, biochemistry and molecular biology, in addition to high applicability in novel drug discovery and regenerative medicine.

**Cryoinjury model of MI**

**Metabolic adaptations to dysfunctional mitochondria in Barth Syndrome cardiomyopathy**

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**Funding Acknowledgements:** The work in the laboratory of the authors is supported by the Deutsche Forschungsgemeinschaft (DFG; DU1839/2-1), the Bundesministerium für Bildung und Forschung (BMBF) and the Barth Syndrome Foundation

As one of the most energy consuming organs in the human body, the heart strongly depends on mitochondrial oxidative phosphorylation to cover its large energy demand. The largest contributor to energy metabolism is the β-oxidation of fatty acids. The compartmentalization of mitochondria by two membranes structurally organizes the enzymes of β-oxidation, Krebs cycle and the respiratory chain. The essential functions of the inner membrane critically depends on the hallmark phospholipid cardiolipin (CL). Inherited defects in the biogenesis of CL causes Barth Syndrome (BTHS), which is associated with mitochondrial dysfunction in respiratory and the Krebs cycle and cardiomyopathy. CL deficiency not only affects energy conversion but also also abrogates redox homeostasis, another essential function of the mitochondria. Increased consumption of reduction equivalents under conditions of elevated workload, must be compensated by an increase in mitochondrial metabolism. Calcium signals ensure the energetic coupling of mitochondrial and cytosolic metabolism. In BTHS, this coupling is disrupted by a defect in the mitochondrial calcium uniporter (MCU), which transmits calcium emitted from theryanodine receptor into the mitochondrial matrix.

Using a mouse model for BTHS and patient iPSC derived cardiomyocytes, we show here, that defects in energy and redox homeostasis causes significant changes in cardiac metabolism. Using in vivo PET-CT imaging, we find evidence for an extensive metabolic remodeling, including a considerable reduction in fatty acid oxidation and an increase in glucose metabolism in Barth syndrome. The change in metabolism precedes changes in cardiac function in the mouse model. Transcriptome analyses of the BTHS heart muscle revealed the upregulation of a stress-induced retrograde signaling pathway. Independent of the change in carbohydrate metabolism,
this pathway induces a unique compensatory transformation of metabolism. We describe changes in folate metabolism and alterations in the glutamate metabolism and glutathione biosynthesis, which serve to compensate the defects in mitochondrial energy and redox homeostasis. Targeted interventions in the cell metabolism will enable new strategies for therapeutic intervention in cardiomycopathies with mitochondrial dysfunctions.

Methods: We used CRISPR/Cas9 transcriptional modulation based on enzymatically inactive Cas9 (dCas9) fused to transcriptional activators (VPR). The dCas9-VPR complex is recruited by guide RNAs (gRNAs) to the transcriptional start site of genes (CRISPRa) in both, mouse cardiomyocytes and in human induced pluripotent stem cell derived cardiomyocytes and in human induced pluripotent stem cell derived cardiomyocytes and in human induced pluripotent stem cell derived cardiomyocytes in which Myh6 promoter as well as II) in hiPSC and III) hiPSC-CM driven by Cag promoter. No adverse effects were observed in all conditions and transgene expression was maintained along the course of experiments. KLF15 expression was significantly enhanced by application of both, single guide RNAs (gRNAs) and multiple gRNAs (1.8 fold change for single and 2.6 fold change for triple gRNA compared to non-targeted gRNAs and control clone). We further used an improved genetically engineered dCas9-VPR expression resulting in enhanced KLF15 activation of up to 5.5 fold (n = 2 experiments, 2 independent CRISPRa clones).

Conclusion: We showed feasibility to modulate gene expression by CRISPR/Cas9/VPR in adult mouse and human cardiomyocytes. The titratability of expression levels based on gRNA selection as well as dCas9/VPR levels allows for precise gene activity modulation to physiologically relevant expression levels not achievable by classical overexpression approaches. These tools allow us to validate novel therapeutic targets, such as KLF15, to prevent heart failure progression.

Basic Science – Cardiac Diseases

Vitamin D binding protein as a potential biomarker for heart failure in myocarditis: translational animal model reveals mechanisms

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Funding Acknowledgements: None

Introduction: An estimated 3.1 million cases of myocarditis/cardiomypathy were diagnosed in 2017. Myocarditis, inflammation of the myocardium, is a leading cause of sudden death in children and young adults worldwide. Patients can progress to dilated cardiomypathy, heart and heart transplant. Studies have found that Vitamin D binding protein (DBP) could be instrumental in response to tissue injury and activation of the immune response. This chemotaxis activation has been shown to be due to activation of the complement cascade via C5a. The involvement of DBP in disease has never been assessed for myocarditis or other cardiovascular diseases.

Purpose: We hypothesize that DBP activates the immune response by altering the complement cascade in a sex-specific manner leading to increased myocarditis.

The overall goal of this study is to examine the role of DBP and complement in patients with myocarditis and determine the mechanism whereby DBP alters cardiac inflammation in a translational animal model of viral myocarditis.

Methods: We utilized a translational animal model of viral myocarditis to assess the mechanism whereby DBP could alter disease pathway activation and increase acute myocarditis. We also utilized stored serum samples from myocarditis patients and patient data to assess the relationship between DBP levels in patient serum vs. left ventricular (LV) ejection fraction (EF) by sex and age.

Results: We found that DBP was upregulated 45-fold in men with myocarditis compared to women and correlated to %EF in women (P = 0.0004). Additionally we found that men with myocarditis had significantly higher levels of complement C3, C4b, C9 (P <0.0001) in their sera than women with disease, suggesting sex differences in DBP and complement influence cardiac inflammation. We found that DBP exacerbates myocarditis using DBP deficient (DBP−/−) mice in our animal model of CBV3 myocarditis in both female (P = 0.009) and male mice (P = 0.003). Increased disease was attributed to shifts in cytokines; IL-17 (P = 0.0004) in female mice and IFNγ (P = 0.04) and IL-1β (P = 0.04) in male mice and increased activation of the complement cascade; C5a (females P = 0.03, males P = 0.02), CR1 (females P = 0.04), C3 (females P = 0.008, males P = 0.04), C3bR1 (males P = 0.02), C4b (males P = 0.03), and C3aR1 (males P = 0.03) in a sex specific manner. We also found in our myocarditis mouse model that sex differences in complement C3 and CR1 may increase susceptibility to myocarditis in males.

Conclusion: Our translational animal model of viral myocarditis has revealed that DBP can increase inflammation, myocarditis, by upregulating the complement cascade. We found that having a mechanistic understanding of circulating DBP in patients and mice could be used to predict disease severity in patients with myocarditis and potentially other forms of heart failure.

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Tenascin C promotes cardiac fibrosis, inflammation and endothelial dysfunction in a mouse model of diabetes

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Funding Acknowledgements: Stiftung Aktion Österreich-Ungarn

Background: Cardiovascular dysfunction in diabetes is characterized by the exessive fibrosis and endothelial dysfunction. More recently, Tenascin-C (TN-C) upregulation in the myocardium and serum predicts worse outcome in diabetic and heart failure patients. However, the causative role of TN-C in fibrosis and vascular dysfunction remains elusive in diabetes.

Methods: AJ and TNC-KO adult male mice were repeatedly injected with streptozocin. Cardiac function was assessed by echocardiography at baseline and at 18-20 weeks follow-up. Vascular endothelial function was performed by using wire myography in isolated aorta segments. Cardiac fibrosis was assessed by histochemistry. In addition, the hemodynamic effects of recombinant human TN-C (rhTN-C) on isolated rat hearts were evaluated on erythocyte-perfused, isolated working heart system. Furthermore, human ventricular cardiac fibroblasts (HCF) were cultured, then starved and treated with 1 TGF-β; 2 rhTN-C (10μg/ml) and TLR4 inhibitor in combination with TN-C and subsequently mRNA expression of α-SMA, TN-C, Col-1, Col-3, LOX-1 and ACE1 were assessed by RT-qPCR. In addition, human umbilical vein endothelial cells (HUVEC) were treated with either rhTN-C (10μg/ml) or combination with TLR4 inhibitor (TAK-242, 50μM) and analyzed the expression of NADPH oxidase 1 and 4 (NOX1, NOX4) and interleukin-6 (IL-6).

Results: Blood glucose levels of AJ and TNC-KO animals did not, however lack of TN-C was accompanied by preserved ejection fraction (P < 0.05) and endotheliump-dependent relaxation in compared to diabetic AJ mice, respectively at 12 weeks (P < 0.001 and P < 0.01). Histology revealed less cardiac and perivascular fibrosis in TN-C-KO diabetic animals in the AJ diabetic group (P < 0.01, respectively). In addition, cumulative dosage of rhTN-C (80 ng/ml) resulted in a significant reduction in cardiac output (P < 0.01) and LV systolic pressure (P < 0.05) in association with a massive upregulation of TNF-α and IL-1β in isolated rat hearts. TGF-β1 upregulation in TN-C-KO mice resulted in HCF (P < 0.01). Notably, HCF were exposed to rhTN-C promoted both α-SMA and Col 1 and 3 as well as LOX-1-α mRNA expression, respectively (P < 0.05). In addition, HUVEC incubated with rhTN-C showed increased expression of IL-6 and oxidative stress-related markers (NOX4) and TLR4 inhibitor pre-treatment markedly reversed these changes.

Conclusions: These findings uncover a novel mechanism that TN-C contributes to cardiovascular dysfunction in diabetes. TN-C created an intracellular environment that facilitated fibrosis and oxidative stress, which, in turn, resulting in cardiomyocyte and endothelial cell dysfunction. Thus, TN-C may be a critical mediator of the progression of cardiovascular dysfunctions in diabetes as well as potential target for future therapy.

Characterization of molecular mechanisms underlying dilated cardiomyopathy with ataxia (DCMA) using pluripotent stem cell (iPSC)-derived cardiomyocytes

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Funding Acknowledgements: 01EO15004 Federal Ministry of Education and Research, Germany (BMBF)

Background: Dilated cardiomyopathy with ataxia (DCMA) is a autosomal recessive disorder characterized by life threatening early onset cardiomyopathy associated with a metabolic syndrome. DCMA is caused by mutations in the DNAJC19 encoding an inner mitochondrial membrane (IMM) protein with a presumed function in mitochondrial biogenesis and cardiolipin remodeling via direct interaction with prohibitin 2 (PHB2).

Methods and Results: A novel human iPSC-based in vitro platform was generated to investigate DCMA by reprogramming of two patient-derived lines of siblings with discordant cardiac phenotypes and a CRSIPR/Cas9-edited isogenic Mutant (DNAJC19tv). Mutant and control iPSC-derived cardiomyocytes (iPSC-CMs) were characterized at a mature stage. The mutation was predicted to cause a loss of the DNA interaction domain, which was confirmed by loss of full-length DNAJC19 protein in all mutant cell lines. Subcellular investigation of DNAJC19 demonstrated a nuclear restriction in mutant iPSC-CMs. The loss of DNAJC19 co-localization with mitochondrial structures was accompanied by enhanced fragmentation, an overall reduction of mitochondria size and smaller cardiomyocytes. Ultrastructural analysis yielded decreased mitochondria sizes and abnormal cristae providing a link to defects in mitochondrial biogenesis and cardiolipin remodeling. Examination of mitochondrial function revealed an overall higher oxygen consumption rate (OCR) in all mutant iPSC-CMs compared to controls indicating a higher electron transport chain activity. Double radioactive tracer uptakes (18F-FDG, 125I-BMIPP) showed decreased fatty acid uptake that was in line of patient cell line accompanied by increased glucose uptake, functional measurements (Indo-1 AM) were performed to get insights on Ca2+ kinetics, contractility and arrhythmogenic potential on >120 days matress-matured iPSC-CMs. Significantly increased beating frequencies, elevated diastolic Ca2+ concentrations and a shared trend towards reduced sarcromere shortening was observed in mutant cell lines based on and upon isoproterenol stimulation. Extended speed of recovery was seen in all mutant iPSC-CMs but most striking the male patient cells additionally showed significantly prolonged relaxation times. Investigations of Ca2+ transient shapes unraveled enhanced arrhythmogenic features in mutant cells, comprised by both the occurrence of DADs/EADs and fibrilation-like events with discordant preferences.

Conclusions: We suggest that loss of full-length DNAJC19 impedes PH2β-complex stabilization within the IMM, thus hindering PHB-rings from building IMM-specific phospholipid clusters. These clusters are essential to enable normal cardiolipin remodelling during triase morphogenesis. Disturbed cardial function and mitochondrial morphology and biogenesis. Moreover, excess OCRs, altered substrate utilization and abnormal Ca2+ kinetics enables insights into the pathogenesis of DCMA.

HDAC inhibition regulates cardiac function by increasing myofilament calcium sensitivity and decreasing diastolic tension

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Funding Acknowledgements: European Research Area Network on Cardiovascular Diseases (ERA-CVD)/I/v/Austrian Society of Cardiology

Background/Introduction: Heart Failure with preserved Ejection Fraction (HFpEF) accounts for approximately 50% of all HF diagnoses with no proven effective therapies. We established a large animal model of slow progressive pressure overload that recapitulates key clinical features of HFpEF. We then tested the effects of the pan-HDAC inhibitor suberanoxihydroxy acid (SAHA) in the model and found that SAHA reversed and prevented the development of diastolic, systolic, and pulmonary dysfunction.

Purpose: Evaluate the effects of SAHA at the level of cardiomyocyte and contractile protein function to understand how it modulates cardiac function in parallel studies using cardiac tissue from humans and large mammals with similar physiological features (i.e. long action potential, similar myosin heavy chain isoform).

Methods: Adult feline ventricular cardiomyocytes (AFVM) were isolated from male donor cats and cultured with 2 μM SAHA or vehicle (DMSO) for 90 min, then incubated with a calcium (Ca2+) indicator (Fluo-4AM) and electrically stimulated (0.5Hz) to record Ca2+ transients and contractions. Skinned muscles were isolated from treated AFVM and functional experiments were performed to assess myofilament Ca2+ sensitivity and passive stiffness. The effects of SAHA on human cardiac tissue was assessed using left ventricle (LV) trabeculae isolated from non-failing donor hearts treated with 10μM SAHA or vehicle for 120 min while being electrically stimulated (1Hz). Developed force and relaxation parameters were recorded. Skinned myocytes were then isolated for calcium sensitivity and passive stiffness experiments.

Results: SAHA treated AFVM had a significant increase in contractility (fractional shortening) and improved relaxation kinetics (time to 50% baseline, return velocity), but no difference in peak Ca2+ transients (Figure A, B). These findings are indicative of an increase in myofilament Ca2+ sensitivity. Skinned muscles, used to assess myofilament function, were isolated from treated AFVMs. There was a significant increase in Ca2+ sensitivity and passive stiffness with SAHA (Figure C, D). Extending these findings to human cardiac tissue, SAHA treated trabeculae isolated from non-failing hearts had decreased diastolic tension and increased developed force, with a similar systolic peak force. Skinned myocytes isolated from these trabeculae had a similar response to AFVMs, with an increase in myofilament Ca2+ sensitivity and decrease in passive stiffness (Figure E, F).

Conclusions: These findings suggest that SAHA has an important role in the direct control of cardiac function at the level of the cardiomyocyte and myofilament in human and feline myocardium by increasing myofilament calcium sensitivity and reducing diastolic tension. These changes are consistent with functional data.
Mechanistic insights of the p.L13R mutation in the inner nuclear membrane protein Lemd2 leading to cardiomyopathy associated with arrhythmias

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Funding Acknowledgements: REFORM research grant from the University of Regensburg

Background: Clinical significance of tachycardioamyopothy increased with trials on catheter ablation therapy. Human neutromyoccardial biopsies show alterations of mitochondrial architecture.

Purpose: To investigate mitochondrial function in the pathophysiology of tachycardioamyopothy.

Methods: Pacemaker implantation was performed in 14 rabbits. 7 underwent tachypacing with 380 bpm (TCM), while in 7 rabbits the pacemaker remained inactive (SHAM). Left ventricular tissue was subjected to histologic examination, transmission electron microscopy, mass-spectrometry-based metabolomic profiling (LC-HRMS), and targeted transcriptomics of 168 genes of mitochondrial metabolism. Results from the animal model were evaluated for their translational potential using a human-based model. Induced pluripotent stem cell cardiomyocytes (iPS-CM) of healthy donors underwent electric field stimulation (7 days, 120 bpm, TACH vs. SHAM, CTRL) in a paired design. Fluxometry (Amplex Red) and flow cytometry (MitoSOX Red, MitoTracker Green) were performed. For functional evaluation, high-resolution respirometry was established in intact tissue and cells, measuring oxidative capacity while avoiding artefacts caused by mitochondrial isolation.

Results: TCM animals showed left ventricular dilation and systolic dysfunction (ΔLVEDD +5.3 ± 0.2 mm; ΔFS -19 ± 8%; TCM-SHAME; P < 0.001). Histology revealed cardiomyocyte hypertrophy (cross-sectional area 519 ± 32 vs. 413 ± 27 μm², P<0.01) without fibrosis (hydroxyproline content, P=0.52). Findings in electron microscopy included mitochondrial pleomorphy and increased cristae density in TCM animals, suggesting a disturbance of mitochondrial membrane organization. Oxidative phosphorylation capacity in TCM animals was decreased (133 ± 13 vs. 170 ± 16 pmol O2·s·mg−1 tissue, P < 0.05), which was also observed in IPS-CM after tachypacing (995 ± 738 vs. 1383 ± 901 pmolO2·s·mg−1·litre−1·s−1 nitrate synthase activity, TACH vs. CTRL, P < 0.01). Principal component analysis of LC-HRMS-based metabolomics revealed clustering of SHAM and TCM in two distinct groups, underlying profound alterations of metabolism in TCM. As mitochondrial content in IPS-CM remained unchanged after tachypacing (MitoTracker Green-FACS, P > 0.59), these findings hinted towards mitochondrial dysfucntion. Mitochondrial transcriptomics were characterized by altered expression of components of ROS homeostasis, including downregulation of antioxidant enzymes (e.g. glutathione peroxidase 3, fold change (FC) TCM/SHAME 0.5, P < 0.05) and upregulation of the ROS-producing enzyme NOX4 (FC 2.1, P < 0.05). On a functional level, both H2O2 and mitochondrial superoxide emission were increased in IPS-CM after tachypacing (H2O2 Amplex Red 1.65 ± 0.20 vs. 1.03 ± 0.11 μM/160 cells; MitoSOX-FACS MFI 439 ± 166 vs. 279 ± 99, TACH vs. CTRL, P < 0.05).

Conclusion: Tachycardioamyopothy implicates a disturbance of two mitochondrial key functions: oxidative phosphorylation capacity is reduced, while ROS emission increases.

Role and regulation of unconventional motor proteins myosin 5a and 5b in the heart

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Funding Acknowledgements: KFO311

Introduction: The myosin (Myo) superfamily consists of motor proteins involved in cell motility, contraction and intracellular transport. Myo5a and Myo5b perform directional movement along cytoplasmic actin filaments, thereby acting as cargo transporters of cell organelles, vesicles, receptors and signalling molecules.

Purpose: We aimed to analyse the regulation and function of Myo5a and 5b in cardiomyocytes and in the adult heart muscle.

Methods and Results: mRNA expression analysis revealed a developmental specific regulation of Myo5a and 5b expression in cardiomyocytes with Myo5a as the major isoform in embryonic/fetal and Myo5b as the predominant isoform in adult cardiomyocytes. Myo5b expression is reduced in human failing hearts and in neonatal rat cardiomyocytes stimulated by proinflammatory cytokines (TNFa and IFNY). To analyse the role of Myo5b, mice with a cardiomyocyte-specific knockout of Myo5b (Myo5b-KO) were generated. They were born at the expected Mendelian ratio with no indication of morphological abnormalities and normal contractility (FAC) up to the age of 12 weeks (w). However, electrocardiogram recordings (HOLTER analyses) revealed intermittent bundle branch blocks, alterations in the periods between QRS-complexes, supraventricular extra-systoles and atrial fibrillation. Thereafter, male and female Myo5b-KO mice gradually developed a dilated cardiomyopathy (FAC 24w: WT male: 50 ± 15% vs. Myo5b-KO male: 27 ± 12%; P < 0.0001; n=20–22; WT female: 52 ± 12% vs. Myo5b-KO female 38 ± 13%;
Haematopoietic and cardiac GPR55 synchronize post-myocardial infarction remodelling

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Funding Acknowledgements: STE-1053/5-1 Deutsche Forschungsgemeinschaft

Background: Long-term prognosis after myocardial infarction (MI) is predominantly determined by the extent of ischaemia triggered inflammation and remodelling. Experimental targeting of either the immune- or the endocannabinoid-system revealed promising strategies to improve post MI outcome.

Purpose: We aimed at characterizing the role of the cannabinoid-sensitive receptor GPR55 in post MI cardiac inflammation and remodelling.

Methods: Cardiac cell-specific GPR55 gene expression was assessed in cardiomyocytes, macrophages, endothelial cells and fibroblasts sorted from adult wild-type (WT) (flow cytometry, qPCR). Global GPR55−/− (KO) and WT mice were basally characterized or obtained to MI for 1, 3, 5 or 28 days and subsequently analysed via pro-inflammatory (flow cytometry, qPCR, ELISA) and pro-fibrotic early MI parameters (gravimetry, histology, echocardiography, qPCR).

Results: GPR55−/− mice exhibited a volume-overloaded heart, indicated by increased diastolic left ventricular (LV) cavity, volume, heart weight and an immune response resembling the stretch-induced inflammation. After MI, acute LV chemokine expression was attenuated in GPR55−/− mice at day 1, yet, potentiated at day 3 and even prolonged until day 5 post MI, despite initial infarct sizes comparable to WT. While having no effect on LV neutrophil or monocyte infiltration, 3 days post MI, global GPR55 knockout (KO) increased cardiac abundance of macrophages expressing manose-receptor (CD206) and myeloid-epithelial-reproductive tyrosine kinase (MERTK) - hallmarks of pro-reparative and phagocytic macrophages. This was accompanied by potentiated up-regulation of matrix metalloproteinase (Mmp) 9 and collagens (Col) I and II in the infarcted heart. In support of a role for GPR55 in cardiac macrophages, we detected its gene expression in macrophages characterized or assigned to MI for 1, 3, 5 or 28 days and subsequently analysed via pro-inflammatory (flow cytometry, qPCR, ELISA) and pro-fibrotic early MI parameters (gravimetry, histology, echocardiography, qPCR). To elicit the haematopoietic role of GPR55, KO>WT bone marrow chimera were generated, subjected to 3 days MI and characterized regarding cardiac inflammation. WT>WT chimera served as controls.

Conclusion: Our study alludes for the first time in vivo toward a contributory role of GPR55 to development of LV volume-overload, synchronization of post MI wound healing and regulation of LV remodelling.

The glycoprotein Dickkopf-3: a new player for the maintenance of vascular homeostasis

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Funding Acknowledgements: Project code: GR-2011-02350132, 2011. This work was supported by the Italian Ministry of Health

Dickkopf-3 (Dkk3) is a secreted glycoprotein known for its proapoptotic and angiogenic activity. We used both mice and rats to unravel a role for Dkk3 in the regulation of blood pressure (BP). Genetic deletion of Dkk3 in mice enhanced systolic BP and impaired endothelium-dependent acetylcholine (ACh)-induced relaxation

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of mesenteric arteries. Hypertension occurring in Dkk3-/- mice was rescued by lentivirus-encoded Dkk3 expression both in the periphery and within the CNS. Exogenous Dkk3 also rescued Ach-induced vasorelaxation. Endogenous Dkk3 was required for constitutive expression of vascular endothelial growth factor (VEGF), and its BP-lowering effect was mediated by VEGF induction followed by activation of type-2 VEGF receptors, stimulation of the phosphatidylinositol-3-kinase pathway, and Akt-driven phosphorylation of endothelial nitric oxide synthase (eNOS). This novel function of Dkk3 was confirmed in spontaneously hypertensive rats (SHRs), in which central overexpression of Dkk3 lowered BP, whereas Dkk3 silencing amplified the hypertensive phenotype. Interestingly, stroke-prone SHRs (SHRsp rats) feature a severe reduction in Dkk3 levels within the lower brainstem, a brain region that is critically involved in BP regulation. When SHRsp rats are challenged with a hyperosmotic diet, overexpression of Dkk3 in the CNS counters the progression of malignant hypertension and delays the occurrence of stroke.

Cardiac pericytes can be pharmacologically directed towards a smooth muscle phenotype to enhance the revascularisation of the ischemic heart

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Funding Acknowledgements: RM/17/3/33381 British Heart Foundation (BHF) Centre for Regenerative Medicine Award (II) - "Centre for Vascular Regeneration"

Background: Pericytes (PC) are abundant cells that wrap around the whole vascular lumen. To date, it is not known if PC contribute to the maturation of new arterioles in the ischemic heart.

Purpose: we verified whether cardiac PC differentiate into contractile vascular smooth muscle cells (VSMC) in vitro, and if this potential can be pharmacologically induced to enhance the revascularisation of the heart post ischemia in vivo.

Methods and Results: PC were extracted from adult myocardial tissue, expanded in vitro, and characterised for angiogenic profile and function. At baseline, PC do not express contractile VSMC antigens αSMA, CALP, SM22α, SM-MHC and Smoothern B. We discovered that the inhibition of MEK1/2 activity and ERK1/2 signalling using the small molecule PD0325901 redirects the PC towards a VSMC phenotype. Contraction, calcium flux and migration assays confirmed that differentiated PC became stationary contractile cells, phenocopying control coronary artery SMC (CASMC). We further verified this finding using next-generation RNA-sequencing, which showed that differentiated PC expressed a cluster of contractile VSM transcripts similar to control CASMC. Moreover, treated PC acquired a unique pro-angiogenic transcriptional profile, upregulating pro-angiogenic genes LEP and PDGFβ while downregulating potent angiogenesis inhibitors ANGPT2, TIE1 and SERPINF1. We also verified that human PC acquire contractile VSM markers when injected subcutaneously in mice. We next validated this innovative approach in vivo. Healthy C57BL6/J mice given the drug orally for two weeks, showed an increase in the small arteriole density along with an improved myocardial perfusion, when compared with mice given vehicle. Last, administration of PD0325901 to mice with myocardial infarction improved ventricular function and induced an increase in both capillary and arteriole density in the peri-infarct area, when compared with controls given vehicle. Improved revascularisation resulted in reducing infarct expansion in PD0325901-treated mice.

Conclusion: We propose a novel therapeutic approach based on MEK inhibition to promote the revascularisation of the infarcted heart reawakening the plasticity of resident PC. This approach could benefit the treatment of patients with coronary artery disease.

In vivo proteomics identifies lumican as an inhibitor of angiogenesis in the adult mammalian heart

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Funding Acknowledgements: AIRC

Background: Mammalian heart loses its spontaneous regenerative potential during the first week after birth. One of the major differences between regenerating and non-regenerating hearts is adaptation of extracellular matrix (ECM) to injury. Whereas in regenerating hearts ECM remodelling after injury favours cellular migration and invasion of the injured site, in non-regenerating hearts increased collagen deposition and scars prevent cardiac regeneration. Angiogenesis is a prerequisite for regeneration and, consistent with its low regenerative capacity, the adult heart does not form new vessels in response to pro-angiogenic stimuli.

Purpose: The work aims at profiling the proteome of vascular cells and the surrounding ECM of neonatal and adult heart in vivo to identify proteins controlling cardiac angiogenesis.

Methods: To label proteins accessible from the cardiac vasculature in vivo, we perfused neonatal and adult hearts with a biotin-containing solution. The hearts were then homogenised and biotinylated proteins were enriched using streptavidin-conjugated beads for their identification with mass spectrometry. Bioinformatic analysis provided a list of proteins differentially expressed in neonatal and adult hearts from which we chose a candidate, lumican, for further analysis. We investigated the function of lumican in ECM organization and vessel formation both ex vivo and in vivo. Additionally, we identified MMP-14 as an essential mediator of lumican activity in the adult heart.

Results: In vivo proteomic analysis revealed higher levels of lumican and its receptor integrin beta 1 in vessels of the adult heart. We verified the expression of lumican around cardiac arteries by immunofluorescence and confirmed its higher expression by cardiac fibroblasts in adult compared to neonatal hearts. Overexpression of lumican by NIH 3T3 fibroblasts resulted in ECM remodelling and formation of perpendicular structures, which are known to contrast angiogenesis, resulting in reduced proliferation of primary cardiac endothelial cells. In addition, by Western blot we identified different glycosylation patterns of lumican in the adult and neonatal heart. Since the binding of lumican to the active site of MMP-14 is known to be modulated by its glycan chains, we compared MMP-14 activity in the adult and neonatal heart. Consistent with higher levels of lumican glycosylation in the adult heart, we registered lower levels of MMP-14 activity in this organ. The inhibitory activity of lumican on cardiac angiogenesis was confirmed in lumican knockout mice, which have more vessels in the heart compared to wild type animals.

Conclusion: We identified lumican as an ECM protein that exerts a potent anti-angiogenic effect in the adult heart. These results shed light on the importance of the vascular ECM in regulating cardiac angiogenesis and pave the way for novel regenerative therapies.