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Abstracts

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Contents

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WESLEY ABPLANALP, Single cell sequencing reveals profound changes in monocytic cell clusters in patients with mutations associated with clonal hematopoiesis

Wesley Abplanalp1, David John1, Birgit Assmus2, Lena Dorsheimer3, Tina Rasper2, Khalil Abou-El-Ardat3,4, Michael A. Rieger3, Andreas M. Zeiher2,6, Mariuca Vasa-Nicotera2, Stefanie Dimmeler1,6

1 Institute for Cardiovascular Regeneration, Goethe University Frankfurt 2 Department of Medicine, Cardiology, Goethe University Hospital, Frankfurt 3 Department of Medicine, Hematology/Oncology, Goethe University Hospital, Frankfurt 4 German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg 5 Institute for Biochemistry I, Goethe University Hospital, Frankfurt 6 German Center for Cardiovascular Research DZHK, Berlin, Germany, partner site Frankfurt Rhine-Main

Background: Inflammation contributes to cardiovascular diseases. Yet, specific signatures of circulating monocytes are missing. Single cell sequencing provides a novel opportunity to define subsets of hematopoietic cells mediating inflammation in humans. Objectives: Therefore, we determined the inflammatory burden driven by heterogeneity in monocytes in subjects with chronic ischemic heart failure (CHF). Methods and Results: We sorted circulating CD31+ cells of patients with CHF versus healthy controls (n=4 per group) and performed single-cell RNA-sequencing. Unsupervised clustering of the entire 37679 cells generated 19 clusters with unique transcriptional profiles. Cells of patients with CHF showed a strikingly different pattern and individual unique clusters as well as several dysregulated pathways compared to healthy controls. Among the specific dysregulated genes were S100A8, interleukin 1b, thrombospondin-1 and matrix metalloprotease-1, which were confirmed by FACS and qRT-PCR in a validation cohort. Because of the profound differences in each of the individual patients, we evaluated the occurrence of somatic mutations associated with clonal hematopoiesis of indeterminate potential (CHIP), which recently was shown to be increased in patients with atherosclerosis. Indeed, two of the four patients revealed a mutation in the DNA methyltransferase DNMT3A. DNMT3A mutations were associated with a change in known DNMT3A target genes such as the pro-inflammatory genes CXCL1, CXCL2 and IL6 in circulating monocytic cells. Conclusions: This study shows that circulating cells derived from patients with CHF have unique and patient-specific phenotypes. This may be in part due to mutations that are enriched in CHIP-driver genes.

ALESSIO ALOGNA, Non-invasive evaluation of blood oxygen saturation in the heart using blood-oxygen-level-dependent T2 magnetic resonance imaging in a porcine model of acute systemic hyper- and hypoxemia.

Alessio Alogna1, Alessandro Faragli1, Carolin Kolp1, Christian Stehning2, Eike Nagel3, Burkert M Pieske1,4 , Heiner Post1,5, Sebastian Kelle1,4

1 Charité - Universitätsmedizin Berlin, Dept. of internal medicine and Cardiology, Campus Virchow Klinikum - Berlin Institute of Health; 2 Philips Research Hamburg; 3 University Hospital Frankfurt; Institute for Experimental and Translational Cardiovascular Imaging; DZHK Centre for Cardiovascular Imaging; 4 Department of Internal Medicine and cardiology German Heart Institute Berlin; 5Department of Cardiology Contilia Heart and Vessel Centre, St. Marien-Hospital Mülheim

Background Quantification of blood oxygen saturation (O2 saturation) is essential for the clinical evaluation of patients with cardiovascular diseases. The blood-oxygen-level-dependent (BOLD) effect has recently emerged as an effective way to non-invasively assess the O2 saturation by exploiting the paramagnetic properties of hemoglobin through T2 magnetic resonance imaging (T2-MRI). Aim of this study was to evaluate the accuracy of BOLD-T2-MRI against cardiac catheterization in a porcine model of acute systemic hyper- and hypoxemia. Methods Ten anaesthetized, healthy Landrace pigs (53±9 kg) were acutely instrumented with arterial and right atrial catheters for invasive blood gas analysis. After baseline, following hyper- and hypoxemia protocol steps were performed: I) ventilation on 100% O2 (100% O2) II) dobutamine-induced systemic hyperperfusion (Dob), III) verapamil-induced systemic hypoperfusion (Vera) IV) room air ventilation (21% O2). At each protocol step, arterial and central venous blood O2 saturation (ScvO2) were measured by invasive catheter sampling before and after the acquisition of T2 MRI data. Blood T2 was measured in the ventricles by means of T2 maps. Results Baseline ScvO2 (84±6%) significantly increased during hyperemia (90±5% at 100% O2), while decreased during the hypoxemia steps, Vera and 21% O2 (64±5% and 33±8%, both $p<0.05$). Dob failed to recruit a further ScVO2 reserve (87±4%). Baseline heart rate (106±14 min-1) and cardiac output (6.4±1 l/min) substantially increased during Dob (145±12 min-1 and 8,9±2 l/min), while decreased during Vera (91±12 min-1 and 3.8±1 l/min, all $p<0.05$). T2-relaxation time in the RV increased during hyperemia (146±45 ms vs 167±38 ms, bl vs 100%O2, $p<0.05$) and decreased during Vera (91±29 ms, $p<0.05$). When estimating ScvO2 from T2 times, Dob and 21% O2 data showed a high variability, presumably due to the sensitivity of T2 maps to pronounced hemodynamic changes. Linear regression analysis including all the other measurement steps showed a significant correlation between BOLD-derived and catheter derived O2 saturation ($p<0.01$, r2=66%). Conclusion BOLD-T2-MRI mapping shows a significant correlation with cardiac catheterization in a clinically relevant range of O2 saturations. However, pronounced hemodynamic changes negatively impacts on the accuracy of BOLD-T2-MRI. This technique may add important information in the clinical evaluation of patients with heart failure as well as pulmonary hypertension.

MORAD ASADI, Endothelial TSAd and endothelial connections during sepsis

M. Asadi1, T. Ziegler1, S. d'Almeida1, R. Hinkel1, M. Hellström2, K-L Laugwitz1, C. Kupatt1

1 I. Medizinische Klinik und Poliklinik, Klinikum rechts der Isar, München, 2 Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Systemic inflammation during sepsis is frequently followed by a decompensated hemodynamic state due to the volume shift from the vasculature to the extracellular space. This effect is partially mediated by the widespread disintegration of the endothelial barrier. Studies in mice have shown that decreasing vascular leakage can alleviate the hemodynamic decompensation occurring during sepsis. The vascular endothelial growth factor (VEGF) family and its corresponding receptors are potent regulators of vascular permeability. VEGF-Receptor 2 (VEGFR2) constitutes the predominant VEGFR family member on vascular endothelial cells which, in part, mediates the increase in vascular permeability via the downstream adaptor T-cell specific adaptor (TSAd) via binding to phosphorylation sites on the VEGFR2 cytoplasmic tail, specifically the tyrosine residue 951 (human) / 949 (mouse). To investigate the role of the VEGFR2 signaling on vascular permeability during sepsis in vivo, we induced systemic inflammation in mice and assessed hemodynamic parameters, vascular permeability, survival and followed molecular pathways downstream of TSAd that are involved in vascular permeability. Furthermore, we carried out a systemic analysis of the involved signaling molecules downstream of the VEGFR2 under in vitro septic conditions. For investigating the role of TSAd under inflammatory conditions in vitro, we designed TSAd-shRNA coding lentiviruses and created a TSAd-knockdown cell line, which was later exposed to inflammatory media.

ANDREA BÄHR, LEA29Y expression facilitates acceptance of human engineered heart tissue in transgenic pigs

Andrea Bähr 1, Florian Weinberger 2, Stefan Eichhorn 3, Stefanie Egerer 4, Katharina Klett 1, Nikolai Klymiuk 4, Marina Reinsch 2, Liesa Castro 2, Eckhard Wolf 4, Markus Krane 3, Thomas Eschenhagen 2, Christian Kupatt 1

1 Innere Medizin I, Klinikum Rechts der Isar, TU München, Munich, Germany 2 UKE, Hamburg, Germany 3 Deutsches Herzzentrum, Munich, Germany 4 Institute for Molecular Animal Breeding and Biotechnology, LMU München, Munich, Germany

Background: Inhibiting the B7/CD28 co-stimulatory pathway of T-cell activation by blocking agents such as the CTLA4-Ig variant LEA29Y might alleviate cellular rejection mechanisms in xenotransplantation settings. Pigs that express LEA29Y systemically under the control of a ubiquitously active CAG promote have been established and characterized. These pigs show a significantly impaired immune system and present with a defect in T cell maturation processes and an almost complete absence of B cells and NK cells. Thus, LEA29Y transgenic pigs might allow for the characterization of the regenerative properties of human engineered heart tissue (EHT) by serving as recipients of such material. Methods: Large mesh-structured fibrin-based human EHTs are generated from induced pluripotent stem cell derived cardiomyocytes and are cultured for 21 days before transplantation. Human EHT patches are sutured to the epicardium of the left ventricle of CAG-LEA29Y transgenic pigs. Pigs are additionally immune suppressed by daily intravenous or oral application of corticosteroids, Mycophenolat-Mofetil and Tacrolimus. Serum levels of immunosuppressive medication are determined on a regular basis. Follow up period is up to 7 days after which the graft area is explanted and investigated for integrity and cell infiltration by histology,

immunofluorescence and immunohistochemistry. Results: EHTs can be fixed sufficiently to epicardium of left ventricle. Surgical procedure and aftercare can be performed successfully, including reliable application of immunosuppression. At explantation, recovery of the graft is possible. No signs of cellular infiltration into the graft can be detected at present. Conclusions and outlook: An experimental set up that facilitates investigation of xenogeneic graft implantation into an immunosuppressed transgenic pig model has been established successfully. Follow up period will be increased to examine cellular rejection processes in more detail. Pigs will be additionally subjected to an impairment in heart function to investigate the regenerative properties of the EHT grafts on the heart.

BISHWAS CHAMLING, Sugars make the difference – Glycosylation of cardiodepressant antibodies regulates their activity in dilated cardiomyopathy

*Yvonne Reinke1,5+, PhD; Stephanie Könemann1,5+, MD; Bishwas Chamling1,5+, MD; Stefan Gross1,5, PhD; Kerstin Weitmann2,5, PhD; Wolfgang Hoffmann2,5, MD; Karin Klingel3, MD; Matthias Nauck4,5, MD; Jens Fielitz1,5, MD; Marcus Dörr1,5, MD; Stephan B. Felix, MD1,5**

1) Department of Internal Medicine B, Cardiology, University Medicine Greifswald, Germany 2) Institute for Community Medicine, University Medicine Greifswald, Germany 3) Cardiopathology, Institute for Pathology and Neuropathology, University Hospital Tübingen, Germany 4) Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Germany 5) DZHK (German Centre for Cardiovascular Research), partner site Greifswald, Germany

Background Cardiodepressant antibodies contribute to cardiac dysfunction in dilated cardiomyopathy (DCM). Changes in immunoglobulin G (IgG) glycosylation modulate the activity of various autoimmune diseases and influence disease activity as well as severity of various autoimmune diseases. We hypothesized that alterations in IgG glycosylation are involved in the disease course of DCM. Methods and Results IgG glycosylation was analyzed in plasma samples of 50 DCM patients using a lectin-based ELISA. Negative inotropic (cardiodepressant) activity (NIA) of antibodies was assessed by measuring the effect of purified DCM-IgG on the shortening of isolated rat cardiomyocytes by means of a video-edge detection system. IgG obtained from plasma of healthy blood donors served as control. DCM-IgG contained significantly less sialic acid (25%) and galactose (-16%; both $P < 0.001$), but showed no significant differences in core-fucosylation compared to controls. Interestingly, IgG with NIA displayed a lower percentage of sialylation (16%, $P < 0.001$) core-fucosylation (-15%, $P = 0.015$) and galactosylation (10%, $P = 0.129$) than IgG without NIA. The extent of NIA was directly associated with IgG sialylation ($r = 0.68$; $P < 0.001$) and galactosylation ($r = 0.37$; $P = 0.001$). Conclusion Reduced sialylation and galactosylation of IgGs enhances their cardiodepressant activity in DCM indicating that changes in IgG glycosylation may be involved in the pathogenesis of DCM. Keywords Dilated cardiomyopathy, cardiodepressant antibodies, IgG glycosylation, Negative Inotropic Activity (NIA).

JAN CHRISTOPH, Electromechanical Vortex Filaments during Cardiac Fibrillation

Jan Christoph 1 2, Mohammed Chebbok 1 2, Johannes Schröder-Schetelig 3, Claudia Richter 1 2 3, Flavio Fenton 4, Gerd Hasenfuß 1 2, Stefan Luther 1 2 3

1 University Medical Center Göttingen, 2 German Center for Cardiovascular Research, 3 Max Planck Institute for Dynamics and Selforganization, 4 Georgia Institute of Technology

Background: Mapping of atrial or ventricular fibrillation aims at visualizing the complex electrical wave activity responsible for the rapid, irregular beating of the heart muscle. Objective: Because catheter-based approaches provide only surface mapping of the electrical cardiac activity, we have developed an ultrasound-based imaging approach, with which we analyze the three-dimensional mechanical deformation of the fibrillating heart at high speeds, such that we are able to resolve electromechanical rotors and the 3D organization of the electromechanical rotor activity during ventricular fibrillation. Methods and Results: Using high-resolution 4D ultrasound ex vivo in intact, isolated pig hearts, we show that it is possible to identify mechanical filament-like phase singularities within the deforming, fibrillating heart muscle, which like fingerprints of electrical vortex filaments evolve through the volume of the tissue and presumably indicate the core regions of three-dimensional electrical rotors. Simultaneous panoramic optical mapping of the deforming heart surface shows that electrical action potential vortices and spiral waves visible on the surface create vortex-like mechanical deformations and mechanical rotor patterns, which similarly rotate and whose core regions co-exist and co-localize with the core regions or phase singularities of action potential rotors. Simultaneous tri-modal optical mapping of voltage, calcium and strain in fibrillating hearts also indicates that the excitation-contraction coupling mechanism during ventricular fibrillation leads to closely coupled electromechanical turbulence, with a high congruency of voltage and calcium spiral vortex waves and according rotating deformation patterns on the surface of the heart. Conclusions: Overall, the data suggests that cardiac fibrillation can be characterized through mechanical phase singularities and contraction vortex filaments, and that ultrasound can, similar to optical mapping, provide highly detailed maps of complex arrythmias. However, ultrasound superseedes optical mapping and catheter mapping in that it can visualize the wave dynamics underlying ventricular fibrillation within the entire tissue beneath the surface. We expect that our findings will significantly enhance the understanding of cardiac fibrillation, and will stimulate the development of novel instrumentation for arrhythmia imaging and may lead to novel diagnostic and therapeutic approaches.

KASHAN DAVID, Effect of Ionizing Irradiation on Engineered Heart Muscle for Cardiac Repair

Kashan David1,2, Buntaro Fujita1,2, Susanne Schlick1,2, Paul Balfanz1,2, Wolfram-Hubertus Zimmermann1,2, Malte Tiburcy1,2

1Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Göttingen, Germany 2German Center for Cardiovascular Research (DZHK), partner site Göttingen

Introduction Implantation of Engineered Heart Muscle (EHM) from human pluripotent stem cells is a promising strategy to remuscularize the failing heart. Residual pluripotent stem cells may pose a risk of unwanted growth. Here, we tested the hypothesis that residual pluripotent cells can be effectively removed from EHM by ionizing irradiation. Methods and Results First, the response to increasing doses of irradiation (0, 3, 10, 30, 50, 100 Gy) was tested in pluripotent stem cells (PSC) and PSC-derived cardiomyocytes (CM). Cell number was monitored by bioluminescence (BLI) using a PSC line with constitutive Luciferase (Luc) expression. Irradiation of PSC induced significant cell death after 48 hrs at all doses applied as evidenced by a significant loss of BLI signal (29148±3103 p/s/cm2/sr in 0

Gy to 72±249 p/s/cm2/sr at 10 Gy, n=8, p<0.05) and an increase in LDH release (n=4). Interestingly, BLI signal 7 days after irradiation indicated PSC growth in 0 and 3 Gy groups, but not with higher doses. CM were surprisingly resistant to irradiation (BLI: 24169±2349p/s/cm2/sr at 0 Gy vs. 15975±2293p/s/cm2/sr at 100 Gy, n=8) without LDH increase 24 hrs after irradiation. To test the effect on muscle function 2 week-old EHM (loop format: 1.25 million cardiomyocytes and stroma cells at 2:1 ratio) were irradiated with 30 Gy and cultured for another 5 weeks without evidence for functional impairment (Force of contraction: 0.7±0.2 mN in irradiated vs 0.7±0.1 mN not irradiated EHM, n=8). Finally, irradiated and non-irradiated EHM patches (10 million cardiomyocytes and stroma cells at 9:1 ratio) were implanted onto the epicardium of nude rats with chronic myocardial infarction induced by permanent LAD ligation (2 weeks before implantation). Consistent with the data from EHM loops, irradiation with 30 Gy did not affect baseline patch bioluminescence or function. Interestingly, 3 months after implantation there was only evidence of stable grafts in the non-irradiated group (18±9% of the baseline BLI signal, n=8) while irradiated EHM patches did not persist (1±1% of the baseline BLI signal, n=5, p<0.05). Conclusion Ionizing irradiation effectively kills residual stem cells without compromising the function of postmitotic cardiomyocytes and EHM. However, EHM survival after epicardial implantation is reduced after irradiation. Further experiments need to be conducted to optimize survival of fully post-mitotic EHM after irradiation.

DANIEL FINKE, Epigenetic memory of metabolic stress - Identification of regulators for cardiac stress susceptibility

Finke D. 1,3, Schanze L. 1,3, Heckmann M.B. 1,3, Meßmer T. 2, Katus, H.A. 1,3, Lehmann L.H. 1,3

1 Department of Internal Medicine III: Cardiology, Angiology & Pulmonology, University Hospital Heidelberg; 2 German Cancer Research Center (DKFZ); 3 German Centre for Cardiovascular Research (DZHK)

Heart failure is an emerging problem in western societies. However, despite growing knowledge about diagnostics and therapies, individual predisposing factors are widely unknown. Obesity directly contributes to impaired cardiac function, but the underlying mechanisms as a sustained cardiac risk factor remain elusive. To assess potential sustained transcriptional epigenetic events, we treated animals with either High Fat Diet (HFD) for 10 weeks or Low Fat Diet (LFD) as a control. Further, we included a third group on HFD for 5 weeks and 'reverse feeding' on LFD for 5 weeks. Based on bodyweight we found a complete recovery (LFD: 27.76g ± 0.37g, HFD: 32.93g ± 0,59g, Reverse: 28.01g ± 0.67g; mean ± SEM). Functionally, echocardiography showed a slight worsening in HFD, whereas reverse feeding did not result in a significant change compared to control (LFD) (LFD: 77.22% ± 1.53%, HFD: 71.64% ± 1,53%, Reverse: 75.01% ± 1,28%; Ejection fraction, mean ± SEM). Cardiomyocyte hypertrophy was not completely normalized in the reverse group (LFD: 411.0µm2 ± 6.15µm2, HFD: 572µm2 ± 9.59µm2, Reverse: 506.8µm2 ± 8.97 µm2; area, mean ± SEM). Fibrosis was not increased significantly in HFD in comparison to LFD. RNAseq analysis of isolated adult cardiomyocytes (HFD, n=9; LFD n=10; Reverse, n=10; n, animals/group) revealed specific gene programs, sustained upregulated after reverse feeding, which mainly play part in lipid and fatty acid consumption. The most differentially regulated target was the secreted cytokine midkine (mdk), a heparin-binding protein. Mdk was highly suppressed after HFD and serves as a potential sustained regulator of the cardiac stress response. By a specific analysis of the cardiomyocytes chromatin (ChIPseq for H3K27ac, H3K4me1 and H3K27me3), using isolated adult cardioymyocytes of LFD (n=3), HFD (n=3) and Reverse treated animals (n=3), we aimed to understand potential regulation of cardiac enhancers. By a stringent bioinformatical approach, we identified 282 novel 'active' enhancer after HFD, whereas only two of them stayed 'active' after reverse feeding. Genetic manipulation of these novel 'metabolic enhancer' will give us further insights about their specific biological role and their function in epigenetic memory. Our approach, by overlapping transcriptional and epigenetic changes

on a genome wide level has the potential to uncover a fundamental and previously unrecognized link between individual risk and epigenetic stress memory.

YOUSSEF FOUANI, The endothelial-enriched lncRNA NTRAS regulates vessel permeability by controlling alternative splicing of tight junction gene

Youssef Fouani1, Nicolas Jaé1, David John1, Ilka Wittig2, Oliver Rossbach3, Albrecht Bindereif3, Stefan Liebner4, Kavi Devraj4 and Stefanie Dimmeler1, 5

1 Institute for Cardiovascular Regeneration, University of Frankfurt, Germany 2 Functional Proteomics Core Unit, Faculty of Medicine, University of Frankfurt, Germany 3 Institute of Biochemistry, Justus Liebig University of Giessen, Germany 4 Institute of Neurology, Faculty of Medicine, University of Frankfurt, Germany 5 German Center for Cardiovascular Research, Rhein/Main, Germany

The discovery of long noncoding RNAs (lncRNAs), together with evidence for a predominantly noncoding human transcriptome, initiated a new era in RNA biology. LncRNAs exhibit various mechanisms of action including the recruitment of chromatin modifiers and modulation of splicing or mRNA stability. Although the role of some distinct lncRNAs have been explored, our knowledge on lncRNA functions in vascular biology is still limited. Here, we analyze the functional role of the hypoxia-induced and endothelial-enriched lncRNA NTRAS (Non-coding Transcript Regulating Alternative Splicing). This nuclear localized transcript was identified in RNA deep sequencing data from hypoxia-exposed HUVECs and functional assays revealed that NTRAS silencing significantly impairs in vitro sprouting (to 36 ± 1%, $p<0.05$), cell growth, cell cycle progression (66 ± 4% reduction of S-phase cells) and induces permeability (196 ± 7%). Using antisense selection, we enriched for endogenous NTRAS-protein-complexes (32 ± 16 fold over Ctl, $p<0.05$). Analysis of co-purified proteins by mass spectrometry revealed the splicing regulator hnRNPL to be bound to NTRAS. In line, NTRAS exhibits a bona fide hnRNPL binding motif and RNA immunoprecipitation confirmed the observed interaction. Next, we assayed for a functional involvement of NTRAS in alternative splicing by RNA deep sequencing. Strikingly, silencing of NTRAS or hnRNPL lead to altered exon usage in 103 common cases, among those the tight junction protein ZO1. Since hnRNPL silencing is known to activate ZO1 exon 20 inclusion, we assessed the effect of NTRAS silencing and observed enhanced exon 20 skipping ($p<0.05$). Altered splicing of ZO1 upon NTRAS silencing was confirmed by using ZO1 minigenes ($p<0.05$). Functionally, ZO1 is required for tight junction assembly. Currently we investigate the role of the "α-motif" encoded by exon 20 in the context of vascular permeability. First results in mice, in which NTRAS and its splicing regulatory feature is conserved, show that NTRAS silencing by GapmeRs significantly enhances vascular permeability in heart (58.5%), brain (45.7 %) and muscle tissues (152.9%) (all $p<0.05$), and induces lethality of mice compared to controls. In summary, we characterized NTRAS as novel endothelial-enriched lncRNA, which controls vascular integrity and permeability. Mimicking NTRAS functions e.g. by target site blockers may be therapeutically be used to preserve barrier function in systemic inflammatory syndromes.

ROBIN HINDMARSH, Exploring the role of LZTR1 for the development of cardiac hypertrophy in genome-edited iPSC-derived cardiomyocytes

Robin Hindmarsh 1, Ulrich Hanses 1, Malte Tiburcy 2, Mandy Kleinsorge 1, Lennart Roos 1, Wolfram-Hubertus Zimmermann 2, Gerd Hasenfuß 1, Bernd Wollnik 3, Lukas Cyganek 1

1 Clinic for Cardiology and Pneumology, University Medical Center Göttingen, 2 Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Germany, 3 Institute of Human Genetics, University Medical Center Göttingen, Germany

Noonan Syndrome (NS) is a multisystemic developmental disorder. It is characterized by its clinical variability with common symptoms such as congenital heart disease, reduced growth, facial dysmorphism, auditory deficits and mild mental disability. NS is caused by mutations in genes that regulate the RAS-MAPK pathway including PTPN11, SOS1, RAF1, RIT1 and KRAS that account for roughly 80% of all NS cases. Recently, we identified a family displaying the autosomal recessive form of NS with severe hypertrophic cardiomyopathy in two siblings with unaffected parents. Whole exome sequencing revealed a compound heterozygous mutation of Leucine zipper like transcription regulator 1 (LZTR1) in both children representing the first identified genetic cause of the autosomal recessive form of NS. However, the functions of LZTR1 and its pathogenic role for the development of cardiac hypertrophy are largely unknown. The recent development of induced pluripotent stem cell (iPSC) technology allows the study of human diseases using patient-specific cells in vitro. In this project, we aim to investigate the disease through patient-specific as well as genome-edited iPSC-derived cardiomyocytes (iPSC-CMs) with particular focus on the role of LZTR1 as a cause for NS. Patient-specific iPSC lines from both children with pronounced NS and from the parents were successfully generated from skin biopsies. In addition, LZTR1-knockout (KO) iPSC lines were generated via CRISPR/Cas9 genome editing. Initial data indicate that the hypertrophic cardiac phenotype can be recapitulated in patients' iPSC-CMs. Until now, it is not clear if the identified LZTR1 mutations cause NS due to a loss or a gain of function. Custom-tailored LZTR1-KO cell lines, mimicking the maternal and paternal inherited mutations separately enable us to conquer this question through analysis of calcium cycling, cellular hypertrophy as well as RAS-MAPK signaling activity. By combining functional and molecular approaches, we aim to get insights into the underlying disease mechanisms leading to the severe phenotype of NS as well as to improve the current diagnostics and to enhance treatment strategies.

MELANIE HULSHOFF, Aortic stiffness in diabetes is contributed by endothelial-to-mesenchymal transition

Melanie S. Hulshoff 1,2,3,‡, Isabel N. Schellinger 4,‡, Xingbo Xu 1,2, Fang Cheng Wong 1, Guido Krenning 3, Uwe Raaz 4, ŧ, Elisabeth M. Zeisberg 1,2, ŧ.

1 Department of Cardiology and Pneumology, University Medical Center Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany, 2 German Center for Cardiovascular Research (DZHK), Partner Site Göttingen, Germany, 3 Department of Pathology and Medical Biology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, Netherlands, 4 University Heart Center, Göttingen, Germany.

Background – The incidence of diabetes mellitus is increasing considerably worldwide and is currently affecting more than 422 million people. The major cause of death in type 2 diabetes is cardiovascular disease. Accelerated aortic stiffening is an independent predictor of cardiovascular disease and mortality in diabetes patients. We previously showed that aortic stiffness precedes hypertension in db/db mice (a mouse model of diabetes), making aortic stiffness an early contributor

to cardiovascular disease development. Purpose – Elucidating how aortic stiffening develops is urgently needed to halt the pathophysiological process at an early time point. We now identified a novel regulator of aortic stiffness: the endothelial-to-mesenchymal transition (EndMT). Methods – To assess the occurrence of EndMT, aortic sections of db/db mice were co-immunofluorescent stained with the endothelial marker CD31 and the mesenchymal markers α-SMA or S100A4. Also, mRNA expression of the EndMT transcription factors Snail, Slug and Twist was examined in aortic tissue from db/db mice as well as in aortic tissue from diabetic patients. We overlapped the micronome of aortic tissue from db/db mice with high glucose treated human umbilical vein endothelial cells (HUVECs) in order to identify the underlying mechanism by which EndMT contributes to aortic stiffening. Results – We demonstrated a robust co-localization of CD31 with either α-SMA or S100A4 in aortic sections of db/db mice which was almost absent in control mice. Moreover, we showed that the mRNA levels of the EndMT transcription factors were significantly upregulated in aortic tissue of both db/db mice and diabetic patients when compared to controls. As underlying regulator, we identified miR-132-3p as the most significantly downregulated miR in both the micronome of aortic tissue from db/db mice and of high glucose treated HUVECs. In aortic tissue from diabetic patients, miR-132-3p was also significantly downregulated. We identified Kruppel-like factor 7 (KLF7) as a target of miR-132-3p and show that KLF7 is significantly upregulated in aortic tissue of both db/db mice and diabetic patients as well as in high glucose treated HUVECs. Conclusion(s) – We demonstrate that aortic stiffness in diabetes is contributed by EndMT. We identified miR-132-3p and KLF7 as novel regulators of EndMT in this context. Altogether, this gives us new insights in the development of aortic stiffening in type 2 diabetes.

KRISTIN KRAEKER, Pravastatin as a potential treatment to reduce long-term cardiovascular risk after preeclamptic pregnancy

Kristin Kräker 1-5, Jamie M O'Driscoll 6, Till Schütte 2-4, Florian Herse 1,2,4,5, Stefan Verlohren 4, Dominik N Müller 1-5, Basky Thilaganathan 6, Nadine Haase1-5, Ralf Dechend 1-4,7

1 Experimental and Clinical Research Center, Berlin, 2 Berlin Institute of Health (BIH), Berlin, 3 DZHK (German Centre for Cardiovascular Research), partner site Berlin 4 Charité – Universitätsmedizin Berlin, Berlin 5 Max-Delbrueck Center for Molecular Medicine, Berlin, 6 Fetal Medicine Unit, St. George's University Hospitals NHS Foundation Trust, London, 7 HELIOS-Klinikum, Berlin

Preeclampsia is a disease during pregnancy characterized by hypertension and proteinuria and is the main cause of maternal and fetal mortality worldwide. It is associated with an increased long-term risk of cardiovascular disease, although the underlying functional and structural mechanisms are unknown. We investigated maternal cardiac changes after preeclamptic pregnancy and the effect of pravastatin treatment in an established and comparable rat model. Female Sprague-Dawley (SD) rats harboring the human angiotensinogen gene [TGR(hAogen)L1623] develop a preeclamptic phenotype during pregnancy when mated with male SD rats carrying the human renin gene [TGR(hRen)L10J]. Using advanced echocardiography, including speckle tracking analysis, we were able to show that this model shows similar cardiovascular changes compared to the human situation. For possible intervention, we treated preeclamptic dams with pravastatin. Untreated former preeclamptic rats show cardiac hypertrophy after pregnancy, described in both echocardiography and histologic staining. In addition, an increase in interstitial and perivascular fibrosis was observed. Hearts of preeclamptic rats also showed refinement of myocardial capillaries in CD31 staining and advanced 3D vascular imaging using Light Sheet Fluorescence Microscopy. This pathological cardiac modelling may be associated with an increased cardiovascular risk in later life and is predisposed to long-term structural and functional heart defects. Pravastatin treatment improved functional and structural remodeling and increased postpartum cardiac output. The offspring of preeclamptic pregnancies also benefit from this treatment.

MIN-CHI KU, Cardiac MRI for in vivo quantification of myocardial perfusion deficits in a hypertrophic cardiomyopathy mouse model

Min-Chi Ku1,2, Andreas Pohlmann1 , Qadri Fatimunnisa3, Michael Bader2,3, Lucie Carrier4,5 and Thoralf Niendorf1,2,6

1Berlin Ultrahigh Field Facility (B.U.F.F.), Max Delbrueck Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany 2DZHK (German Centre for Cardiovascular Research), partner site Berlin, Germany 3Molecular Biology of Peptide Hormones, Max Delbrueck Center for Molecular Medicine in the Helmholtz association (MDC), Berlin, Germany 4Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany 5DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Germany 6Experimental and Clinical Research Center, Charite Medical Faculty and the Max Delbrueck Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

Current diagnosis of hypertrophic cardiomyopathy (HCM) is based on unexplained thickening of left ventricular wall detected by non-invasive imaging such as cardiac MRI (CMR). This morphological change does not reflect the underlying subclinical myocardial ultra-structural changes including the kinetics of hypertrophy, fibrosis and microvasculature deficits that usually dictate disease prognosis. Notwithstanding the substantial progress of CMR in assessing changes in the myocardial microstructure, in vivo evaluation of such microstructural changes and their mechanisms underlying disease progression is still missing. This significantly limits the prognosis of HCM and disease management. Based on our recent findings in patients with HCM, we hypothesize that factors related to impairment in myocardial micro-perfusion may contribute to the microstructural changes in HCM. To test this hypothesis, we used state-of-the-art CMR to quantify the myocardial blood flow (MBF) throughout the cardiac cycle in HCM mouse model. We found that in 3 month old mice, left ventricular wall was significantly thicker in HCM mice. Mean perfusion in the mid-ventricular slice is significantly lower in HCM (end-diastole: 6.8±1.0 mL g-1 min-1, $P<0.05$; end-systole: 4.6±0.5 mL g-1 min-1, $P<0.01$). Sirius Red staining revealed mild fibrosis was shown in HCM mice. Our study is the first one to measure myocardial perfusion in a mouse model with HCM features. We showed that the myocardial micro-perfusion change associates with the signs of myocardial remodeling such as hypertrophy and fibrosis. In human studies, myocardial fibrosis is progressive in some HCM patients and the perfusion abnormality is one of the mechanisms of the fibrotic process. Furthermore, the progression of myocardial remodeling is usually associated with an increased risk of clinical events in HCM. Our work provides adequate ground for measuring perfusion deficit in HCM and will be further validated with more sophisticated microvasculature analysis in the HCM mouse model. To conclude, our results help to fill in the missing link between in vivo detection of subclinical perfusion deficits and histological hallmarks of myocardial remodeling therefore provides a translational aspect. Ultimately, we wish to establish effective CMR marker to better assess microstructural changes in HCM and translate our knowledge into improving early cardiomyopathy treatment.

MARIYA M. KUCHERENKO, Vascular remodeling in Pulmonary Hypertension due to Left Heart Disease

Mariya M. Kucherenko1,2,3, Pengchao Sang1,2, Dag Wulsten4, Georg Duda4, Wolfgang M. Kuebler2,3, Christoph Knosalla1,3**

*1 DHZB, Klinik für Herz, Thorax- und Gefäßchirurgie, Augustenburger Platz 1, 13353 Berlin 2 Charité - Universitätsmedizin Berlin, Institut für Physiologie, Charitéplatz 1, 10117 Berlin 3 DZHK: German Centre for Cardiovascular Research 4 Charité - Universitätsmedizin Berlin, Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Augustenburger Platz 1, 13353 Berlin; *equal contribution*

Pulmonary hypertension (PH) is a frequent complication of left heart disease (LHD) and the lack of treatment options ultimately results in right heart failure as a cause of death. Insight into the reactive components of PH that lead to disease progression is still limited. Pulmonary arterial stiffening has recently been identified as both diagnostic marker and pathomechanism in pulmonary arterial hypertension, yet its presence, extent, and role in PH-LHD is so far unclear. We therefore aimed to characterize vascular remodeling (VR) in pulmonary arteries (PA) of PH-LHD patients leading to PA stiffening. We assessed physical, structural and cellular characteristics of VR on tissue samples of conducting PA collected from LHD patients and donors during heart transplantation. Patients were classified into two major categories – LHD (with normal PA pressure) and PH-LHD, while donors' samples served as controls. PA stiffness was determined by a tensile test and calculation of Young's modulus which was significantly higher in PH-LHD (3.12±0.85 kPa, n=3) as compared to control (1.80±0.33 kPa, n=8) or LHD alone (1.24±0.12 kPa, n=5). Next, we combined computed tomography imaging and histological analyses to assess size parameters of PA, PA-wall, and PA-lumen. PH-LHD patients showed increased: (i) diameters of the pulmonary trunk (32.27±1.60 mm) and right and left PAs (24.11±0.85 and 24.90±0.82 mm, n=19) versus LHD (26.8±1.6 mm, 19.97±1.25 and 19.78±1.4 mm respectively, n=9), (ii) PA-wall thickness (1.33±0.09 mm, n=12 versus 1.16±0.08 mm, n=7 in controls), and (iii) PA-lumen diameter (29.71±2.43 mm, n=11 versus 24.22±2.40 mm, n=6 mm in LHD alone). Analysis of extracellular matrix (ECM) showed 7-fold increase in collagen/elastin ratio in PH-LHD samples versus controls (n=9 and 8 respectively) and altered ECM-fibrillary-network. PH-LHD samples also displayed changes in ECM and cellular composition within the inner PA region with excess smooth muscle cells, remodeled endothelial cells, and an 80% downregulated inner elastic membrane elastin. Based on a detailed characterization of human PA samples, we identified PA stiffening in PH-LHD and characterized underlying changes in vascular morphology and PA wall composition. In combination with in-depth analysis of clinical data on PH-LHD patients these findings provide a basis for subsequent in vitro studies and animal models to elucidate molecular mechanisms of and novel therapeutic strategies for VR in PH-LHD.

ANJA MÄHLER, Increased Salt Intake Decreases Postprandial Energy Expenditure in Healthy Volunteers - a Randomized Clinical Study

Anja Mähler1,2,3, Nicola Wilck1,2,3,4, Samuel Klamer1,3, Andras Balogh1,2,4, Lars Klug1,2, Michael Boschmann1,2, Dominik N. Müller1,2,3,4

1 Experimental & Clinical Research Center, a cooperation between Charité Universitätsmedizin Berlin and Max Delbruck Center for Molecular Medicine, Berlin, Germany 2 Berlin Institute of Health, Berlin, Germany 3 DZHK (German Centre for Cardiovascular Research), partner site Berlin, Germany 4 Max Delbruck Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

High salt intake is a potential risk factor for obesity independent of energy intake, though underlying mechanisms remain unclear. Diet-induced thermogenesis (DIT) accounts for about 10% of total energy expenditure. We hypothesized that high salt intake decreases DIT in healthy volunteers. We enrolled 40 healthy subjects (sex ratio 1:1) in a randomized, double-blind, placebo-controlled, parallel-group study (NCT03024567). They received either 6 g salt or placebo daily in capsules over 14 days on top of their habitual diet. Before and after the intervention, resting and postprandial energy expenditure, ambulatory blood pressure, bioelectrical impedance analysis (BIA), and food intake from 3-day food records were obtained. Energy expenditure was measured by indirect calorimetry (canopy hood) after a 12h overnight fast and a standardized 440 kcal, high-protein meal. In both groups, 19 subjects completed the study (placebo: nine men, 29 ± 6 years, BMI 23.1 ± 0.5 kg/m2; salt: ten men, 32 ± 7 years, BMI 23.3 ± 0.7 kg/m2). Salt intake from foods was 6 g/d in both groups, both before and after the intervention. Resting energy expenditure did not change in either group. DIT was significantly decreased after salt ($P = 0.049$) but not after placebo (NS). Decreased DIT was accompanied by a decreased fat and therefore increased carbohydrate oxidation after salt ($P = 0.03$). However, this was also the case after placebo ($P < 0.0001$). Surprisingly, systolic blood pressure was increased in four and 11 subjects after salt and placebo, respectively (both NS). Diastolic blood pressure was higher in seven subjects, both after salt and placebo (both NS). Body composition and hydration did not change due to increased salt intake or placebo. In conclusion, a moderate short-term increase of salt intake decreased the thermic effect of a high-protein meal. This could contribute to the observed weight gain in populations consuming a Western diet high in salt.

Day 2, Thursday, 10 January 2019, Posters 16-35

JIRKO KÜHNISCH, Identification of a therapeutic approach targeting the PRDM16 associated cardiomyopathy

J. Kühnisch1,2, J. Dartsch1, A.K. Kahlert3, C.A. MacRae4, N. Hubner2,5, S. Klaassen1,2,6

1 Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Experimental and Clinical Research Center (ECRC), a joint cooperation between the Charité Medical Faculty and the Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany; 2 DZHK (German Centre for Cardiovascular Research), partner site Berlin, Berlin, Germany; 3 TU Dresden, Institut für Klinische Genetik, Dresden, Germany; 4 Max Delbrück Center for Molecular Medicine, Berlin, Germany; 5 Harvard Medical School and Harvard Stem Cell Institute, Cardiovascular Division, Brigham and Women's Hospital, Boston, USA; 6 Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Pediatric Cardiology, Berlin, Germany

Cardiomyopathy and heart failure caused by genetic defects are frequent and life-threatening conditions. Recently, we linked mutation of the gene PR/SET domain 16 (PRDM16) to human cardiomyopathy. Moreover, ablation or mutation of Prdm16 in zebrafish leads to cardiac dysfunction, diminished cardiomyocyte proliferation and increased cardiomyocyte apoptosis. Here, we identify a specific class of peptide molecules that rescue the Prdm16 induced phenotype in zebrafish and we establish a preclinical mouse model for the Prdm16 associated cardiomyopathy. Using a small molecule library, we screened for compounds that potentially rescue the cardiac phenotype caused by deactivation or mutation of the Prdm16 gene in zebrafish. Selective antagonists of the melanocortin 4 receptor (MC4R) were shown to normalize the cardiac output phenotype and cardiomyocyte proliferation. The identified MC4R antagonists are short peptides. Consistently, morpholino mediated silencing of the MC4R transcript rescued the cardiac phenotype in Prdm16 mutant zebrafish. Of note, the selective MC4R agonist THIQ failed to normalize cardiac failure in Prdm16 zebrafish mutants. In order to establish a mammalian animal model to study PRDM16 associated cardiomyopathy, we conditionally deactivated murine Prdm16 by using the Nkx2.5Cre driver model. Resulting Nkx2.5Cre;Prdm16floxflox mice with homozygous Prdm16 deactivation are generally viable but 25% of them decease within the first 2 months of postnatal life. Nkx2.5Cre;Prdm16floxflox mice develop severe cardiomyopathy at the 4 month stage that is characterized by approx. 50% reduction of the cardiac ejection fraction. Moreover, the left ventricular mass and the heart/body mass ratio is increased by more than 30% in Nkx2.5Cre;Prdm16floxflox mice compared to controls. This work identifies a potential therapeutic approach for the PRDM16 associated cardiomyopathy and establishes a novel preclinical cardiomyopathy mouse model. Application of the therapeutic approach targeting MC4R may lead to a novel treatment for cardiomyopathy.

SARA LELEK, Evaluation of analgesic treatment after cryoinjury in zebrafish heart

Sara Lelek1,2, Mariana G. Simões1, Daniela Panáková1,2

1 Max Delbrück Center for Molecular Medicine 2 German Center for Cardiovascular Research (DZHK)

Heart failure is one of the most common causes of death worldwide. While humans are not able to regenerate their heart after myocardial infarction (MI), zebrafish heart can fully recover after an injury, making it an excellent model organism to study how to overcome limited regenerative response in humans. To mimic human infraction, cryoinjury method has been broadly used in order to induce an 'ischemia-like' injury in the ventricle of adult zebrafish. This procedure has been shown to be well tolerated by animals, which recover fast after the injury. Recently it has been reported, however, that fish can perceive pain, indicating that current protocols need to be revised, assuring that any possible pain and discomfort can be reduced to minimum. In this study, we investigated the effect of different analgesic (lidocaine and morphine) treatments after cryoinjury, addressing their effects on the alleviation of possible pain and the heart regenerative process. Our data shows that lidocaine treatment does not have an impact on the zebrafish behaviour and pain alleviation, furthermore it has a negative effect on the regeneration by slowing down the process. Interestingly, morphine treatment does not have an influence on the regeneration speed, however a significant improvement in the behaviour was determined after 2h post injury.

CHRISTOPH LIPPS, Extracellular vesicles mediated small non-coding RNAs in the progression of chronic thromboembolic hypertension

Christoph Lipps1,2,7, Philipp Northe1,7, Ricardo Figueiredo3, Manfred Rohde4, Alexandra Brahme5, Eva Krämer-Albers5, Christoph Liebetrau2,7, Till Keller2,7, Oliver Dörr2,6,7, Holger Nef6,7, Christian Hamm2,6,7, Christian Troidl1,2,7

1 Medical Clinics I - Experimental Cardiology, Justus-Liebig-University Giessen, Giessen; 2 Department for Cardiology, Kerckhoff Clinics GmbH, Bad Nauheim; 3 GenXPro GmbH, Frankfurt am Main; 4 Central Unit for Microscopy, Helmholtz Centre for Infection Research, Braunschweig, 5 Molecular Biology, Johannes-Gutenberg-University Mainz; 6 Medical Clinics I - Cardiology and Angiology, University Clinics Giessen, Giessen,7 DZHK-partner site RheinMain

Background: Extracellular vesicles (EVs), such as microvesicles and exosomes, are continuously released into the bloodstream. EVs are essential for the maintenance of intercellular communication. Importantly, small non-coding RNAs (sncRNAs) are selectively packaged into EVs so that the profile of these molecules varies according to the pathological situation. They regulate basic biological functions in almost all cell types and have been shown to play a role in cardiovascular diseases. Purpose: mThe aim of the study was to identify profiles of sncRNAs derived from extracellular vesicles in order to identify novel patterns potentially useful for diagnosis or prognosis of chronic thromboembolic pulmonary hypertension (CTEPH) and that might serve as novel potential drug targets. Methods: We isolated EVs from serum of CTEPH patients and healthy individuals. We used a column-based method for EV purification followed by the isolation of the EV-mediated total RNA. A small RNA library was prepared to obtain a global profile of small non-coding RNAs using TrueQuant technology, which eliminates PCR-derived reads from the gene expression profiles. In addition, a polymer-based isolation method was used for purification of small EVs. EVs were characterized by transmission electron microscopy (TEM), Nanoparticle tracking analysis and biochemical means. Total RNA was isolated and qPCR analysis was performed to validate the quantification of small non-coding RNA identified in EVs. Results: TEM and NTA analysis revealed MV subpopulations isolated by the different methods: the polymer-based method comprised a subpopulation of small EVs (30-150

nm in diameter) and the column-based method EVs with 50-300 nm. We identified 18 microRNAs (miRNAs) and various PIWI-interacting RNAs (piRNAs) clusters that are differentially abundant in EVs from CTEPH patients and healthy subjects. The piRNA DQ593039 correlated with various clinical parameters. Prediction of target genes revealed a contribution of piRNAs in cardiac and vascular remodelling. Conclusion: The isolation method defines the subpopulation of EVs and has an impact on the outcome of miRNA and piRNA species analysis. TrueQuant technology enables the identification of disease-specific small non-coding RNA profiles. This information assists in the discovery of the cellular mechanisms and reprogramming processes involved in CTEPH progression and may aid the establishment of novel diagnostic markers and therapeutic strategies.

ELISA MASTANTUONO, Riboflavin treatment improves mitochondrial cardiomyopathy due to ACAD9 deficiency

E. Mastantuono 1, 2, B.M. Repp 1, 2, C.L. Alston 3, M. Schiff 4, T.B. Haack 1,2, A. Rötig 5, C.B. Catarino 6, G. Schottmann 7, J. Poulton 8, J.A. Mayr 9, M. Schuelke 7, M. Zeviani 10, R. McFarland 3, T. Klopstock 6, T. Meitinger 1, 2, 16, T. M. Strom 1,2, U. Herberg 11, W. Sperl 9, M.C. , H.Ling 12, P. Freisinger 13, R., R.W. Taylor 3, J. Häberle 14, J. Vockley 15, H. Prokisch 1,2, S. Wortmann 1,2,9.

1 Inst. of Human Genetics-Technische Univ. München 2 Helmholtz Zentrum München. 3 Wellcome Trust Centre for Mitochondrial Research 4 INSERM-Univ. Paris Diderot 5 Univ. Paris Descartes 6 Ludwig-Maximilians-Univ. München 7 NCRC-Charité-Universitätsmedizin Berlin 8 Univ. of Oxford 9 Depart. of Pediatrics Salzburg 10 MRC-Mitochondrial Biology Unit Cambridge 11 Depart. of Pediatric Cardiology, Univ. of Bonn 12 Depart. of Pediatric Cardiology, Beijing Anzhe Hospital 13 Depart. of Pediatrics, Klinikum Reutlingen 14 Univ. Children Hospital Zurich 15 Depart. of Pediatrics, Univ. of Pittsburgh School of Medicine 16 DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany.

Background Patients with cardiomyopathy are routinely diagnosed with gene panels in which mitochondrial genes are underrepresented. However, more than 100 mitochondrial disease genes are known to be associated with cardiomyopathy and the gene list is constantly growing. For most of these disorders there is no effective treatment, but cofactor metabolism deficiencies represent an exception as promising treatment options are available. Methods In this study, we investigated the genetic, clinical and biochemical findings in a cohort of 70 patients with ACAD9 deficiency, one of the most frequent causes of mitochondrial cardiomyopathy. We further evaluated the effect of riboflavin treatment in patients and focused on its effect on survival rates in patients with onset in the first year of life as they were particularly severe. We finally assessed the riboflavin effect in patient-derived fibroblast cell lines. Results The clinical presentation of ACAD9 deficiency was dominated by cardiomyopathy (85%) with 30% of the cases showing sudden cardiac death (SCD) in neonatal period. In the majority of the patients, cardiomyopathy presented in the first year of life and determined a shorter survival as compared to cases with a later presentation. Oral riboflavin treatment significantly improved the survival rate of treated patients (p=5.34 10-05). Remarkably, our results show that riboflavin treatment also improved complex I activity for most of patient-derived fibroblasts tested. Conclusions Mitochondrial dysfunction represents a significant cause of cardiomyopathy and the increasing use of WES has unmasked such cases promoting precision treatment. The clinical benefit of ACAD9 deficient patients after riboflavin intake justifies a trial with riboflavin (20 mg/kg/day, maximum 200 mg/day) in every patient with this genetic diagnosis. For these patients, early diagnosis and therapeutic intervention could represent the difference between life and death.

SÖREN MEYER, The role of FAM129B in monocyte/macrophage function during myocardial infarction

Sören Meyer 1,2 , Sophie Engelskircher 1, Susann Werkmeister 1, Florian Leuschner 1,2

1 Heidelberg University Hospital, Heidelberg, Germany. 2 DZHK, Partnersite, Heidelberg/Mannheim, Germany

Exaggerated inflammatory processes are associated with poor prognosis and increased tissue damage following myocardial infarction (MI). A growing body of evidence demonstrates that monocytes and their lineage descendant macrophages are key players in the immune response to cardiac ischemia. A tight balance of their pro- and anti-inflammatory properties is an absolute prerequisite for optimal cardiac healing. In RNAseq analysis Family with sequence similarity 129 member B (FAM129B) was identified as a potential regulator of monocyte function during the inflammatory response after MI. FAM129B is strongly upregulated in monocytes at the site of injury compared to monocytes in the bone marrow in a mouse model of MI. In addition, in vitro data show that FAM129B knockdown influences the phenotype of monocytes/macrophages.

IRENE MÜLLER, Serum levels of the damage-associated molecular pattern S100A8/S100A9 as a diagnostic and monitoring biomarker in patients with a recent onset of myocarditis

1,2,3Irene Müller, 1Uwe Kühl, 4Thomas Vogl, 5Alexander Krannich, 1,2,3Sophie Van Linthout, 1,2,3Carsten Tschöpe

1 Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Internal Medicine and Cardiology, Campus Virchow Klinikum, Berlin, Germany; 2 DZHK (German Center for Cardiovascular Research), partner site Berlin, Germany; 3 Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin-Brandenburg Center for Regenerative Therapies, Campus Virchow Klinikum, Berlin, Germany; 4 Department of Immunology, University of Münster, Münster, Germany; 5Biostats, Berlin, Germany

BACKGROUND: S100A8/A9 is important in cardiovascular disorders. We recently showed its relevance in acute Coxsackievirus B3-induced myocarditis (MC). However, the role of S100A8/A9 serum levels in acute MC patients has not been unraveled so far. PURPOSE: We aimed to evaluate serum S100A8/A9 levels as a diagnostic and monitoring tool in patients with a recent onset of MC. METHODS: Serum S100A8/A9, hsCRP, and NT pro-BNP levels were analyzed in patients with a recent onset of MC (≤ 30 days (d), n=29; ejection fraction (EF): 43.8%±14%), dilated cardiomyopathy patients with inflammation (DCMi: n=112; EF: 28.8%±12%) or without inflammation (DCM: n=58; EF: 26.7%±9%), and controls (co: n=51; EF: 60%±5%). Blood samples and endomyocardial biopsies (EMBs) were collected at time point (T1). In a subgroup, S100A8/A9 serum levels and EMBs were available at T1 (n=10) and follow-up (T2, n=10, mean follow-up 8 months). RESULTS: MC ≤ 30 d patients showed a 4.5-fold ($p<0.0001$), 19.3-fold ($p<0.0001$), and 4.0-fold ($p<0.0001$) increase in S100A8/A9, hsCRP, NT pro-BNP levels vs co, respectively. S100A8/A9 levels correlated with the disease activity, demonstrated by EMB counts of inflammatory cells (CD3: $r=0.464$, $p=0.0128$, LFA-1: $r=0.551$, $p=0.002$, Mac-1: $r=0.418$, $p=0.026$), and the EF ($r=0.545$, $p=0.0027$). Serum S100A8/A9 levels were increased by 3.0-fold ($p<0.0001$) and 1.8-fold ($p=0.0005$) in DCMi (n=112), and DCM (n=58) patients vs co, respectively. However, the S100A8/A9 levels of DCMi and DCM patients were 1.5-fold ($p=0.07$) and 2.5-fold ($p<0.0001$) lower vs MC ≤ 30 d patients. hsCRP correlated with EMB inflammation and EF. NT pro-BNP showed no relation to the EMB inflammation, but a negative correlation with EF. ROC analyses of S100A8/A9 in MC ≤ 30 d provided a cut-off value of 538 ng/ml

with a specificity=92%, sensitivity=86.2%, a PPV=92.6%, a NPV=85.2%, and an AUC=0.934 vs co, which was superior to hsCRP (cut-off=5 mg/l): specificity=95.8%, sensitivity=58.6%, a PPV=94.4%, a NPV=65.7%, AUC=0.885. In the subgroup, S100A8/A9 levels decreased after heart failure medication (T1: 2454 ± 1931 ng/ml vs T2: 934.4 ± 552 ng/ml; p=0.002), reflected by a decrease of EMB inflammatory markers. Baseline serum S100A8/A9 levels predicted the change in EMB CD3 and Mac-1. CONCLUSIONS: Serum S100A8/A9 levels are important in recent onset MC. These results support an additional value for S100A8/A9 serum levels as a potential diagnostic biomarker and as a tool to monitor the course of the disease.

PAULA MÜNKLER, High resolution mapping of focal VT from the papillary muscle identifies rate dependency of activation recovery interval

Paula Münkler1, Niklas Klatt1, Katharina Scherschel1, Pawel Kuklik1, Christiane Jungen1, Ulrike Pape1, Torsten Christ2, Christian Eickholt1 Stephan Willems1, Christian Meyer1

1University Heart Center Hamburg, University Hospital Hamburg-Eppendorf, Martinistraße 52, 20642 Hamburg, Germany

Background: Ventricular arrhythmia (VA) originating from papillary muscle can be malignant. Activation recovery interval (ARI) is a surrogate for action potential duration and can identify a propensity to arrhythmia. Methods: We established an ovine model of focal VA . Focal VA were induced by injecting aconitine into the left anterior papillary muscle in 10 female domestic sheep under general anesthesia. Endocardial high-density electroanatomic mapping was performed using a 64-electrode basket catheter. Epicardial mapping was performed simultaneously using a custom-made 104-electrode sock. Unipolar signals were analyzed for local activation time (LAT) and ARI during incremental pacing and during VA. Results: Endocardial mapping of 47 sustained VA showed focal activation with the earliest activation at the injection site. ARI showed a heart-rate dependent decrease independent of the anatomical region. The regression curve of ARI and cycle length during VA was characterized by a steeper slope (slope 0.50, R2=0.72) compared to the regression curve of ARI and cycle length during ventricular pacing (slope 0.22, R2=0.89). Conclusion: In structurally healthy hearts, there is a regionally homogeneous adaptation of ARI to increasing heart rate in VA. During VA, changes in cycle length are associated with a more pronounced change in ARI compared to during ventricular pacing. This is represented by a steeper slope of the regression curve and may reflect the greater propensity to wavebreak and degeneration of VA compared to ventricular pacing.

NIKOLETA PAVLAKI, Gene therapy with phosphodiesterase 4B in a murine model of pressure overload-induced cardiac hypertrophy

Nikoleta Pavlaki, Viacheslav O. Nikolaev

1 University Medical Center Hamburg-Eppendorf

Background: Heart failure (HF) is typically characterized by chronic neurohormonal hyperactivation and increased production of a second messenger 3’,5’-cyclic adenosine monophosphate (cAMP). Paracrine factors and hormones such as nitric oxide (NO) and natriuretic peptides (NPs) counterbalance the cAMP-induced effects by stimulating 3’,5’-cyclic guanosine monophosphate (cGMP) synthesis, and the hydrolysing enzymes called phosphodiesterases tightly regulate intracellular levels of both cyclic nucleotides. At the early onset of HF, the interplay among β-AR and PDEs is compromised leading to disease-associated alterations in cAMP/cGMP compartmentation. Purpose: To restore cyclic nucleotide compartmentation in ryanodine receptor microdomains by

cardiac-targeted overexpression of PDE4B in a mouse model of pressure overload-induced heart failure. Methods: Transgenic (TG) mice expressing localized cAMP biosensors for distinct membrane microdomains at the sarcolemma and sarcoplasmic reticulum were subjected to Transverse Aortic Constriction (TAC) and gene therapy with phosphodiesterase PDE4B. After 8 weeks, the animals were sacrificed and their hearts were enzymatically digested in a Langendorff set-up. Ventricular cardiomyocytes were isolated and plated on laminin-coated coverslips for live imaging with Förster Resonance Energy Transfer (FRET). FRET measurements were performed upon β-AR stimulation with the non-selective β-AR agonist isoproterenol and subsequent pharmacological inhibition of PDE4 by rolipram. Results & Conclusions: Preliminary data on healthy TG mice showed that PDE4 tightly controls cAMP levels stimulated by the β-adrenergic receptor agonist in the ryanodine receptor domains. Upon β-AR stimulation, cAMP activates cAMP-dependent protein kinase, which, in turn, phosphorylates and enhances the catalytic activity of PDE4 at high isoproterenol concentrations, while at submaximal β-AR stimulation, the effect is far less strong. Since in pressure-overload hypertrophy model, PDE4 levels are selectively decreased at the ryanodine receptor, the effect of PDE overexpression remains to be explored in both subcellular microdomains of this disease model.

TOBIAS PETZOLD, Rivaroxaban exerts a direct antiplatelet effect on platelet activation and arterial thrombosis

Tobias Petzold MD1, Manuela Thienel MD1, Lisa Dannenberg MD2, Joachim Pircher MD1, Andreas Ehrlich VMD1, Christian Schulz MD1, Malte Kelm MD2, Steffen Massberg MD1, and Amin Polzin MD2

1 Medizinische Klinik und Poliklinik I, Klinikum der Universität München, Ludwig-Maximilians-University Munich, Germany 2 Department of Cardiology, Pulmonology and Vascular Medicine, Medical Faculty of the Heinrich Heine University Düsseldorf, Düsseldorf, Germany; Cardiovascular Research Institute Düsseldorf (CARID)

Abstract Aims: Non-vitamin K anticoagulants (NOACs) have become first choice in patients with atrial fibrillation (AF) requiring oral anticoagulation (OAC) for stroke prevention. Besides that, a reduced frequency of myocardial infarction (MI) has been observed in patients treated with factor Xa (FXa) inhibiting NOACs including rivaroxaban (RIVA). Along this line low dose (RIVA) improves outcome in coronary artery disease (CAD) patients. By now, the reason for this reduction in MACCE is unknown. In this study we hypothesized, that RIVAs antithrombotic potential is linked to a hitherto unknown antiplatelet effect that impacts on platelet reactivity and arterial thrombosis. Methods and Results: Arterial thrombus formation under RIVA treatment, was analysed in a rodent model of arterial thrombosis and revealed a reduced thrombus stability. For in vitro studies patients on permanent RIVA treatment for stroke prevention due to AF, respective controls and patients with new onset AF prior and after first intake of RIVA (time-series analysis) were recruited. Thrombus formation under arterial flow conditions on collagen and atherosclerotic plaque material as well as platelet aggregation responses were attenuated under RIVA, independent of its anticoagulatory capacity. Low-dose RIVA (2.5mg) treatment reduced platelet activation but not platelet aggregation or thrombus formation. Conclusion: This study identified a so far unknown direct-antiplatelet effect of RIVA that together with its well-known potent anticoagulatory capacity might lead to reduced frequency of myocardial infarction and improved outcome in patients on high risk for myocardial infarction.

ANCA REMES, Prevention of myocardial hypertrophy following AAV9-mediated NFAT hairpin decoy ODNs delivery into the myocardium

Anca Remes1, Nesrin Schmiedel1, Lin Ding1, Andreas Jungmann2, Nina Ullrich3, Susanne Hille1, Hugo A. Katus2, Norbert Frey1, Andreas H. Wagner3, Oliver J. Müller1, Markus Hecker3

1 Department of Internal Medicine III, University Hospital Kiel, Kiel, Germany, and German Centre for Cardiovascular Research (Hamburg, Kiel, Lübeck site), Kiel, Germany, 2 Internal Medicine III, University Hospital Heidelberg, Germany 3 Institute of Physiology and Pathophysiology, Heidelberg University, Heidelberg, Germany

Introduction: Cardiac hypertrophy represents the myocardial response to stress stimuli and increased workload resulting in impaired cardiac function and heart failure. An increased calcineurin–nuclear factor of activated T-cells (NFAT) signaling was shown to play an essential role in pathological cardiac hypertrophy and thus represents a powerful therapeutic target. Purpose: Development of an approach for NFAT neutralization using adeno-associated virus (AAV)-mediated delivery of NFAT-neutralizing decoy oligodeoxynucleotides (dODNs) and its validation in a model of left ventricular hypertrophy. Methods: HL-1 cells and primary neonatal cardiomyocytes (NCMs) were treated with a hairpin consensus (hp cons) dODN with high affinity for NFAT and a mutated dODN as control. To achieve continuous expression of hpNFAT dODNs, we generated AAV vectors for expression of the nucleic acid drug as shRNA (RNA dODN, AAV6 in vitro, AAV9 in vivo). Pro-hypertrophic gene program was induced by endothelin-1 (ET-1) application. Fetal gene expression was analyzed using qPCR and BNP protein levels were measured using ELISA. In vivo delivery of dODNs was achieved by tail vein injection of AAV9 vectors prior to inducing myocardial hypertrophy by transverse aortic constriction (TAC). After 6 weeks, cardiac hypertrophy markers (heart weight/tibia length, HW/TL) and heart failure parameters (lung weight/tibia length, LW/TL) were quantified. RNA decoy ODNs were detected by in situ hybridization. Heart function and left ventricular mass were evaluated by echocardiography. Additionally, fibrosis in cardiac tissue sections was assessed by Masson's Trichrome staining. Results: Treatment with hp cons dODNs and transduction with AAV6 expressing hp cons RNA dODNs led to a significant downregulation of ET-1 induced fetal gene program in both HL-1 cells and NCMs. Moreover, we observed a reduction in protein translation level following NFAT neutralization and a significant decreased BNP protein concentration. The mutated dODN had no effect on these parameters. In vivo AAV9-mediated hp cons RNA dODNs expression prior to TAC led to a reduction in the HW/TL and LW/TL ratios and a pronounced decrease in the LW mass. Importantly, heart function was markedly improved and fibrosis was reduced by NFAT neutralization. Conclusion: AAV-mediated delivery of NFAT-neutralizing dODNs into cardiomyocytes prevented cardiac hypertrophy in mice subjected to TAC, suggesting a potential therapeutic perspective.

LENNART ROOS, Exploring LZTR1-associated Noonan Syndrome in patient-specific and CRISPR-corrected iPSC-derived cardiomyocytes

Lennart Roos 1,2, Mandy Kleinsorge 1,2, Ulrich Hanses 1,2, Malte Tiburcy 2,3, Robin Hindmarsh 1,2, Wolfram-Hubertus Zimmermann 2,3, Gerd Hasenfuß 1,2, Bernd Wollnik 2,4, Lukas Cyganek 1,2

1 Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany, 2 DZHK (German Center for Cardiovascular Research), partner site Göttingen, 3 Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Göttingen, Germany, 4 Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany

Noonan syndrome (NS) is a multisystemic disorder characterised by a variable set of clinical symptoms including craniofacial dysmorphia, short stature and congenital heart defects, the latter of which are found in the majority of patients. NS affects 1 in 1000 to 2500 newborns and is caused by germline mutations in genes that encode components of the RAS/MAPK signalling pathway, leading to its overactivation. In this study, we present a family displaying a novel autosomal recessive form of NS with two sibling children suffering from severe hypertrophic cardiomyopathy (HCM) with outflow obstruction caused by compound heterozygous mutations within the Leucine Zipper Like Transcription Regulator 1 (LZTR1). However, the function of LZTR1 and its contribution to the development of HCM is still obscure. We generated patient-specific iPS cell-derived cardiomyocytes (iPSC-CMs) from the affected siblings, which were then deeply investigated on the molecular and functional level. Our data demonstrate that the patient-specific iPSC-CMs recapitulate the hypertrophic disease phenotype in vitro by displaying hypercontractility in 'engineered heart muscle' as well as a malfunction in calcium handling. Furthermore, we could show that LZTR1 is indeed involved in increased RAS/MAPK signalling activity and could gain first insights into the cellular function of LZTR1 and its pathological relevance. We corrected the paternal SNP in the patient's iPSCs via CRISPR/Cas9 genome editing in order to consolidate the family's mutations as disease-causing and, moreover, to evaluate the possibility for gene therapy as a treatment option. Through the combination of functional and molecular approaches, this study will ultimately lead to a better understanding of the underlying disease mechanisms leading to this severe phenotype of NS and thus enable a more effective diagnosis and treatment.

SINA SCHULTZ, Hyperglycemia-induced α-dicarbonyls in diabetes-related vascular dementia

Sina Schultz, Markus Schwaninger

Institute for experimental and clinical pharmacology and toxicology, University of Luebeck

Aim Diabetes mellitus is the most common metabolic disorder in humans, approaching epidemic proportions with increasing incidence and prevalence. Thereby, chronic hyperglycemia displays a strong correlation with the development of dementia. Although it is well established that chronic hyperglycemia leads to vascular complications, the molecular basis of the development of cognitive impairment due to diabetes is still unclear. A possible link could be the production of highly reactive and toxic metabolites of glucose. Hence, we investigated the production of a distinct class of such molecules, which share an α-dicarbonyl structure. In addition, we studied their effects on the cerebrovascular system and cognitive decline. Method Diabetes was induced in C57BL/6 mice by injecting 50 mg/kg streptozotocin on 5 consecutive days. After 12 weeks, mice were tested for cognitive impairment by an object-place recognition test and sacrificed for analyzing the vascular system and determining glucose metabolites by mass spectrometry. Results We provide evidence that chronic hyperglycemia leads to an increase of α-dicarbonyls in blood plasma and brain tissue of diabetic mice. This was associated with an increase of 4-hydroxynonenal positive vessels, indicating

oxidative stress. Further analysis of the vascular system revealed higher abundance of string vessels, reflecting the degeneration of the vascular integrity. Furthermore, we detected an increased extravasation of IgG into the brain and signs of cognitive impairment of diabetic mice performing in an object-place recognition test. Conclusion Our data indicate that vascular pathology, associated with α-dicarbonyl-induced oxidative stress, could be an essential factor promoting cognitive impairment and dementia in diabetes.

YASMINE SEIBEL, The role of serotonin in atherosclerosis, angiogenesis and remodelling of the vessel wall

Yasmine Seibel 1,2,3,4,5; Natalia Alenina 1,5; Michael Bader 1,3,4,5,6

1 Max Delbrück Center for Molecular Medicine, 2 Humboldt University Berlin, 3 Charité Berlin, 4 BSRT, 5 DZHK, 6 BIH

Introduction: My project aims at investigating the role of peripheral serotonin (5-HT) in the development of atherosclerosis and regenerative processes after myocardial infarction. As an autacoid 5-HT has great potential to be a key player in tissue remodelling, especially in fibroblast rich tissues like the adventitia. Here, 5-HT might mediate secretion of proinflammatory and adhesive proteins or it might support endogenous healing processes after vessel injury and so prevent atherosclerotic processes. On the other hand, 5-HT seems to be essentially involved in atherosclerosis development and calcification of arteries. To investigate its role in the pathogenesis of this common disease might give us a great tool to design novel therapy approaches. Material & Methods: The ApoE deficient mouse is the commonly used model for atherosclerosis. In our lab we generated double-knockout mice for ApoE and TPH1 or SERT; both lacking 5-HT in the periphery. Male and female mice were either fed with a high cholesterol diet (Western Diet, WD) to induce atherosclerosis within few weeks or left to age and develop atherosclerotic plaques (AP) naturally. Subsequently, all experimental groups were dissected and organs were analyzed by HPLC, RT-PCR, Histological methods, IHC and ELISA. A special focus was put on the analysis of the aortic arch and brachiocephalic artery, sites of initial stages of AP formation. Results: Using biochemical methods we first confirmed that 5-HT is absent (SERT/ApoE) or drastically reduced (TPH1/ApoE) in double knockout mice in comparison to control ApoE-deficient animals. By analyzing blood profile we found that feeding the animals WD significantly increases blood cholesterol levels. However, ApoE/TPH1 double knockout animals showed lower cholesterol levels than ApoE knockouts. We could also prove that all animals with ApoE deficiency develop APs. ApoE/SERT males showed a significantly reduced AP size compared to ApoE males. The AP burden in ApoE/TPH1 males also tended to be decreased but surprisingly, ApoE/TPH1 females showed the opposite effect. Conclusion: My data confirmed that serotonin levels influence AP formation. However, the effects of 5-HT absence are weak and probably confined to males. Further analysis of different tissues (heart, brachiocephalic artery) and different components of the AP (collagen, macrophages and monocytes, calcium) will be necessary to complete the picture of the role of 5-HT in atherosclerotic processes.

NICOLINE SMIT, Promoting transparency in preclinical research: preregistration of animal studies on www.preclinicaltrials.eu

Nicoline W. Smit 1, Mira van der Naald 2, Kim E. Wever 3, Dirk J.G.M Duncker 1, 4, Steven A.J. Chamuleau 2

1 Netherlands Heart Institute, Utrecht, The Netherlands. 2 University Medical Center Utrecht, Utrecht, The Netherlands. 3 Radboud Medical Center, Nijmegen, The Netherlands. 4 Erasmus Medical Center, Rotterdam, The Netherlands

Background: Publication bias, selective outcome reporting, and risks of bias limit the validity and reproducibility of animal studies and threaten the translational value. Preregistration is a promising process to reduce these limitations and create transparency. For clinical trials preregistration is standard however, this is not the case for animal study protocols, whilst these form the basis for clinical research. On preclinicaltrials.eu we have developed an online platform to preregister animal study protocols on. Objectives: An expert group on preclinical evidence synthesis designed the registration form which consists of 34 fields. Details of the study's hypothesis, design, outcome measures (primary and secondary), measures to reduce bias and sample size rational are asked for. Authors need to indicate whether their study is exploratory or confirmatory. Reference to publication(s) or data repositories can be provided. Protocols can be made publicly directly after submission, or after an embargo period. Preclinicaltrials.eu aims to provide an overview of all executed animal studies, including those that remain unpublished, and thus contribute to a reduction of publication bias and unnecessary duplication. It allows reviewers and researchers to access additional information on the study design. Finally, preclinicaltrials.eu aims to increase awareness and transparency concerning risk of bias and selective outcome reporting and potentially reduce these biases. Future perspectives: We encourage all researchers to preregister their (confirmatory) animal study protocols on preclinicaltrials.eu. We believe all stakeholders involved in animal research should encourage preregistration and call upon researchers, institutes, medical journals, funding bodies, policy and law makers, scientific societies and others involved parties to make prospective registration the standard and mandatory in animal research.

ELISABETH STRÄSSLER, Functional analysis of iPSC-derived endothelial cells from risk-stratified CAD patients and comparison with primary endothelial cells

Elisabeth Tamara Strässler 1,2; Katriina Aalto-Setälä 4; Mostafa Kiamehr 4; Nicolle Kränkel 1,2; Ulf Landmesser 1,2,3

1 Charité – Universitätsmedizin, 2 Deutsches Zentrum für Herz-Kreislauf Forschung, 3 Berlin Institute of Health, 4 University of Tampere

Cardiovascular disease remains the leading cause of death in industrialised countries and has long since been accepted as a multifactorial disease with both genetics and lifestyle impacting on the development and course of the disease. In order to quantify functional differences caused by variation in genetic background, we used induced pluripotent stem cell-derived endothelial cells of risk stratified coronary artery disease patients for comparison with primary endothelial cells and functional analysis. Skin samples from three risk populations (healthy, stable CAD, acute myocardial infarction) were collected and reprogrammed to induced pluripotent stem cells (iPSCs) using Sendai Virus. These iPSCs were then differentiated to endothelial cells (iPSC-ECs). Primary endothelial cells (HAEC = human aortic endothelial cells, HMVEC-C = human microvascular cardiac endothelial cells) and iPSC-ECs were then compared in their functionality as well as in their response to different inflammatory stimuli (e.g. TNF-α, oxLDL). Inflammatory stimulation was assessed under static as well

as pro- and anti-atherogenic flow conditions (laminar vs turbulent flow, high and low shear stress). Angiogenesis was assessed using a Matrigel-based sprouting assay. All iPSC-ECs showed typical endothelial characteristics such a cobblestone morphology and expression of endothelial markers (CD31, CD34, CD146, CD309) and were able to form typical capillary-like structures when plated to Matrigel. Induced pluripotent stem cell-derived endothelial cells showed an inflammatory response more similar to HMVEC-Cs than to HAECs as the latter demonstrated a much stronger upregulation of typical inflammatory markers such as ICAM1 and VCAM1 ($p<0.0001$). This observation holds true under both static and pro-atherogenic flow conditions. Although HMVEC-Cs showed a higher angiogenic capacity than iPSC-ECs ($p<0.001$). Induced pluripotent stem cell-derived endothelial cells from different donor populations showed comparable inflammatory response under static and flow conditions. However, iPSC-ECs from CAD patients showed a weaker VCAM1 upregulation compared to iPSC-ECs from healthy donors ($p<0.001$) under inflammatory stimulation. In conclusion, iPSC-ECs are comparable to primary endothelial cells in their basic functions although there are significant differences in inflammatory response between subtypes of primary endothelial cells as wells as between iPSC-ECs from different donor groups.

STEPHANIE TENNSTEDT, Soluble Guanylyl Cyclase: SNP Analysis - a Step Toward Drug Discovery

Stephanie Tennstedt1/2/3, Viviana Luisa Vedder1/2/3, Jeanette Erdmann1/2/3

1 Institute for Cardiogenetics, Universität zu Lübeck; 2 DZHK (German Centre for Cardiovascular Research), Partner Site Hamburg/Kiel/Lübeck, Germany; 3 University Heart Centre Lübeck, 23562 Lübeck, Germany

We previously showed that genetic alterations in the gene GUCY1A3, which encodes the α1 subunit of soluble guanylyl cyclase (sGC), are strongly associated with coronary artery disease (CAD). Moreover, we found a relationship between coding variants of GUCY1A3 and reduced cGMP levels. In vitro studies showed that reduced cGMP levels triggered by genetic alterations in GUCY1A3, can be rescued by the sGC stimulator BAY 41-2272. Treating patients carrying genetic alterations in GUCY1A3 with sGC stimulators such as BAY 41 2272 might be a novel approach to reduce the risk of cardiovascular diseases. Stimulators of sGC are emerging therapeutic agents for cardiovascular diseases, but in-depth studies are lacking. However, 7,802 SNPs are known for GUCY1A3, 408 of which are missense variants; of these, 110 are located within the catalytic domain, the binding site of BAY 41-2272. It is likely that certain SNPs influence the binding mode of the stimulator, which might impact the success of treatments based on sGC stimulators. Now we have established a screening pipe line to characterize the effects of each individual SNP on protein structure and function. Moreover, if we want to develop new therapeutics targeting sGC, it is crucial to understand the way in which BAY 41-2272 binds to specific sGC variants. Finally, the obtained data will be used to generate a genetic landscape which is a first step towards the development of novel therapeutics for patients with CAD carrying genetic variants of GUCY1A3.

VIVIANA VEDDER, High-content screening using zebrafish as a model for angiogenesis

Viviana Luisa Vedder1/2/3, Zouhair Aherrahrou1/2/3, Jeanette Erdmann1/2/3

1 Institute for Cardiogenetics, Universität zu Lübeck; 2 DZHK (German Centre for Cardiovascular Research), Partner Site Hamburg/Kiel/Lübeck, Germany; 3 University Heart Centre Lübeck, 23562 Lübeck, Germany

Angiogenesis, a natural process that forms new blood vessels from preexisting ones, is also associated with a variety of diseases. Irregularities in the formation of secondary blood vessels can lead to conditions, such as atherosclerosis, diabetes and hypertension, major risk factors of cardiovascular diseases (CVDs), the leading cause of death worldwide. Nowadays zebrafish are well established for high-content screenings and provide a small, transparent model organism that allows angiogenesis to be tracked quickly and efficiently. For this purpose, the common transgenic zebrafish line Tg(fli1a:eGFP) was used, which expresses eGFP in vessels and thus allows visualization of the blood vessels in the developing embryo in a multiwell plate format. Here drug screening for compounds affecting blood vessel formation and patterning can be rapidly used to find new targets and at the same time offer a therapy through drug repositioning. In this project we will screen a drug library of nearly 1300 compounds, to identify clinically applicable drugs regulating angiogenesis.

TIM WILHELMI, Serelaxin alleviates cardiac fibrosis through inhibiting endothelial-to-mesenchymal transition via RXFP1

Tim Wilhelmi 1,3, Xingbo Xu 1,3, Xiaoying Tan 2,3, Melanie S. Hulshoff 1,3,4, Michael Zeisberg 2,3, Elisabeth M Zeisberg 1,3

1 Department of Cardiology and Pneumology, University Medical Center of Göttingen, Georg-August University, Göttingen, Germany, 2 Department of Nephrology and Rheumatology, University Medical Center of Göttingen, Georg-August University, Göttingen, Germany, 3 DZHK (German Centre for Cardiovascular Research, partner site Göttingen, Germany), 4 Laboratory for Cardiovascular Regenerative Medicine, Department of Pathology and Medical Biology, University of Groningen, Groningen, The Netherlands

Aims: Cardiac fibrosis is an integral constituent of every form of chronic heart disease, and persistence of fibrosis reduces tissue compliance and accelerates the progression to heart failure. Relaxin-2 is a human hormone, which has various physiological functions such as mediating renal vasodilation in pregnancy, and its recombinant form Serelaxin has recently been tested in clinical trials as a therapy for acute heart failure but did not meet its primary endpoints of reduction in cardiovascular death after 6 months or reduced worsening heart failure after 5 days. The aim of this study is to test whether Serelaxin could have a long-term beneficial anti-fibrotic effect in the heart. Methods and Results: We utilized two different mouse models of pressure overload (ascending aortic constriction (AAC) and Angiotensin II (AngII) administration via osmotic minipumps). Our results demonstrate a significant and dose-dependent anti-fibrotic effect of Serelaxin in the heart. We further show that this effect is mediated, at least in part, through inhibition of endothelial-to-mesenchymal transition (EndMT) via activation of the NOTCH signaling pathway by Serelaxin through the endothelial receptor RXFP1, which is commonly downregulated in diseased hearts. Conclusions: We demonstrate in cell culture experiments that upon downregulation of RXFP1 via TGFβ1, Serelaxin induces gene activating histone modifications, thereby reactivating RXFP1 expression. In summary, this study identifies Serelaxin to alleviate EndMT and cardiac fibrosis by reactivating RXFP1 expression through epigenetic mechanisms and suggests that Serelaxin may have a long-term beneficial effect as anti-fibrotic therapy in chronic heart failure.

JULIA WINTER, PCSK9 deficiency is not associated with impaired cardiac repair capacity early after myocardial infarction

Julia Winter1,2; Maria Luisa Barcena de Arellano2,3; Matthias Lohmar1,2; Jenny Jansen2,3; Ellen Na1; Sabine Knüppel1; Vera Regitz-Zagrosek2;3; Ulf Landmesser1,2,4

1 Department of Cardiology, Charité – Universitätsmedizin Berlin, Germany, 2DZHK (German Centre for Cardiovascular Research), partner site Berlin, Germany; 3 Institute of Gender in Medicine, Center of Cardiovascular Research, Charité – Universitätsmedizin Berlin, Germany;4 BIH (Berlin Institute of Health) Berlin, Germany

Background: Proprotein convertase PCSK9 plays a crucial role in LDL cholesterol metabolism. PCSK9 inhibitory antibodies target circulating PCSK9, thereby preventing LDLR degradation in liver cells, ultimately decreasing serum LDL levels. Recently, they have been introduced as a potential novel treatment option in patients after a myocardial infarction. However, knockout (KO) of PCSK9 was accompanied by impaired liver regeneration after hepatectomy in mice. Here we therefore investigate the impact of PCSK9 deficiency on the cardiac repair response early after myocardial infarction (MI). It is of pivotal importance to understand the safety of PCSK9-Inhibition early after an acute MI. Methods: We analysed PCSK9 KO as well as wildtype (WT) mouse hearts by echocardiography before and after permanent ligation of the left anterior descending coronary artery (myocardial infarction, MI). Sirius red staining of tissue sections was performed to analyse fibrosis in the remote area and to determine thickness of remaining left ventricular wall of the infarcted hearts. Results: Our study revealed, that PCSK9 KO itself did not cause significant changes in cardiac output (CO), left ventricular end systolic or diastolic volume (LVESV, LVEDV), stroke volume (SV) or ejection fraction (EF) compared to WT mice. Four weeks after MI, no significant differences in cardiac function (CO, EF, SV, LVESV, LVEDV) could be observed between WT and PCSK9 KO mice, either. These results indicate, that reduction of PCSK9 in patients with MI is not a risk factor. Additionally we observed, that the remaining left ventricular wall in the infarct area tends to be thicker in mice lacking PCSK9 (p=0,054). This was accompanied by reduced fibrosis in the remote area of infarcted hearts, even if infarct size determined by echocardiography did not show any differences between KO and WT mice. These data suggest less maladaptive remodelling in PCSK9 KO. Conclusion: Taken together, our study shows for the first time, that knockout of PCSK9 is not associated with impaired repair capacity of the heart after tissue damage during MI. Less maladaptive remodeling was observed after MI, supporting the concept that PCSK9 inhibition is safe for application early after MI.

JASMIN ZERNIKOW, 17β-Estradiol regulates periostin in a sex-specific manner in human cardiac fibroblasts

J. Zernikow1,2, V. Regitz-Zagrosek1,2, S. Diecke2,3, S. Lutz4,5, E. Dworatzek1,2

1 Institute of Gender in Medicine and Center for Cardiovascular Research, Charité-Universitaetsmedizin Berlin, Germany; 2 DZHK (German Centre for Cardiovascular Research), partner site Berlin; 3 Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany; 4 Institute of Pharmacology and Toxicology, University Medical Center Goettingen, Germany; 5 DZHK (German Centre for Cardiovascular Research), partner site Goettingen

Background: Cardiac fibrosis contributes to heart failure progression. Men with aortic stenosis (AS) exhibit more fibrosis and significant higher periostin (Postn) protein levels compared to women. 17β-Estradiol (E2) and estrogen receptors (ER) contribute to sex differences in cardiac fibrosis in mice. So far, there are no data available whether E2 and ER regulate Postn in cardiac fibroblasts (CF).

Therefore, we analyzed the effects of E2 and ER on Postn expression and secretion in male and female human CF.
Methods: Postn mRNA levels were measured in heart samples from AS-patients (male: n=7; female: n=6) and healthy donors (male: n=7; female: n=4). CF from healthy donors (n=3 per sex) were treated with 10nM E2, ERα- (10nM PPT), ERβ-agonist (100nM KB5), or vehicle for 6, 12, 24h. Postn mRNA and protein were measured in whole-cell lysates and cell culture supernatants (CCS). Human cardiomyocytes (CM; induced pluripotent stem cells) were 24h incubated with CCS from CF+/-6h E2-treatment. NPPA, NPPB, MYH6, and MYH7 mRNA were measured as marker for CM-activation.
Results: Only male AS-patients showed induction of Postn mRNA compared to healthy controls ($p<0.001$). 12h E2-treatment down-regulated Postn mRNA in female ($p<0.05$), but increased Postn mRNA level in male CF compared to vehicle ($p<0.05$). 12h ER-agonist treatment showed no effect on Postn mRNA. 6h E2-treatment increased Postn protein in whole-cell lysate samples of both sexes ($p<0.01$), while secreted Postn protein was regulated in a similar sex-specific manner as the mRNA levels. CCS-treatment, derived from male and female CF+/-E2-treatment, activated CM in a sex-specific manner. CCS-treatment down-regulated MYH6 and MYH7 mRNA compared to control ($p<0.05$) only in female CM. NPPA mRNA was induced upon CCS derived from vehicle-treated CF ($p<0.05$) in CM of both sexes. However, NPPA induction with E2-derived CCS was only present in male CM ($p<0.05$). As NPPB mRNA induction in female CM could be observed after CCS-treatment from all groups ($p<0.05$), we found an increase in NPPB in male CM only after treatment with CCS derived from E2-treated male CF ($p<0.05$).
Summary: The E2-induced sex-specific regulation of Postn mRNA and secretion in CF might be one possible mechanism for sex-differences in cardiac fibrosis as observed in the clinic. A better knowledge of how E2 and ER mediate sex-specific cardiac fibrosis development could help to design pharmacological interventions according to the sex of patients.