Session 7: Novel insight into pathomechanisms of cardiomyopathy and heart failure

Lectures

L7.1

Understanding the genetic architecture of cardiomyopathies: challenges & opportunities

James Ware

ICTEM, Imperial College London, UK
James Ware <j.ware@imperial.ac.uk>

It has been more than 30 years since the first genes responsible for inherited cardiomyopathies were identified, and genetic testing is a routine part of the management of these conditions. Despite this, the majority of cardiomyopathy cases in the clinic do not receive a molecular diagnosis, and remain genetically unexplained.
This talk aims to summarise our current understanding of the genetic architecture of cardiomyopathies, to explore contemporary challenges and opportunities in understanding the genetic basis of these conditions, and to discuss how advances in our understanding of their architectures promises to translate to the clinic, through target identification and patient stratification.

L7.2

Heart failure with preserved ejection fraction – are we seeing the light at the end of the tunnel?

I. Falcão Pires

Department of Surgery and Physiology, Cardiovascular R&D Centre, Faculty of Medicine of the University of Porto, Portugal
Inês Falcão-Pires <ipires@med.up.pt>

Changes in lifestyle and population ageing are leading to a distressing increase in cardiovascular diseases and heart failure (HF). Characterized by an insufficient capacity of the heart for ejecting blood to the systemic circulation, this syndrome is subdivided in HF with reduced (HFrEF) or preserved ejection fraction (HFpEF), requiring different treatment approaches. In this regard, and despite its increasing epidemiological relevance, HFpEF has been considered the 'poor cousin' of HF, as opposed to HFrEF, whose therapeutic options increase patients' survival. HFpEF prognosis has remained unchanged, and therapy is inefficient as assessed by a large number of HF clinical trials that have systematically failed to show any prognostic benefits. This is partly ascribed to the lack of a robust animal model of HFpEF. As a result, treatment guidelines are limited to targeting the comorbidities and ameliorating patient well-being, rather than treating the failing heart itself.
HFpEF is a complex syndrome, but the consensus is that ageing and comorbidities, such as hypertension, type 2 diabetes and obesity are critical to the disease onset, which results from the interplay and additive injury imposed by systemic inflammation, endothelial dysfunction and neurohumoral dysregulation. Cumulative evidence suggests that energetic unbalance may account for the development of HFpEF.
Until recently, the lack of effective therapies hampered the prognosis of HFpEF patients; however, the latest findings from cardiovascular outcome trials showed a 34% reduction in hospitalization for HF or cardiovascular death in patients whose glucose reabsorption by the kidney is blocked upon sodium/glucose cotransporter-2 inhibitors (SGLT2i) treatment compared to those with placebo or with the standard of care conventional antidiabetic drugs. These outstanding results call for discussion of the clinical implications and encourage in-depth studies of "reverse pharmacology", taking research from the bedside to the bench to dissect the mechanisms of action of SGLT2 inhibitors and other nutritional interventions that lead to activation of pathways like those involved in SGLT2 effects, mostly focusing on metabolic and inflammatory pathways. This presentation aims to provide an overview of several therapeutic interventions that have been attempted using a robust model of HFpEF, the obese ZSF1 rat.

L7.3

Pathomechanisms underlying cardiomyopathy due to *TTN* truncation

Andrey Fomin[1], Anna Gärtner[2], Lukas Cyganek[1], Malte Tiburcy[3], Marion von Frieling-Salewsky[4], Andreas Unger[4], Anna Hucke[4], Wolfram H. Zimmermann[3], Hendrik Milting[2], Wolfgang A. Linke[4]

[1]Clinic for Cardiology and Pneumology, University Medical Center, Göttingen, Germany; [2]Erich and Hanna Klessmann Institute, Heart and Diabetes Centre NRW, University Hospital of the Ruhr-University Bochum, Bad Oeynhausen, Germany; [3]Institute of Pharmacology and Toxicology, University Medical Center, Göttingen, Germany; [4]Institute of Physiology II, University of Münster, Münster, Germany
Wolfgang A Linke <wlinke@uni-muenster.de>

Heterozygous truncating variants in *TTN* (TTNtv) cause dilated cardiomyopathy (DCM) but the underlying pathomechanisms are unclear. We aimed to elucidate the key pathomechanisms of TTNtv-DCM. We studied LV tissues from 14 nonfailing donor hearts and 113 endstage failing DCM patients and identified a TTNtv in 22 DCM patients (19.5%) by next generation sequencing. By titin protein analysis, we found titin haploinsufficiency in TTNtv-DCM hearts. Strikingly, all adult TTNtv-DCM hearts showed stable expression of truncated titin proteins. Expression was variable, up to one-half of the total titin protein pool, and negatively correlated to patient age at heart transplantation. Truncated titin proteins were not present in sarcomeres but instead in intracellular aggregates. Deregulated ubiquitin-dependent protein quality control was apparent. Next, we studied hiPSC-CMs with a patient-derived A-band TTNtv or CRISPR/Cas9-edited M-band TTNtv. TTNtv hiPSC-CMs showed reduced wildtype titin expression compared to control cells and they contained truncated titin proteins, whose proportion increased upon inhibition of proteasomal activity. In engineered heart muscle generated from hiPSC-CMs, depressed contractility caused by TTNtv could be reversed by mutation-correction using CRISPR/Cas9, thus eliminating truncated titin proteins and raising wildtype titin. Functional improvement also occurred when titin proteins were increased by proteasome inhibition. In conclusion, the major pathomechanisms of TTNtv-DCM include titin haploinsufficiency and truncated titin protein enrichment with aggregate formation, along with aberrant protein quality control. Results can be exploited for new therapies of TTNtv cardiomyopathies.

L7.4

Precision medicine in HCM: evidence for mutation-specific pathomechanisms and negative inotropic drug efficacy

C. Ferrantini

Department of Clinical and Experimental Medicine, University of Florence, Italy
Cecilia Ferrantini <cecilia.ferrantini@unifi.it>

Cardiovascular therapy has dramatically evolved in the last 50 years, thanks to ground-breaking advances in antihypertensive and heart failure agents, lipid-lowering drugs and anticoagulants. A major exception is represented by inotropic drugs. Most agents in current use were developed decades ago and are limited by non-selectivity, pro-arrhythmogenicity and narrow therapeutic ranges, especially in the context of structural heart diseases, including genetic-based cardiomyopahties and particularly HCM.

A large biorepository of genotyped myocardial samples has been created from Florence HCM patients with pathogenic mutations in sarcomere proteins. Mutations in these proteins primarily cause changes of myofilament mechano-energetic function, but are also associated with derangement of intracellular calcium handling and indeed behave as 'acquired' channelopathies. To attempt a better biophysical comprehension of the crosstalk between contractile protein defects and membrane protein dysfunction, this talk will focus on: (i) repositioning of drugs acting on intracellular calcium handling and sarcoplasmic reticulum stability (e.g. Na current blockers such as Ranolazine and disopyramide); (ii) novel compounds acting on sarcomere cross-bridge cycling and myofilament Ca-sensitivity (e.g. small molecules such as Mavacamten).

As HCM pathophysiology appears to be mutation- rather than disease-specific, the role of individual genotypes in the prediction of drug efficacy will be discussed.

Oral Presentations

O7.1

The role of miR-378a in development of cardiomyopathy of dystrophic mice

U. Florczyk-Soluch, K. Polak, R. Sabo, J. Dulak

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
Urszula Florczyk-Soluch <urszula.florczyk@uj.edu.pl>

Dystrophin deficiency lays at the root of Duchenne muscular dystrophy (DMD). Although skeletal muscle symptoms define characteristic of DMD, it is the heart problem the biggest challenge that usually develop in the form of dilated cardiomyopathy. Current standards of DMD management have improved median life expectancy to 4thdecade of patient's age. Nonetheless cardiac causes of death increase and prognoses remain poor. Due todifficulties in direct targeting of dystrophin deficiency, strategies addressing secondary pathways are essential.
MicroRNA-378a, a regulator of metabolism and muscle biology, particularly abundant in skeletal and cardiac muscle, reported to protect against ischemic injury and heart failure, might be of particular importance in DMD. As we showed, dystrophic mice lacking miR-378a (*mdx-miR-378a*$^{-/-}$) exhibit better physical performance and attenuated skeletal muscle damage. The cardiac involvement in those mice is now verified by us.
To model cardiomyopathy we used the strategy to hasten the onset of cardiac problems by physical activity.We applied 12-week treadmill regimen to gradually accelerate cardiac damage, as typical for DMD patients, moderately increasing workload of the heart. During exercise regimencardiac parameters were monitored by transthoracic echocardiography. Changes in *mdx-miR-378a*$^{-/-}$hearts were detected including inflammation and fibrosis. Results suggest that miR-378a can be considered as potential modulator of DMD severity.

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Virtual Posters

P7.1

Investigation of immune-mediated mitochondrial damage using functional iPS-cardiomyocytes derived from the blood of rheumatoid arthritis patients

J. Wolnik[1], K. Andrysiak[1], M. Skoczyńska[2], P. Wiland[2], G. Kubiak[3], J. Dulak[2], M. Biniecka[1,3]

[1]Departament of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland; [2]Department of Rheumatology and Internal Medicine, Medical University of Wroclaw, Wrocław, Poland; [3]Silesian Park of Medical Technology, Kardio-Med Silesia, Zabrze, Poland
Monika Biniecka <monika.biniecka@uj.edu.pl>

Objectives: To generate functional cardiomyocytes (CMs) from peripheral blood mononuclear cells (PBMCs) of rheumatoid arthritis (RA) patients using induced pluripotent stem cell (iPSc) technology and to determine if pro-inflammatory mediators may promote mitochondrial dysfunctions to iPSC-CMs.
Methods: PBMCs were isolated from whole blood of RA patients and heathy controls (HC). PBMCs were transduced with reprogramming Sendai vectors. Matured iPSC colonies were analysed for the presence of pluripotency markers and subjected to differentiation towards CMs. Established iPSC-CMs were purified, examined by Seahorse, analysed for the presence of CMs-associated markers, and stimulated with RA fibroblast cells (RASFCs) conditioned media or TNF-α.
Results: PBMC were successfully reprogrammed into iPSCs and the pluripotency of iPSCs was confirmed. Spontaneously beating CMs were efficaciously generated from iPSCs. Magnetic separation of iPSC-CMs allowed to enrich positive selection of mature CMs, all of which demonstrated sarcomeric structure, the presence of CMs-associated markers and greater oxygen consumption rate when compared to iPSC-derived noncardiomyocytes. iPSC-CMs stimulated with conditioned media showed increased IL-1β and IL-6 expression. iPSC-CMs stimulation with TNF-α led to the loss of the mitochondrial membrane potential and elevated accumulation of reactive oxygen species.
Conclusions: Functional iPSC-CMs can be easily obtained from the blood of RA patients. Inflammation-induced deterioration in mitochondrial function may lead to CMs dysfunction and cardiac complications.

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P7.2

Is7 domain of titin is essential for cardiac function in mice

Ariane Biquand[1,2], Simone Spinozzi[1,2], Paola Tonino[3], Joshua Strom[3], Jérémie Cosette[1], Zaher Elbeck[4], Ralph Knöll[4,5], Henk Granzier[3], William Lostal[1,2], Isabelle Richard[1,2]

[1]Genethon, 91000, Evry, France; [2]Université Paris-Saclay, Univ Evry, Inserm, Généthon, Integrare research unit UMR_S951, 91000, Evry-Courcouronnes, France; [3]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ 85721, USA; [4]Department of Medicine, Integrated Cardio Metabolic Centre (ICMC), Heart and Vascular Theme, Karolinska Institutet, 141 57 Huddinge, Sweden; [5]Bioscience Cardiovascular, Research and Early Development, Cardiovascular,Renal and Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca, 431 50 Gothenburg, Sweden

Ariane BIQUAND <abiquand@genethon.fr>

Titin is the largest and the third most abundant protein in striated muscle, with a primary role in sarcomere organization and stifness. Mutations in the titin gene are now recognized as an important cause of cardiac diseases. The titin gene undergoes various alternative splicing events, but in the M-line region, the only exon that can be spliced out is Mex5, which encodes for the is7 domain. Interestingly, the majority of titin isoforms are Mex5+ in the heart. However, little is known about the cardiac function of the is7 domain. Our data demonstrate that the is7 domain is indispensable for titin function in the heart. A comprehensive functional, histological, transcriptomic, microscopy and molecular analysis of a mouse model lacking the Mex5 exon in the titin gene (ΔMex5) showed: left ventricular dilation, altered cardiac function, massive fibrosis, sarcomere and mitochondria ultrastructural alterations and abnormal expression of excitation-contraction coupling proteins. To our knowledge, this is the first time that a possible relationship between the is7 domain and excitation-contraction coupling is reported, providing important insights for the identification of new targets in the treatment of titinopathies.

P7.3

Burst-like transcription of sarcomeric genes is associated with cell-to-cell allelic and functional heterogeneity in Hypertrophic Cardiomyopath

V. Burkart[1], K. Kowalski[1], J. Beck[1], D. Aldag-Niebling[1], T. Holler[1], A. Radocaj[1], B. Keyser[2], J. van der Velden[3], S. Lal[4], C. dos Remedios[4], D. Hilfiker-Kleiner[5], T. Kraft[1], J. Montag[1]

[1]Molecular and Cell Physiology, Hannover Medical School, Hannover, Germany; [2]Department of Human Genetics, Hannover Medical School, Hannover, Germany; [3]VU University Medical Centre, Amsterdam, Netherland; [4]Department of Anatomy and Histology, Bosch Institute, University of Sydney, Sydney, Australia; [5]Clinic of Cardiology and Angiology, Hannover Medical School, Hannover, Germany

Valentin Burkart <burkart.valentin@mh-hannover.de>

Transcriptional bursting is a common expression mode for most genes. Alleles are transcribed independently from each other, leading to different ratios of allelic transcripts from cell to cell. In heterozygous patients, this could cause a heterogeneous phenotype among cells within a tissue and contribute to disease development.

Here, we analyzed transcription of three commonly affected genes in Hypertrophic Cardiomyopathy (HCM); myosin binding protein C (cMyBP-C, *MYBPC3*), β-myosin heavy chain (β-MyHC, *MYH7*) and cardiac troponin I (cTnI, *TNNI3*). Using RNA-fluorescence *in situ* hybridization, we found that these genes are transcribed burst-like. Along with that, we showed unequal allelic ratios of *MYH7* and *TNNI3*-mRNA among individual CMs and unequally distributed wildtype cMyBP-C protein across tissue sections from HCM-patients.

The mutations led to opposing functional alterations, e.g. increasing (cMyBP-$C_{c.927\text{-}2A>G}$) or decreasing (β-$MyHC_{A200V}$, β-$MyHC_{R723G}$, $cTnI_{R145W}$) calcium sensitivity. However, all patients showed a large variability in calcium-dependent force generation among individual CMs, indicating contractile imbalance is common in HCM-patients.

Together we present evidence, that three commonly affected genes in HCM are transcribed in bursts, causing different ratios of mutant per wildtype mRNA and protein among individual CMs. Mutation induced functional alterations may thus lead to variable force generation in the myocardium which could contribute to HCM-development.

P7.4

Investigating novel roles for nesprin-1 and the LINC complex in Dilated Cardiomyopathy

S. A. De Silva, C. Zhou, N. Catibog, C. M. Shanahan, Q. P. Zhang

King's College London British Heart Foundation Centre of Research Excellence, Cardiovascular Division, London, UK
Shanelle Amelia de Silva <shanelle.de_silva@kcl.ac.uk>

Cardiomyopathies are an important cause of heart failure and sudden cardiac death. Recent studies have demonstrated the importance of the "passive" mechanical components of cardiomyocytes (CMs) as new causes for dilated cardiomyopathy (DCM). Nesprin-1/-2 are highly expressed in skeletal and cardiac muscle and together with SUN (Sad1p/UNC84)-domain containing proteins form the LInker of Nucleoskeleton and Cytoskeleton (LINC) complex at the nuclear envelope (NE), which, in association with lamin A/C and emerin, mechanically couples the nucleus to the cytoskeleton. We have recently identified novel nesprin-1 mutants in DCM patients, which cause LINC complex disruption, leading to defects in nuclear organisation and myogenesis *in vitro*. We aim to investigate mechanisms through which these mutations lead to DCM. Therefore, we have generated a nesprin-1 mutant R8253Q knock-in (KI) mouse line (equivalent to human SYNE1 R8272Q) as the first clinically relevant animal model. Preliminary mouse echocardiography data showed significantly reduced thickness of left ventricle posterior wall in diastole, and reduced % ejection fraction in the KIs at 15 weeks after birth, suggesting LV dysfunction and a tendency of DCM. Immunofluorescence showed elongated nuclei in KI hearts and clustered nuclei in KI skeletal muscle. Therefore, we propose to explore novel roles of nesprin-1 and the LINC complex in cardiomyocyte mechanotransduction using this KI model and are currently characterizing this mouse model to explore if nesprin-1 mutants cause defective nuclear positioning and microtubule organization, leading to defective mechanotransduction and nuclear homeostasis.

P7.5

KBTBD13 is a novel cardiomyopathy gene

Josine M. de Winter[1], Karlijn Bouman[2], Maarten P. van den Berg[3], Joshua Strohm[4], Jan D. Jongbloed[5], Wilma van der Roest[5], Jan van Wijngaarden[6], Janneke Timmermans[7], Robin Nijveldt[7], Frederik van den Heuvel[7], Erik Jan Kamsteeg[8], Baziel G. van Engelen[2], Ricardo Galli[1], Sylvia J. P. Bogaards[1], Robbert-Jan van der Pijl[1,4], Henk Granzier[4], J. Peter van Tintelen[9,10], Karin van Spaendonck-Zwarts[5,9*], Nicol C. Voermans[2*], Coen A. C. Ottenheijm[1,4*]

[1]Department of Physiology, Amsterdam UMC, location VUmc, Amsterdam, The Netherlands; [2]Department of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands; [3]Department of Cardiology, University Medical Centre, Groningen, The Netherlands; [4]Department of Cellular and Molecular Medicine, University of Arizona, Tucson AZ, USA; [5]Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands; [6]Department of Cardiology, Deventer Hospital, Deventer, The Netherlands; [7]Department of Cardiology, Radboud University Nijmegen Medical Centre, The Netherlands; [8]Department of Human Genetics, Radboud University Medical Centre, Nijmegen, The Netherlands; [9]Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherland; [10]Department of Genetics, Unive University Medical Center Utrecht, Utrecht, The Netherlands
Josine de Winter <jm.dewinter@amsterdamumc.nl>

*Contributed equally

Variants in *KBTBD13* cause nemaline myopathy type 6 (NEM6), a congenital myopathy characterized by slowness of movement due to slow relaxation kinetics of skeletal muscles. The majority of NEM6 patients harbors the Dutch founder variant, c.1222C>T, p.Arg408Cys (*KBTBD13*^{R408C}), yet patients are globally dispersed. Although *KBTBD13* is expressed in cardiac muscle, cardiac involvement in NEM6 is unknown. Here, we assessed cardiac structure and function in mice and humans harboring *KBTBD13*^{R408C}.

Cardiac structure was unaffected in *Kbtbd13*^{R408C}-knockin (KI) mice. The end-systolic pressure-volume relation (ESPVR) was unaffected, but the end-diastolic pressure-volume relation (EDPVR) was steeper in KI mice, indicating diastolic dysfunction. *Kbtbd13*-knockout (KO) mice had reduced heart mass, and a blunted increase in heart rate and left ventricular ejection fraction (LVEF) upon dobutamine administration. The ESPVR was less steep in KO mice, indicating systolic dysfunction. Thus, both gain-of-function (KI) and loss-of-function (KO) variants in *KBTBD13* can cause cardiomyopathy. Of the sixty-five patients with the *KBTBD13*^{R408C} variant, 21% presented with structural changes (LV dilatation), 63% with functional abnormalities (LVEF < 50%) and 52% with cardiac arrhythmias. Five patients had received an implantable cardioverter defibrillator. Furthermore, three cases of sudden cardiac death were reported, and five patients died at young age.

Hence, we recommend that (1) *KBTBD13* should be added to the cardiomyopathy genetic panel to facilitate the diagnosis of patients; (2) patients harboring *KBTBD13* variants should be referred to the cardiologist at a young age for cardiac screening.

P7.6

Study of the mechanisms of the cardiac dysfunction in mice lacking myosin VI

Olena Karatsai, Lilia Lehka, Dominika Wojton, Anna I. Grabowska, Maria J. Redowicz

Laboratory of Molecular Basis of Cell Motility, Nencki Institute of Experimental Biology PAS, Warsaw, Poland
Olena Karatsai <o.karatsai@nencki.edu.pl>

Cardiomyopathy is a group of diseases that are characterized by structural and functional myocardial abnormalities such as enlargement of one or both heart ventricles, and reduced blood pump functioning. These alterations can be caused by mutations in genes encoding sarcomere proteins, however, the mechanisms of etiopathogenesis are still poorly studied. Unconventional myosin VI (MVI) is one of the proteins important for the functioning of the striated muscle. It has been previously shown that lack of MVI is associated with the left ventricular hypertrophy in adult mice (Hegan *et al.*, 2015). We decided to study the mechanisms of cardiac dysfunctions of *Snell's waltzer* (MVI-KO) mice of different ages. We observed that the hypertrophy of the left ventricle is age-dependent and is more pronounced in the newborn (P0) and 1-year old MVI-KO mice. Noticeably, the highest content of MVI is in the hearts of both P0 and 1-year old animals. The heart mass increase in the MVI-KO mice seems to be correlated with the increase of the level of GATA4 transcription factor, which is critically involved in inducible gene expression evoked by a variety of hypertrophic stimulations. In addition, the level of proliferation marker protein Ki-67 was also increased in P0 knockout mice. However, the activation status of the Akt/mTOR pathway did not correspond with muscle increase. The mechanism that underlies this phenomenon is still under investigation.

References:

Hegan PS, Lanahan AA, Simons M, Mooseker MS (2015) Myosin VI and cardiomyopathy: Left ventricular hypertrophy, fibrosis, and both cardiac and pulmonary vascular endothelial cell defects in the Snell's waltzer mouse. *Cytoskeleton (Hoboken)* **72**(8): 373-387. DOI: 10.1002/cm.21236.

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P7.7

Information processing in the β-adrenergic signaling pathway by phospholamban pentamers

D. Koch

Randall Centre for Cell and Molecular Biophysics, King's College London, London, UK
Daniel Koch <daniel.koch@kcl.ac.uk>

Cardiac relaxation depends on the reuptake of calcium into the sarcoplasmic reticulum by the calcium pump SERCA. Under resting conditions, SERCA is inhibited by monomers of the micropeptide phospholamban (PLN). During the "fight-or-flight" response, β-adrenergic stimulation leads to phosphorylation of PLN by protein kinase A (PKA), thereby releasing SERCA inhibition and promoting fast calcium reuptake. One of the open questions in the field is the physiological role of PLN homo-pentamers which are mainly regarded as an inactive storage or buffering form for monomeric PLN. Combining mathematical modeling and biochemical experiments, we present evidence that PLN pentamers can delay phosphorylation of PLN monomers via substrate competition. Moreover, simulations and and experiments show that steady-state phosphorylation of PLN is bistable due to cooperative dephosphorylation of pentamers. Further analyses show that both effects could reduce the effect of molecular fluctuations, indicating that PLN pentamers may act as molecular noise-filters to ensure consistent monomer phosphorylation and SERCA activity despite fluctuating PKA-activity in the upstream signaling network. Importantly, these results offer new perspectives on the role of PLN in cardiac arrhythmias resulting e.g. from pathogenic PLN mutations such as R14del.

P7.8

The effect of TPM1 gene mutations associated with congenital heart diseases on the structural and functional characteristics of the alpha- and kappa-tropomyosin

G. Kopylova[1], A. Kochurova[1], V. Berg[1], S. Bershitsky[1], D. Levitsky[2], A. Gonchar[2], A. Matyushenko[2], D. Shchepkin[1]

[1]Institute of Immunology and Physiology, Russian Academy of Sciences, Yekaterinburg, Russia; [2]A.N. Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia

Galina Kopylova <g_rodionova@mail.ru>

Tropomyosin (Tpm) takes part in the Ca^{2+} regulation of heart contraction and cardiac development and myofibril assembly [McKeown et al., Dev Dyn, 2014]. Mutations in the *TPM1* gene, encoding cardiac alpha (Tpm1.1) and kappa (Tpm1.2) Tpm, were shown to be associated with left ventricular non-compact cardiomyopathy (LVNC) and congenital heart defects (CHD) (Kelle *et al.*, 2016, *Am J Med Genet*; England *et al.*, 2017, *J Mol Cell Cardiol*). We studied the effects of the I130V (CHD) and D159N (LVNC) mutations of Tpm, associated with LVNC and CHD, on the properties of Tpm1.1 and Tpm1.2.

With differential scanning calorimetry, we found that the D159N mutation increases the thermal stability of the C-terminus of Tpm1.1 but did not affect it of Tpm1.2. On the contrary, the I130V mutation increased the thermal stability of the C-terminus in Tpm1.2 but did not affect t it in Tpm1.1. The thermal stability of the Tpm1.1 complex with F-actin measured by light scattering was higher than Tpm1.2. The D159N mutation increased the thermal stability of F-actin with Tpm1.1, and I130V increased it with Tpm1.2. The D159N mutation reduced the maximal sliding velocity of thin filaments with Tpm1.1 over cardiac myosin in an in vitro motility assay. Mutation I130V in Tpm1.2 decreased the maximal sliding velocity of thin filaments and Ca^{2+} sensitivity of the filament velocity. Thus, the effect of the LVNC mutations on the Ca^{2+} regulation of actin-myosin interaction depends on the Tpm isoforms.

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P7.9

Unbiased screen of the cardiac proteome in a rat model of heart failure with preserved ejection fraction (HFpEF)

F. Koser[1], M. Abdellatif[2], C. Türk[3], M. Krüger[3], S. Sedej[2], W. A. Linke[1]

[1]Institute of Physiology II, University Hospital Münster, Germany; [2]Division of Cardiology; Medical University of Graz, Austria; [3]Institute for Genetics, University of Cologne, Germany

Franziska Koser <fkoser@uni-muenster.de>

Aims: Despite high public-health importance, the pathomechanism of HFpEF remains elusive and specific therapy is lacking. To confirm the ZSF1 rat as an appropriate HFpEF model, we tested whether previously suggested pathomechanistic pathways of HFpEF are altered, and intended to gain new insights in the HFpEF pathomechanism using unbiased proteomic screens.

Methods & Results: Female and male ZSF1 obese and lean rats were fed with a Purina diet up to the onset of the HFpEF phenotype in the ZSF1 obese rats.

Unbiased proteomic screens revealed the substantial reprogramming of the acetylome, but not the proteome or phosphoproteome, in ZSF1 obese *vs.* lean rats highlighting obesity as a crucial comorbidity in the HFpEF pathogenesis. Proposed features of the HFpEF pathomechanism could only be partially confirmed in ZSF1 obese rats by immunoblot-based quantitation. Neither eNos activity, nor iNos expression were altered, contradicting the presence of oxidative/nitrosative stress. In contrast, low-grade meta-inflammation was confirmed by increased CD68 and P-selectin expression. Moreover, PKG activity and PKG site-specific titin phosphorylation were decreased, potentially increasing titin-based cardiomyocyte stiffness. Beside titin hyperacetylation, which may also contribute to the increased cardiomyocyte stiffness in HFpEF, we propose disturbed Ca^{2+}-handling, indicated by PTM alteration of Ryr2 and SERCA2a, as an additional contributor to diastolic dysfunction in HFpEF patients.

In conclusion, our findings support suggestions that both mechanical and metabolic stress are disease causing elements of HFpEF. We confirm the ZSF1 obese rat is an appropriate model for HFpEF, and reveal new insights in the HFpEF pathomechanism.

P7.10

The R502W mutation in murine cardiac myosin-binding protein C leads to pathogenic myocardial remodeling in the absence of protein haploinsufficiency

Laura Sen-Martín, Ángel Fernández-Trasancos, David Sánchez-Ortiz, María Rosaria Pricolo, Lorena Cussó, Gabriela Guzmán-Martínez, Jorge Alegre-Cebollada

Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain
Laura Sen Martín <laura.sen@cnic.es>

Many myocardial pathologies are caused by inherited genetic mutations that result in anatomical alterations and compromised cardiac function. In humans, the missense variant R502W in cardiac myosin-binding protein C (cMyBP-C) is the most frequent mutation leading to hypertrophy cardiomyopathy (HCM). However, the molecular mechanisms sustaining pathogenicity of variant R502W remain unknown, since both cMyBP-C's mRNA and protein structure have been proposed not to be perturbed by the mutation. Using CRISPR/Cas9-based genetic engineering, we have generated a knock-in mouse model that harbors the R502W mutation in murine cMyBP-C, and characterized the resulting cardiac phenotype. Using echocardiography and magnetic resonance imaging, we detect thicker trabeculae, altered ventricle geometry, reduced left ventricular systolic function and diastolic dysfunction in homozygous p.R502W mice. Interestingly, biochemical analysis shows that cMyBP-C mRNA and protein levels are not altered by the mutation. Since we there is no evidence of cMyBP-C haploinsufficiency in the R502W mice, we propose that pathogenicity of the mutation stems from alternative mechanisms, which we are currently investigating. We expect that further investigation of R502W mice will shed light on the molecular triggers of HCM caused by the many cMyBP-C point mutations that do no lead to protein haploinsufficiency.

P7.11

Role of heme oxygenase-1 in hiPSC-derived atrial cardiomyocytes

Alicja Martyniak, Mateusz Jeż, Kalina Andrysiak, Jacek Stępniewski, Józef Dulak

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
Alicja Martyniak <alicja.martyniak@doctoral.uj.edu.pl>

Atrial fibrillation (AF) represents a serious cardiac condition which increases the risk of heart failure and coronary heart disease. It has been reported that polymorphism in *HMOX1* promoter leading to different level of heme oxygenase-1 (HO-1), a cytoprotective enzyme may constitute a genetic risk factor for AF. The role of HO-1 in atrial cardiomyocytes (aCM), has not been thoroughly investigated.
To evaluate the role of HO-1 in aCM we used hiPSC transduced with lentiviral vectors for GFP or HO-1-overexpression (hiPSC-GFP and hiPSC-GFP-HO-1, respectively). Cells were subjected to cardiac differentiation using small molecules regulating WNT pathway together with either DMSO, to generate predominantly ventricular CMs (hiPSC-vCM) or 1 μM retinoic acid (RA) to enrich for atrial CMs.
Analysis of atrial-specific gene expression, including NPPA, PITX2, COUP-TFI, COUP-TFII confirmed successful generation of hiPSC-aCM upon RA treatment of differentiating hiPSCs. Additionally, immunofluorescent staining indicated presence of Myl7 in hiPSC-aCM. Our preliminary results indicate that HO-1 overexpression results in increased expression of potassium channels (KCNQ1, KCNH2, KCNJ3, KCNA5) and longer (field potential duration) FPD, when measured on multi-array electrode
Obtained results shows that stimulation with 1 μM RA during cardiac mesoderm formation successfully enrich hiPSC-CMs with atrial CMs. Moreover, atrial CMs with HO-1 overexpression show different electrophysiological properties and expression of ion channels. Those results indicate that HO-1 plays important role is atrial CMs physiology and obtained model can be used in studies of atrial-specific disorders.

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P7.12

Endoplasmic Reticulum (ER) Stress in dystrophic mouse myoblasts and enhanced calcification in *mdx* skeletal muscle

Justyna Róg[1], Olga Krupska[1], Aleksandra Oksiejuk[1], Bernadeta Michalska[1], Dariusz C. Górecki[2], Krzysztof Zabłocki[1]

[1]Laboratory of Cellular Metabolism, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK

Justyna Róg <j.jakubczyk@nencki.edu.pl>

Duchenne muscular dystrophy (DMD) is the most common genetic muscle disease caused by mutations in the dystrophin-encoding gene. It leads to a progressive wasting of muscle fibres and finally premature death. Dystrophin appears in myotubes while is undetectable in undifferentiated muscle cells irrespectively of DMD mutation. Despite this fact myoblasts derived from dystrophic mice (*mdx*) exhibit aberrant calcium homeostasis due to affected expression of genes encoding calcium-toolkit proteins. They include receptors, Ca^{2+}-binding proteins, pumps and exchangers. Such changes affect intracellular Ca^{2+}-dependent biochemical processes and influence some cellular functions (e.g. cell motility). Here we have investigated effects of *mdx* mutation on the level of protein markers of the endoplasmic reticulum (ER) stress and unfolded protein response (UPR). We found an elevation of GRP78, IRE1 and ATF6 protein content and a reduced level of P-elf2alfa and CHOP in *mdx* myoblasts. These effects indicate an increased ER stress and an activation of two UPR pathways in these cells. Interestingly, despite increased level of GRP78 (ER stress marker) no markers of UPR were found in the whole *mdx* muscle (Gastrocnemius), while an elevated OSX and RUNX2 protein level confirms stimulated muscle calcification. Possible relations between these changes and affected Ca^{2+} homeostasis in *mdx* muscle cells as well as some pharmacological attempts to modify these effects will be discussed.

P7.13

Differential impact of HCM-associated TnT mutations on atrial function

G. Vitale[1], J.M. Pioner[1], F. Gentile[1], B. Scellini[1], N. Piroddi[1], R. Coppini[2], E. Cerbai[2], J. Tardiff[3], C. Tesi[1] , C. Poggesi[1] , C. Ferrantini[1]

[1]Department of Experimental and Clinical Medicine, University of Florence, Italy; [2]Department of NeuroFarBa, University of Florence, Italy; [3]Department of Medicine, University of Arizona, USA

Giulia Vitale <giulia.vitale@unifi.it>

The pathogenesis of atrial myopathy in Hypertrophic Cardiomyopathy (HCM) remains poorly investigated and no specific association with genotype appear evident so far. In the Florence HCM cohort, patients harboring mutations in the T1 tropomyosin-binding site of Troponin T (i.e R92Q) showed an increased susceptibility to both ventricular arrhythmias and atrial fibrillation compared to patients carrying other cTnT mutations. Whether atrial myopathy results from increased LV filling pressure or specific sarcomere mutation-driven atrial remodeling is unresolved. Here, atrial myocardium from two HCM mouse models carrying different cTnT mutations (R92Q and E163R), was investigated. Echocardiography studies showed a significant left atrial dilation in both models, although more pronounced in the R92Q. In E163R atrial myocardium the energy cost of tension generation was markedly increased, but no changes of twitch amplitude and kinetics nor any increased arrhythmogenic propensity was observed. In the atria of R92Q mice, ATP consumption was normal, but we revealed a marked increase in calcium sensitivity, a reduced amplitude and prolongation of twitch contractions and an increased arrhythmogenic propensity. In HCM atria both mutations induce primary sarcomeric alterations that are similar to those previously observed in the ventricles. However, in the E163R atria, an increased myofilament ATP consumption is compatible with a preserved electromechanical function while in the R92Q atria the severe increase in myofilament calcium sensitivity is associated with E-C coupling remodeling and arrhythmias propensity. The data suggest that genotype-specific remodeling pathways underlie the pathogenesis of atrial myopathy in HCM.

P7.14

Loss of stearoyl-CoA desaturase 4 protects against lipid accumulation in the heart in high fat diet fed mice

M. Wolosiewicz[1], A. Filip[1], A. Olichwier[1], A.Sowka[1], M. Duda[2], P. Dobrzyn[1]

[1]Laboratory of Molecular Medical Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Clinical Physiology, Postgraduate Medical School, Warsaw, Poland

Marcin Wołosiewicz <m.wolosiewicz@nencki.edu.pl>

Stearoyl-CoA desaturase (SCD) is a rate-limiting enzyme, catalyzing the synthesis of monounsaturated fatty acids (FA) from saturated FA. Among four isoforms (SCD1-4) SCD4 is expressed exclusively in the heart, however, its metabolic function is unknown. The aim of the present study was to determine the impact of SCD4 deletion on the heart metabolism and function in the model of high-fat diet (HFD) induced obesity. HFD resulted in increased relative wall thickness and decreased end-diastole diameter in wild type (WT) mice. Interestingly, this effect was not observed in $SCD4^{-/-}$ mice, although interstitial fibrosis was increased in both WT and $SCD4^{-/-}$ HFD fed groups. Next, we established that cardiac lipid accumulation was significantly lower in $SCD4^{-/-}$ mice compared with WT mice fed HFD. NanoString analysis of mRNA levels revealed that HFD in WT mice resulted in: a) higher expressions of genes involved in FA transport and β-oxidation, b) decreased expression of glucose transport and catabolism genes, c) tendency to decrease in FA synthesis genes. In $SCD4^{-/-}$ group effect of HFD was similar, but ω-oxidation genes expression were significantly lower. Western Blot analysis confirmed mRNA-based results and demonstrated that HFD leads to changes in lipolytic proteins level (increased adipose triglyceride lipase [ATGL] inhibitor – G0S2 and decreased ATGL activator – ABHD5) in WT mice. In $SCD4^{-/-}$ mice protein levels of: a) G0S2 and ABHD5 were affected oppositely to WT, b) hormone-sensitive lipase was increased, which suggests increased lipolysis. Summarizing, obtained results show that SCD4 is an important point in the regulation of heart function and its deficiency led to lower lipid accumulation in cardiomyocytes.