9th COST Action CardioRNA MC and WG Meeting

22 - 24 February 2023
Nicosia, Cyprus

MEETING VENUE:
University of Nicosia
Main Campus
UNESCO Amphitheater

LOCAL ORGANIZERS:
Prof. Kyriacos Felekkis
Dr. Christos Papaneophytou

Funded by
the European Union

UNIC | SCHOOL OF LIFE AND HEALTH SCIENCES

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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

MEETING VENUE AND TIMES

UNIVERSITY OF NICOSIA,
MAIN CAMPUS
UNESCO AMPHITHEATER

46 MAKEDONITISSIS AVENUE,
CY-2417, NICOSIA, CYPRUS

WED	22 FEB	14:00-19:00
THU	23 FEB	09:00-17:00
FRI	24 FEB	09:00-15:00

AIRPORTS

1. LARNACA INTERNATIONAL AIRPORT "GLAUKOS KLIRIDIS"
Located in Larnaca, 53km south of Nicosia. There are two options to navigate from the airport to Nicosia; either by taxi, or by the Kapnos Airport Shuttle.

2. PAPHOS INTERNATIONAL AIRPORT
Located in Paphos, 145km west of Nicosia. There are two options to navigate from the airport to Nicosia: either by taxi, or by the Kapnos Airport Shuttle.

NAVIGATION

TAXI
For taxi services you may contact Mr. Antonis at 00357 99529036 (special price with UNIC) or any taxi service from the airport

AIRPORT SHUTTLE
Kapnos Airport Shuttle is a private airport shuttle company that offers daily routes from their station in Nicosia to the two international airports of the island and back at Kyrinias Street, Nicosia, as well as a route between the two airports. Booking your seat is mandatory and you can do it using the following link https://kapnosairportshuttle.com. From their station in Nicosia, you can then take a taxi to navigate within the city.

PUBLIC TRANSPORT
Public transportation in Nicosia is provided by the Cyprus Public Transport company that offers a variety of bus routes within the city as well as to the rural areas of Nicosia. More information, bus routes, tickets and schedules either on the following link www.publictransport.com.cy or by downloading the application "Pame App" that is available for both iPhone and Android.

HOTELS

When booking for the hotels **do not use any booking platform** but rather **contact the hotels directly.**

5* HILTON NICOSIA

00357 22 695 111

Achaion 1, Egkomi 2413, Cyprus

S: €160 D: €188 T: €216

4* CLEOPATRA HOTEL

00357 22 844 000

info@cleopatra.com.cy

8 Florinis Street, 1065, Nicosia, Cyprus

S: €90 D: €115

3* CENTRUM HOTEL

00357 22 456 444

15 Pasikratous Street, 1011, Nicosia, Cyprus

S: €79 D: €97

3* SEMELI HOTEL

00357 22 452 121

hotel@semelihotel.com.cy

10 Petraki Giallourou Street 1077, Nicosia, Cyprus

S: €88 D: €108

HYPNOS BY BED

00357 22 424 244

3 Kanari Street, Agios Dometios, 2368, Nicosia, Cyprus

S: €140 D: €150 T: €180

3* CLASSIC HOTEL

00357 22 664 006

resclassic@gapgroup.com

94 Rigenis Street, 1513, Cyprus

S: €85 D: €100 T: €200

3rd person up to 12 years old

3* CASTELLI HOTEL

00357 22 712 712

38 Ouzounian Street, 1010, Nicosia, Cyprus

S: €67 D: €72

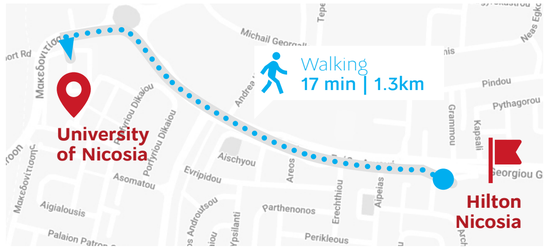
USE PROMOCODE:

CARDIORNA

FROM HOTELS TO VENUE

HILTON NICOSIA

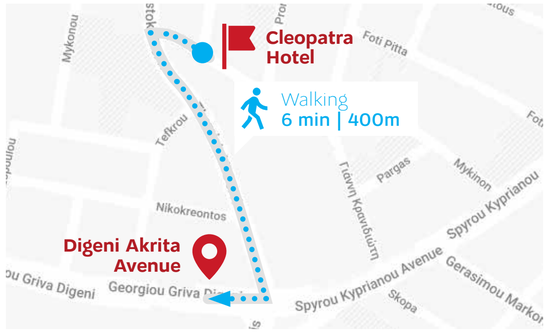
Walk 1.3 km



CLEOPATRA HOTEL

OPTION 1: Walk 400m from Cleopatra Hotel to Digeni Akrita Avenue, take bus number 42 towards Makario Stadium and stop at the University of Nicosia stop.

OPTION 2: Take a taxi to the University of Nicosia (4.3km).



CENTRUM HOTEL

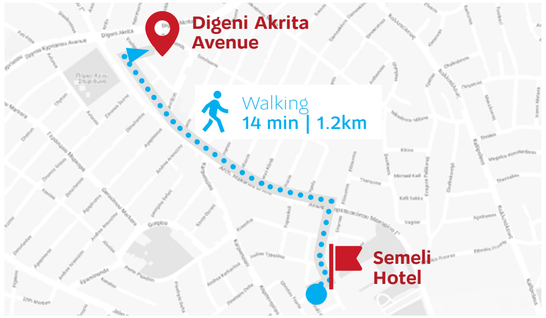
OPTION 1: Walk 550m towards Eleftheria Square (Omirou Avenue), take bus number 41 towards the Makario Stadium and stop at the University of Nicosia stop.

OPTION 2: Take a taxi to the University of Nicosia (4.8km).

SEMELI HOTEL

OPTION 1: Walk 1.2km from Semeli Hotel to Digeni Akrita Avenue, take bus number 42 towards Makario Stadium and stop at the University of Nicosia stop.

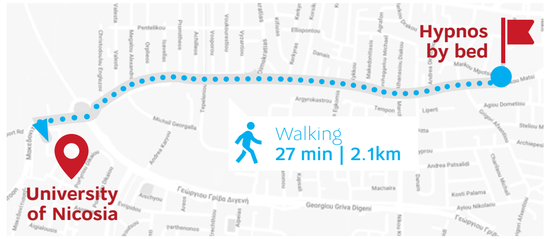
OPTION 2: Take a taxi to the University of Nicosia (6.4km).



HYPNOS BY BED

OPTION 1: Walk 2.1km towards the University of Nicosia.

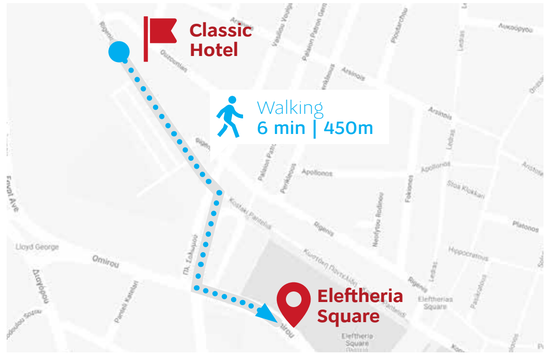
OPTION 2: Take a taxi to the University of Nicosia (2.1km).



CLASSIC HOTEL

OPTION 1: Walk 450m towards Eleftheria Square (Omirou Avenue), take bus number 41 towards the Makario Stadium and stop at the University of Nicosia stop.

OPTION 2: Take a taxi to the University of Nicosia (4.4km).



CASTELLI HOTEL

OPTION 1: Walk 400m towards Eleftheria Square (Omirou Avenue), take bus number 41 towards the Makario Stadium and stop at the University of Nicosia stop.

OPTION 2: Take a taxi to the University of Nicosia (4.8km).

SOCIAL EVENT

FRIDAY,
24 FEBRUARY 2023

NICOSIA OLD CITY TOUR

Social Event fee will be 30 Euros per person
(45 euros for accompanied persons)
Fees will be collected in cash at the registration desk

15:30 - 15:45	Departure from UNIC Meeting point Liberty Monument *Bastion Podocataro at 15:30*
15:50 - 16:15	House of Hadjigeorgakis Kornesios
16:15 - 16:30	Archibishop's Palace, Pancyprium Gymnaeseum
16:30 - 16:50	Pentadaktylou str., Ermou str., Green Line
17:00 - 18:10	**Break at Kafeneio Leoforeio** *For local drinks and delicacies*
18:10 - 18:20	Archaeological site next to the new Nicosia town hall
18:30 - 18:40	Faneromeni square
18:50 - 19:00	Liberty square
19:45	Dinner at "Mezostrati" *Cypriot Meze*










CardioRNA FINAL MEETING - Wednesday 22 February 2023				
Duration (min)	**Time (CEST)**	**Agenda**	**Title**	**Speaker**
60	12:30 to 13:30		LUNCH	
20	14:00 - 14:20	Welcome and introduction	Welcome and Introduction	Yvan Devaux
Working Groups final results				
10	14:20 - 14:30	WG update	WG1 outputs	Fabio Martelli
10	14:30 - 14:40	WG update	WG2 outputs	David de Gonzalo Calvo
10	14:40 - 14:50	WG update	WG3 outputs	Kanita Karaduzovic-Hadziabdic
10	14:50 - 15:00	WG update	WG4 outputs	Emma Robinson (Remote)
SESSION 1: ncRNAs for cardiovascular regeneration - Chairs: Gaia, Bogdan				
15' talk + 5' QA	15:00 - 15:20	Senior researcher talk	Bioinformatic integration of multiomic analyses of clinical samples reveals new targets for therapeutic intervention in ischemic heart disease	Costanza Emanueli
15' talk + 5' QA	15:20 - 15:40	Senior researcher talk	Single-cell RNA-Sequencing analysis of the cardiac long noncoding transcriptome	Thierry Pedrazzini
15' talk + 5' QA	15:40 - 16:00	Senior researcher talk	Development of miRNA therapeutics for cardioprotection	Bence Agg
10' talk + 5' QA	16:00 - 16:15	Senior researcher talk	Epitranscriptomics and nonconventional cardioprotective interventions	Marketa Hlavackova
	16:15 - 16:45		BREAK	
YRI SESSION 2: STSM, Pavia poster winners, Cyprus abstract winners - Chairs: Dimitris B, Fabio				
5' talk + 5' QA	16:45 - 17:00	STSM talk	Bioengineered extracellular vesicles: New source targeting heart	Juliana Ferreira Floriano
5' talk + 5' QA	17:00 - 17:15	STSM talk	miR-210 at the overlap between hypoxia signalling and inflammatory pathways in macrophage biology	Carmen Alexandra Neculachi
5' talk + 5' QA	17:15 - 17:25	Pavia poster winner	Continuous positive airway pressure restores non-coding RNA expression in obstructive sleep apnea patients	Alessia Mongelli
5' talk + 5' QA	17:25 - 17:35	Cyprus abstract winner	Obesity and anxiety associate with inversely regulated immune-related transcripts in zebrafish larvae and adults	Hila Yehuda
5' talk + 5' QA	17:35 - 17:45	Cyprus abstract winner	An alternative splicing signature of genetic and ischemic dilated cardiomyopathy	Marta Furtado
5' talk + 5' QA	17:45 - 17:55	Cyprus abstract winner	Hidden cardiotoxicity of tyrosine kinase inhibitors is associated with a distinct expression pattern of long non-coding RNAs	Riccardo Bernasconi
60	18:00 - 19:00	Round table	NcRNAs for cardiovascular regeneration	Chairs: Gaia, Costanza, Bogdan
	20:00		DINNER	

CardioRNA FINAL MEETING - Thursday, 23 February 2023				
Duration (min)	**Time (CEST)**	**Agenda**	**Title**	**Speaker**
5	08:55 - 09:00	Introduction	Introduction	Yvan Devaux
SESSION 3: New translational models for disease modeling or drug testing - Chairs: Antigone, Dimitris				
15' talk + 10' QA	09:00 - 09:25	Senior researcher talk	HFpEF: Model(s) and Mechanisms	Gabriele Schiattarella
15' talk + 10' QA	09:25 - 09:50	Senior researcher talk	Zebrafish models of cardiovascular disease	Dimitris Beis
15' talk + 10' QA	09:50 - 10:15	Senior researcher talk	Laser Printing for Organ-on-Chip and regenerative medicine applications	Ioanna Zergioti
10' talk + 5' QA	10:15 - 10:30	ECI talk	hiPSC model for the study of cardiac disease and its application to test splicing modulation using ASOs	Sandra Martins
	10:30 - 11:00		COFFEE BREAK	
60	11:00 - 12:00	Round table	New translational models for disease modeling or drug testing	Chairs: Antigone, Dimitris
	12:30 - 14:00		LUNCH	
SESSION 4: Bioinformatics, AI/ML, database, big data, data integration - Chairs: Kanita, Emma				
30' talk + 15' QA	14:00 - 14:45	Introductory lecture	Methods and applications for gene-expression association and causal analyses	Markus Scholz
15' talk + 5' QA	14:45 - 15:05	Senior researcher talk	The Biobank of Cyprus: A research infrastructure to promote next generation biomedical research	Constantinos Deltas
15' talk + 5' QA	15:05 - 15:25	Senior researcher talk	Re-Analyzing Published RNA-seq Data for LncRNAs	Shizuka Uchida
10' talk + 5' QA	15:25 - 15:40	Short talk	RNA editing in resident and peripheral macrophages during neurodegeneration	Korina Karagianni
10' talk + 5' QA	15:40 - 15:55	ECI talk	DIANA-microT Webserver 2023: An update combining predicted miRNA interactomes with expression, variants and disease associations	Spyrus Tastsoglou
	16:00 - 16:30		COFFEE BREAK	
60	16:30 - 17:30	Round table	Discussion on CardioRNAdb CIG	Chairs: Kanita, Yvan
30	17:30 - 20:00		POSTER SESSION - COCKTAIL DINNER	Moderators: Costanza Emanueli, Anne Yael Nossent, Thierry Pedrazzini, Fabio Martelli, Dimitris Beis

CardioRNA FINAL MEETING - Friday 24 February 2023				
Duration (min)	**Time (CEST)**	**Agenda**	**Title**	**Speaker**
5	08:55 - 09:00	Introduction	Introduction	Yvan Devaux
SESSION 5: Circular and new RNAs - Chairs: Anne Yaël, Fabio				
15' talk + 5' QA	09:00 - 09:20	Senior researcher talk	HFpEF: Model(s) and Mechanisms	Fabio Martelli
15' talk + 5' QA	09:20 - 09:40	Senior researcher talk	Zebrafish models of cardiovascular disease	Yael Nossent
15' talk + 5' QA	09:40 - 10:00	Senior researcher talk	Laser Printing for Organ-on-Chip and regenerative medicine applications	Matthias Hackl
5' talk + 5' QA	10:00 - 10:10	ECI talk	hiPSC model for the study of cardiac disease and its application to test splicing modulation using ASOs	Shrey Gandhi
5' talk + 5' QA	10:10 - 10:20	Senior researcher talk	Laser Printing for Organ-on-Chip and regenerative medicine applications	Alessia BIBI
5' talk + 5' QA	10:20 - 10:30	ECI talk	hiPSC model for the study of cardiac disease and its application to test splicing modulation using ASOs	Dominika Lukovic
	10:30 - 11:00		COFFEE BREAK	
60	11:00 - 12:00	Round table	Round table Circular and new RNAs: evolution of the field in the last 5 years and future perspectives based on CardioRNA experience	Chairs: Anne Yaël, Fabio
30	12:00 - 12:30	Closing remarks	New translational models for disease modeling or drug testing	Yvan Devaux
	12:30 - 14:00		LUNCH	
60	14:00 - 15:00	Management Committee meeting only	Summary on Action activities and next steps	Chair, Vice Chair, Project Manager, Grant Holder, WG Leaders, MC members
	From 15:30		**FINAL SOCIAL EVENT**	

ABSTRACTS

Abstract N°13: Role of circular-PVT1 in ischemic heart failure

Alessia BIBI[1,2], Simona GRECO[1], Alisia MADE[1], Anna Sofia TASCINI[3], Jose GARCIA MANTEIGA[3], Przemyslaw LESZEK[4], Adolfo PAOLIN[5], Serenella CASTELVECCHIO[6], Lorenzo MENICANTI[6], Fabio MARTELLI[1]

1. *IRCCS POLICLINICO SAN DONATO, Molecular Cardiology Laboratory, San Donato Milanese, Milan, Italy*
2. *Department of Biosciences, University of Milan, Milan, Italy*
3. *IRCCS San Raffaele Scientific Institute, Center for Omics Sciences, BioInformatics Laboratory, Milan, Italy*
4. *Institute of Cardiology, Department of Heart Failure and Transplantology, Warsaw, Poland*
5. *Fondazione Banca dei Tessuti di Treviso Onlus, Treviso, Italy*
6. *IRCCS POLICLINICO SAN DONATO, Department of Adult Cardiac Surgery, San Donato Milanese, Milan, Italy*

Introduction: Circular RNAs (circRNAs) are an emerging class of noncoding RNAs originating from the splicing and circularization of pre-mRNAs and long non-coding RNAs. CircRNAs have been found deregulated in several cardiovascular diseases, including heart failure (HF). However, incomplete and sometimes contradictory results have been reported on their regulation and function in HF, indicating that our understanding of the regulation and role of cardiac circRNAs is still very limited. We aim to identify new circRNAs candidates deregulated in ischemic HF and to functionally characterize them in HF.

Methods and results: We performed a high-depth RNA-seq of left ventricle (LV) samples of 20 non end-stage ischemic HF patients and matched controls. qRT-PCR results confirmed the differential expression of 3 circRNAs, circSLC6A6, circMLIP and circHDCA9, which were upregulated in HF samples. To complement the unbiased strategy of RNA-seq, we evaluated the modulation of 15 candidate circRNAs identified by a literature analysis as dysregulated in ischemic or not-ischemic cardiomyopathies or in biological mechanisms relevant for ischemic HF. circPVT1, circANKRD17, circBPTF, displaying a concordant deregulation in different regions of LV myocardium (remote and border zone), were chosen for further analysis.

Using siRNAs targeting the back-splice junction, circPVT1 was specifically knockdown in AC16 cells. RNA-seq analysis of circPVT1 knockdown samples identified differentially expressed genes that are mainly involved in the formation of extracellular matrix and in cellular senescence, suggesting the involvement of circPVT1 in these HF-related disease mechanisms. circPVT1 knockdown in primary adult cardiac fibroblasts attenuated the expression of profibrotic markers upon TGF-β1 treatment, indicating a possible role of circPVT1 in cardiac fibrosis. RNA-seq analysis after circPVT1 pull-down identified several miRNAs interacting with circPVT1. In particular, the enrichment of two fibrosis-related miRNAs, miR-125b-5p and miR-369-5p, was validated by qRT-PCR. miRNA pull-down assay further confirmed the interaction between circPVT1 and these miRNAs. The levels of these two miRNAs were not altered upon circPVT1 knockdown in the presence or absence of TGF-β1. However, miR-125b-5p and miR-369-5p respective targets were deregulated upon circPVT1 silencing, indicating that decreased levels of circPVT1 could increase the cellular pool of bioavailable miRNAs.

Conclusions: We identified new deregulated circRNAs in ischemic HF patients that might play a pathogenic role in HF. circPVT1 is a circRNA upregulated in HF and it may play a role in cardiac fibrosis. circPVT1 binds to fibrosis-related miRNAs miR-125b-5p and miR-369-5p, and could modulate their cellular bioavailability.

Abstract N°22: miR-144-3p: a new marker for severity and mortality prediction in COVID-19

Alisia Madè[1]*, Simona Greco[1], Melanie Vausort[2], Marios Miliotis[3,4], Eric Schordan[5], Shounak Baksi[2], Lu Zhang[6], Ekaterina Baryshnikova[7], Marco Ranucci[7], Rosanna Cardani[8], Guy Fagherazzi[9], Markus Ollert[10,11], Spyros Tastsoglou[3,4], Giannis Vatsellas[12], Artemis Hatzigeorgiou[3,4], Hüseyin Firat[5], Yvan Devaux[2], Fabio Martelli[1]
Presenter: Alisia Madè (alisia.made@grupposandonato.it)

1. *Molecular Cardiology Laboratory, IRCCS Policlinico San Donato, San Donato Milanese, 20097, Milan, Italy*
2. *Cardiovascular Research Unit, Department of Population Health, Luxembourg Institute of Health, L-1445 Strassen, Luxembourg*
3. *DIANA Lab, Department of Computer Science and Biomedical Informatics, University of Thessaly, 35131 Lamia, Greece*
4. *Hellenic Pasteur Institute, 11521 Athens, Greece*
5. *Firalis SA, Molecular Diagnostics, Huningue, France*
6. *Bioinformatics Platform, Luxembourg Institute of Health, L-1445 Strassen, Luxembourg*
7. *Department of Cardiovascular Anesthesia and ICU, IRCCS Policlinico San Donato, via Morandi 30, 20097, San Donato Milanese, Milan, Italy*
8. *BioCor Biobank, UOC SMEL-1 of Clinical Pathology, Department of Pathology and Laboratory Medicine, IRCCS-Policlinico San Donato, via Morandi 30, 20097, San Donato Milanese Milan, Italy*
9. *Deep Digital Phenotyping Research Unit. Department of Precision Health. Luxembourg Institute of Health, 1 A-B rue Thomas Edison, L-1445 Strassen, Luxembourg*
10. *Department of Infection and Immunity. Luxembourg Institute of Health, 29, Rue Henri Koch, L-4354 Esch-sur-Alzette, Luxembourg*
11. *Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis (ORCA), University of Southern Denmark, Odense, 5000 C, Denmark*
12. *Greek Genome Center, Biomedical Research Foundation, Academy of Athens, Athens 11527, Greece.*

Background: COronaVIrus Disease 19 (COVID-19) clinical picture may vary between mild, moderate, or critical respiratory symptoms, including multi-organ failure. microRNAs (miRNAs) may play a role in the progression of various diseases, including COVID-19, and their levels in the peripheral blood can be investigated as possible biomarkers. Here, we explored the potential of miRNAs in delineating COVID-19 patient conditions and predicting clinical outcomes.

Methods and Results: In a pilot study, RNA-sequencing was performed on platelet-poor plasma samples derived from the peripheral blood of 3 surviving and 8 nonsurviving COVID-19 patients. For each patient, samples collected on hospital admission (T0) and before discharge or death (T1) were compared. Out of 734 miRNAs detected, miR-144-3p was the top differentially expressed miRNA between T0 and T1 in surviving, but not in nonsurviving patients. Given the dynamic regulation of miR-144-3p levels in response to disease, to further investigate its biomarker potential, miR-144-3p was measured at admission in 179 COVID-19 patients and 29 healthy controls recruited in 3 centers. In hospitalized patients, circulating miR-144-3p levels discriminated between non-critical and critical illness (AUCmiR-144-3p= 0.71; p= 0.0006), acting also as mortality predictor (AUCmiR-144-3p= 0.67; p= 0.004). In non-hospitalized patients, plasma miR-144-3p levels discriminated mild from moderate disease (AUCmiR-144-3p= 0.67; p= 0.03). Since cytokine storm is a major cause of tissue damage in COVID-19 patients, a panel of 45 cytokines was measured in 62 serum samples. Out of 45 detected cytokines, 31 of them passed technical quality checks. Thus, we explored the added value of a miR-144/cytokine combined analysis in the assessment of hospitalized COVID-19 patients. A miR-144-3p/Epidermal Growth Factor (EGF) combined score discriminated between non-critical and critical hospitalized patients (AUCmiR-144-3p/EGF= 0.81; p< 0.0001); moreover, a miR-144-3p/Interleukin-10 (IL-10) score discriminated survivors from nonsurvivors (AUCmiR-144-3p/IL-10= 0.83; p< 0.0001).

Conclusions: Circulating miR-144-3p, possibly in combination with IL-10 or EGF, emerges as a noninvasive tool for early risk-based stratification and mortality prediction in COVID-19.

Abstract N°21: Elucidating the impaired exercise response in aging postmenopausal women through an epitranscriptomic lens.

Andrea Tamariz-Ellemann[1], A. Yaël Nossent[2,3], Ylva Hellsten[1], Lasse Gliemann[1]

1. *Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark.*
2. *Department of Surgery, Leiden University Medical Center, 2300 RC Leiden, The Netherlands.*
3. *Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, 2300 RC Leiden, The Netherlands.*

Exercise is highly beneficial for maintaining vascular health with aging and has thus been suggested as a means to counteract the increased risk of cardiovascular disease in women following menopause. However, the potential to decrease the risk of cardiovascular disease in post-menopausal women via exercise appears only to exist if exercise is initiated early after the menopausal transition compared to later (+10 years), leading to the exercise timing hypothesis (Gliemann & Hellsten, 2019). Knowledge about how the cardiovascular protective effect of exercise is modulated by aging and menopause is however still sparse. Noncoding RNAs are potential molecular modifiers/regulators of the cardioprotective effects of exercise, as noncoding RNAs holds a central role in the regulation of, on one side the cardiovascular system in general, but also the response to exercise (Hakansson et al. 2018). RNAs are highly modifiable, allowing for cells to respond to both acute and chronic changes, such as menopause, aging, and exercise.

This study examines the changes in the transcriptome and epitranscriptome following an 8-week exercise intervention in 18 sedentary women of different menopausal status; premenopausal (PRE), early postmenopausal (EARLY: 3.1±1.6 years past menopause) and late postmenopausal (LATE: 13.5±3.6 years post menopause). Skeletal muscle biopsies (m.vastus lateralis), were obtained from all participants before and after the exercise intervention, which consisted of high-intensity aerobic training on cycle ergometer 3 times a week for 8-weeks. Total RNA was then isolated and RNA sequencing, outsourced to Qiagen, using QIAseq miRNA library preparation, is currently ongoing. Bioinformatics analyses will be done using R. The study will provide mechanistic understanding of the menopause-associated changes in the protective effect of exercise on the cardiovascular system in women. Furthermore, a potential outcome of the study may provide a better understanding of the exercise timing hypothesis, ultimately leading towards better recommendations for initiation of exercise to women at mid-life.

Gliemann, L. and Hellsten, Y. (2019), The exercise timing hypothesis: can exercise training compensate for the reduction in blood vessel function after menopause if timed right?. J Physiol, 597: 4915-4925. doi:10.1113/JP277056
Hakansson KEJ, Sollie O, Simons KH, Quax PHA, Jensen J and Nossent AY. Circulating Small Non-coding RNAs as Biomarkers for Recovery After Exhaustive or Repetitive Exercise. Frontiers in physiology. 2018;9:1136.

Abstract N°9: Alterations of Cardiac Protein Kinases in Cyclic Nucleotide-Dependent Signaling Pathways in Human Ischemic Heart Failure

Chunguang Wang[1]*, Juuso H. Taskinen[1], Heli Segersvärd[1], Katariina Immonen[1], Riikka Kosonen[1], Johanna M. Tolva[2], Mikko I. Mäyränpää[3], Petri T. Kovanen[4], Vesa M. Olkkonen[1,5], Juha Sinisalo[6], Mika Laine1,6, Ilkka Tikkanen[1,7] and Päivi Lakkisto[1,8]*

1. *Minerva Foundation Institute for Medical Research, Biomedicum Helsinki 2U, Helsinki, Finland*
2. *Transplantation Laboratory,Department of Pathology, University of Helsinki, Helsinki, Finland*
3. *Department of Pathology, University of Helsinki andHelsinki University Hospital, Helsinki, Finland*
4. *Atherosclerosis Research Laboratory, Wihuri Research Institute, Helsinki,Finland*
5. *Department of Anatomy, Faculty of Medicine, University of Helsinki, Helsinki, Finland*
6. *Heart and Lung Center,University of Helsinki and Helsinki University Hospital, Helsinki, Finland*
7. *Abdominal Center, Nephrology, University of Helsinkiand Helsinki University Hospital, Helsinki, Finland*
8. *Clinical Chemistry and Hematology, University of Helsinkiand HelsinkiUniversity Hospital, Helsinki, Finland*

**Correspondence:chunguang.wang@helsinki.fi, paivi.lakkisto@helsinki.fi*

Objectives: Impaired protein kinase signaling is a hallmark of ischemic heart disease (IHD). Inadequate understanding of the pathological mechanisms limits the developmentof therapeutic approaches. We aimed to identify the key cardiac kinases and signaling pathways in patients with IHD with an effort to discover potential therapeutic strategies.
Methods: Cardiac kinase activity in IHD left ventricle (LV) and the related signaling pathways were investigated by kinomics, transcriptomics, proteomics, and integrated multi-omics approach.

Results: Protein kinase A (PKA) and protein kinase G (PKG) ranked on top in the activity shift among the cardiac kinases. In the IHD LVs, PKA activity decreased markedly compared with that of controls (62% reduction, p=0.0034), whereas PKG activity remained stable, although the amount of PKG protein increased remarkably (65%,p=0.003). mRNA levels of adenylate cyclases (ADCY 1, 3, 5, 9) and cAMP-hydrolysing phosphodiesterases (PDE4A, PDE4D) decreased significantly, although no statistically significant alterations were observed in that of PKGs (PRKG1andPRKG2) and guanylate cyclases (GUCYs). The gene expression of natriuretic peptide CNP decreased remarkably, whereas those of BNP, ANP, and neprilysin increased significantly in the IHD LVs. Proteomics analysis revealed a significant reduction in protein levels of “Energymetabolism” and “Muscle contraction” in the patients. Multi-omics integration highlighted intracellular signaling by second messengers as the top enriched Reactome pathway.

Conclusion: The deficiency in cAMP/PKA signaling pathway is strongly implicated in the pathogenesis of IHD. Natriuretic peptide CNP could be a potential therapeutic target for the modulation of cGMP/PKG signaling.

Abstract N°14: Myocardial epitranscriptomics in fasting

Benak Daniel[1,2], Holzerova Kristyna[1], Hrdlicka Jaroslav[1], Kolar Frantisek[1], Olsen Mark[3], Karelson Mati[4], Hlavackova Marketa[1]

1. *Laboratory of Developmental Cardiology, Institute of Physiology of the Czech Academy of Sciences, 14220 Prague, Czech Republic;*
2. *Department of Physiology, Faculty of Science, Charles University, 12844 Prague, Czech Republic;*
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Cardiac tolerance to ischemia can be increased by dietary interventions such as fasting, which is associated with significant changes in myocardial gene expression. Among the possible mechanisms of how gene expression may be altered are epigenetic modifications of RNA – epitranscriptomics. N6-methyladenosine (m6A) and N6,2'-O-dimethyladenosine (m6Am) are two of the most prevalent modifications in mRNA. These methylations are reversible and regulated by proteins called writers, erasers, and readers. We found that the expression of several of these regulators is changed in rat hearts after 3-day fasting on transcript and protein levels, including up-regulation of both demethylases – FTO and ALKBH5. In accordance, decreased methylation (m6A+m6Am) levels were detected in cardiac total RNA after fasting. Based on these results, we studied the inhibition of ALKBH5 and FTO in adult rat primary cardiomyocytes and found that inhibition of either demethylase decreased the hypoxic tolerance of cardiomyocytes isolated from fasting rats. Our results suggest that m6A and m6Am epitranscriptomic machinery is regulated in the hearts of fasting rats and that its regulators affect hypoxic tolerance. Thereby, epitranscriptomic regulation induced by fasting may contribute to the ischemia-resistant phenotype.

Abstract N°17: Integrative analysis of circRNA/miRNA/mRNA regulatory network in anthracycline-induced cardiotoxicity

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Abstract: Although Doxorubicin (DOX) is very potent antitumor anthracycline agent, its considerable cardiotoxicity limits the clinical application. Liposomal encapsulation of DOX (Myocet, MYO) overcomes several limitations of DOX and reduces its cardiotoxic effect. Increasing evidence suggests that circular RNAs (circRNAs) play key roles in cancer development and their expression may be affected by chemotherapeutics. In this study, we aimed to search for non-coding RNAs (miRNAs and circRNA) that are involved in anthracycline-induced cardiotoxicity.

Methods: Human doses of Doxorubicin (DOX; n=5) or Myocet (MYO; n=6) was intravenously injected into domestic pigs in 3 cycles in order to induce cardiotoxicity. Animals who received no treatment served as control group (n=3). At day 60 the obduction was performed and mRNA from left ventricle was isolated following RNA sequencing. By using the CIRIquant algorithm we have identified several circRNAs in the myocardium of DOX and MYO treated animals. Most identified circRNAs were exonic, and the most significantly expressed circRNAs in tissue and cells derived from the mitochondrial genome. To validate our results, porcine cardiac progenitor cells (pCPCs) and porcine cardiac fibroblasts (pCFs) were treated with DOX and MYO in vitro. We forecasted circRNA-miRNA-mRNA network which was validated by bulk RNAseq data retrieved from the same animal models.

Results: The study revealed that treatment with DOX and MYO affects the expression of circRNAs and mt-circRNAs in vivo and in vitro. Constructed network suggests 7 novel candidate circRNAs sponging miR-17, miR-15b, miR-130b, family of let-7 and miR125 in MYO group together with their gene targets that play promiment role in cell cycle regulation, RAS processing, and PI3K-signaling. In addition, we identified 2 novel mitochondrial-derived circRNAs (circ-MT:3033|3289 and circ-MT:3070|3478) that were regulated in MYO group that exhibited similar expression pattern in pCPCs and pCFs in vitro.

Conclusion: This study reveals and explored further mechanisms of drug-induced cardiotoxicity applying prediction algorithms, network analysis and cell culture experiments; thus, updating coherent view on anthracycline-induced cardiotoxicity.

Abstract N°4: High glucose downregulates miR-210 resulting in endothelial dysfunction

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Background: The protective function of micro (miR)-210 is alleviated in several cardiometabolic diseases. The expression levels of miR-210 are found to be lower in plasma, erythrocytes, and atherosclerotic plaques in patients with type 2 diabetes. The downregulation of miR-210 in type 2 diabetes is associated with endothelial dysfunction. However, the mechanism behind downregulation of miR-210 in type 2 diabetes and its association with endothelial dysfunction are not yet fully understood. High glucose, oxidative stress, and high insulin levels play a pivotal role in the pathogenesis of type 2 diabetes. In this study, we aim to elucidate whether these factors affect miR-210 expression and function, thereby inducing endothelial dysfunction.

Materials and Methods: Endothelium-dependent relaxation (EDR) was measured with wire myograph in aortic segments isolated from wild type mice following incubation with and without high glucose (25 mM), high insulin (50 μg/mL), and SIN-1 (25 μM), an oxidative stress stimulator, for 48 h. EDR was also examined in aortas isolated from miR-210 transgenic mice (with or without miR-210 overexpression) under the same conditions as described above. miR-210 levels were measured by qPCR in human carotid arterial endothelial cells treated with and without high glucose.

Preliminary results: High glucose and SIN-1, but not high insulin, impaired EDR in aortas isolated from wild type mice. Of note, the impaired EDR induced by high glucose was attenuated in the aortas where the miR-210 was overexpressed, while the impaired EDR induced by SIN-1 was not affected by the miR-210 overexpression. Furthermore, high glucose treatment decreased the expression levels of miR-210 in human carotid arterial endothelial cells.

Conclusions: High glucose downregulates miR-210, resulting in endothelial dysfunction. Future studies are needed to identify key molecules that are affected by high glucose and can act as upstream regulators of miR-210 for the induction of endothelial dysfunction.

Abstract N°7: Differentially expressed microRNAs in the plasma of long-COVID patients compared to healthy controls and ischemic heart failure patients

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Background: Co-regulation of microRNAs (miRs) has a substantial impact on host immune response to SARS-CoV-2 infection. However, the implication of miRNAs in pathogenesis of long-COVID syndrome is poorly understood.

Methods: From our long-COVID patient database we grouped participants into four groups consisting of symptomatic long-COVID patients, oligosymptomatic post COVID study participants, COVID-naïve healthy controls and ischemic heart failure (HF) patients. We applied small RNA-sequencing approach to identify differentially expressed circulating miRs in plasma samples of 20 individuals of each group.

Results: Baseline characteristics of long-COVID patients are shown in Table 1. Compared with healthy controls, 10 miRs were upregulated and 7 miRs were downregulated in patients with long-COVID. The specific differentially expressed miRs were: upregulated: hsa-miR-1277-5p, hsa-miR-3651, hsa-miR-210-3p, hsa-miR-3960, hsa-miR-484, hsa-miR-21-3p, hsa-miR-345-5p, hsa-miR-3615, hsa-miR-1307-3p and hsa-miR-425-3p; downregulated: hsa-miR-126-3p, hsa-let-7i-5p, hsa-miR-146b-5p, hsa-miR-144-5p, hsa-miR-126-5p, hsa-miR-215-5p and hsa-miR-203a-3p. hsa-miR-1227-5p was most upregulated in patients with long-COVID compared to healthy controls, with a logarithmic fold change (logFC) of 1.5 ($p=0.004$, FDR=0.049). Likewise, hsa-miR-1227-5p was upregulated in oligosymptomatic post COVID study participants with logFC 2.8 ($p<0.001$, FDR 0.001) compared to healthy controls. Concurrently, hsa-miR-203a-3p was the most downregulated in long-COVID patients, with a 2.9 logFC compared to that of the healthy controls. Interestingly, hsa-miR-126-5p, which is specific to endothelial cells and found to rise with COVID severity, was downregulated in long-COVID patients compared to healthy controls. No miRs were differentially expressed between oligosymptomatic post COVID study participants and long-COVID patients, while 207 and 161 differentially expressed miRs were found between healthy controls and ischemic HF patients, and long-COVID and ischemic HF patients, respectively.
The upregulated miRs in long-COVID were found to have 158 potential gene targets, which are displayed in Figure 1. Among those genes, VEGF-A, CDKN1A, MECP2, ROCK2 and CREB1 appeared to be frequent targets, while DDX6 and STRN link hsa-miR-1277-5p and hsa-miR-484 together. Gene set enrichment analysis (GSEA) identified four pathways significantly associated with the upregulated miRs in long-COVID patients: Huntington disease, transcriptional misregulation in cancer, human cytomegalovirus (CMV) infection, Kaposi sarcoma-associated herpesvirus (HSV) infection, as seen in Table 2.

Conclusion: Several differentially expressed miRs were found in our study groups. Long-COVID pathways appear to be related to those of other viral infections (CMV, HSV). The role of miRs as potential biomarkers in long-COVID and cardiovascular diseases needs to be solidified by further studies.

Abstract N°7: Differentially expressed microRNAs in the plasma of long-COVID patients compared to healthy controls and ischemic heart failure patients

Emilie Han[1], Ena Hasimbegovic[1], Julia Mester-Tonczar[1], Nina Kastner[1], Katrin Müller-Zlabinger[1], Katharina Schefberger[1], Andreas Spannbauer[1], Denise Traxler[1], Dominika Lukovic[1], Mariann Gyöngyösi[1]

1. *Department of Internal Medicine II, Division of Cardiology, Medical University of Vienna, 1090 Vienna, Austria*

Background: Co-regulation of microRNAs (miRs) has a substantial impact on host immune response to SARS-CoV-2 infection. However, the implication of miRNAs in pathogenesis of long-COVID syndrome is poorly understood.

Methods: From our long-COVID patient database we grouped participants into four groups consisting of symptomatic long-COVID patients, oligosymptomatic post COVID study participants, COVID-naïve healthy controls and ischemic heart failure (HF) patients. We applied small RNA-sequencing approach to identify differentially expressed circulating miRs in plasma samples of 20 individuals of each group.

Results: Baseline characteristics of long-COVID patients are shown in Table 1. Compared with healthy controls, 10 miRs were upregulated and 7 miRs were downregulated in patients with long-COVID. The specific differentially expressed miRs were: upregulated: hsa-miR-1277-5p, hsa-miR-3651, hsa-miR-210-3p, hsa-miR-3960, hsa-miR-484, hsa-miR-21-3p, hsa-miR-345-5p, hsa-miR-3615, hsa-miR-1307-3p and hsa-miR-425-3p; downregulated: hsa-miR-126-3p, hsa-let-7i-5p, hsa-miR-146b-5p, hsa-miR-144-5p, hsa-miR-126-5p, hsa-miR-215-5p and hsa-miR-203a-3p. hsa-miR-1227-5p was most upregulated in patients with long-COVID compared to healthy controls, with a logarithmic fold change (logFC) of 1.5 ($p=0.004$, FDR=0.049). Likewise, hsa-miR-1227-5p was upregulated in oligosymptomatic post COVID study participants with logFC 2.8 ($p<0.001$, FDR 0.001) compared to healthy controls.

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The upregulated miRs in long-COVID were found to have 158 potential gene targets, which are displayed in Figure 1. Among those genes, VEGF-A, CDKN1A, MECP2, ROCK2 and CREB1 appeared to be frequent targets, while DDX6 and STRN link hsa-miR-1277-5p and hsa-miR-484 together. Gene set enrichment analysis (GSEA) identified four pathways significantly associated with the upregulated miRs in long-COVID patients: Huntington disease, transcriptional misregulation in cancer, human cytomegalovirus (CMV) infection, Kaposi sarcoma-associated herpesvirus (HSV) infection, as seen in Table 2.

Conclusion: Several differentially expressed miRs were found in our study groups. Long-COVID pathways appear to be related to those of other viral infections (CMV, HSV). The role of miRs as potential biomarkers in long-COVID and cardiovascular diseases needs to be solidified by further studies.

Abstract N°7: Differentially expressed microRNAs in the plasma of long-COVID patients compared to healthy controls and ischemic heart failure patients

Table 1 - Baseline characteristics of long-COVID patients

Characteristic	Long-COVID (n=20)
Age, in years	43.5 (35.0 - 51.5)
BMI	22.7 (20.4 - 26.9)
Arterial hypertension	2 (11.8 %)
Diabetes mellitus	0
Hyperlipidaemia	2 (11.8 %)
Smoking	2 (12.5 %)
Heart rate, bpm	73 (70 - 85)
Systolic blood pressure, mmHg	125 (120 - 140)
Diastolic blood pressure, mmHg	80 (80 - 85)

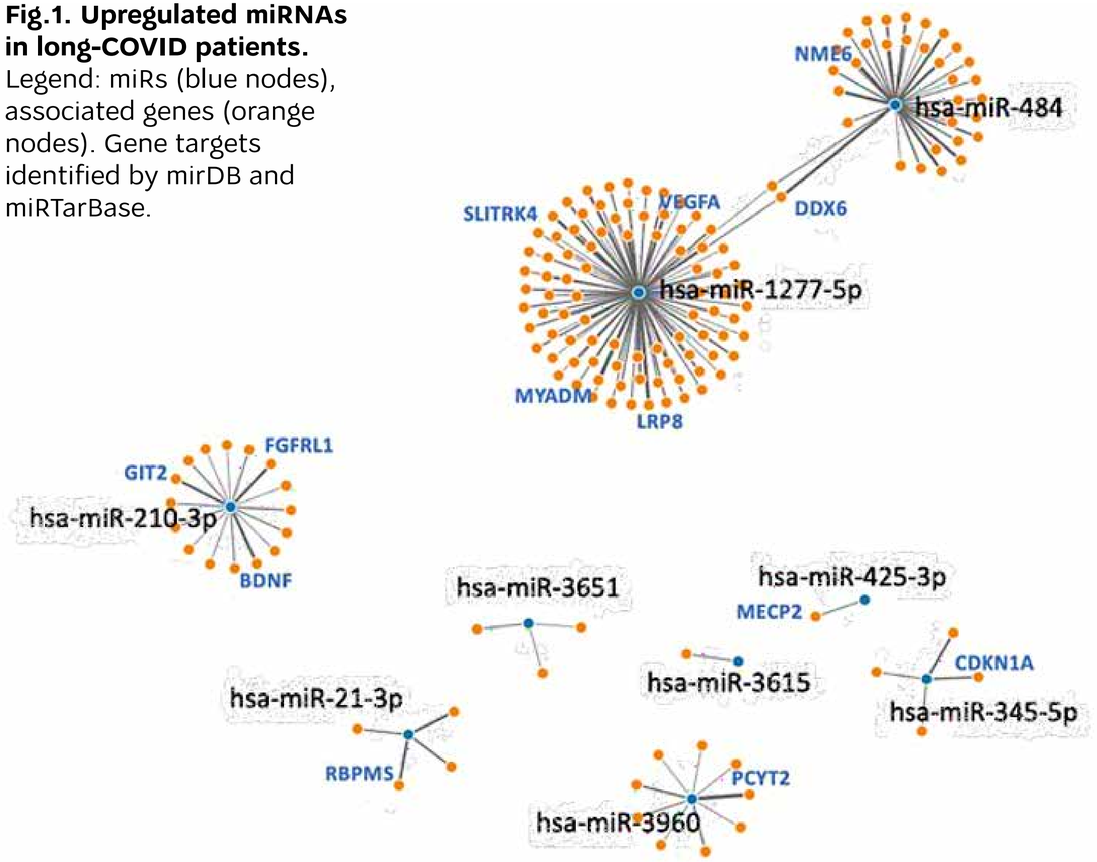
**Continuous data was given as median (IQR) and categorical data as n (%)*

Table 2 - Gene set enrichment analysis (GSEA)

Name	Genes	P-value	Adjusted P-value (BH)
Huntington disease (hsa05016)	BDNF; SIN3A; SP1; SDHD; CREB1; PPARGC1A; NDUFA4	0.006	0.020
Transcriptional misregulation in cancer (hsa05202)	CDKN1A; SIN3A; KMT2A; SP1; MLLT3; HOXA11	0.005	0.022
Human cytomegalovirus infection (hsa05163)	CDKN1A; VEGFA; SP1; CREB1; ROCK2; CALM1	0.011	0.025
Kaposi sarcoma-associated herpesvirus infection (hsa05167)	CDKN1A; VEGFA; IL6ST; CREB1; CALM1	0.018	0.031

**GSEA by miRWalk – KEGG Pathways. BH: Benjamini-Hochberg Procedure*

Fig.1. Upregulated miRNAs in long-COVID patients. Legend: miRs (blue nodes), associated genes (orange nodes). Gene targets identified by mirDB and miRTarBase.



Abstract N°5: Natural Aging Process Is Associated With Increased Mir-29a And Downregulated Serpinh1 In Multiple Organs

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Deregulation of miRNA profile has been reportedly linked to aging process, which is a dominant risk factor for many pathologies. Among the miRNAs with documented roles in aging-related cardiac diseases, miR-18, -21, -22, and -29a were mainly associated with hypertrophy and/or fibrosis; however, their relationship to aging was not fully addressed before. The purpose of this paper was to evaluate the variations in the expression levels of these miRNAs in the aging process. To this aim, multiple organs were harvested from young (2-3-mo-old), old (16-18-mo-old) and very old (24-25-mo-old) mice and the abundance of the miRNAs was evaluated by quantitative RT-PCR. Our studies demonstrated that miR-21a, miR-22 and miR-29a were up-regulated in the aged heart. Among them, miR-29a was highly expressed in many other organs, i.e., brain, skeletal muscle, pancreas and kidney, and its expression was further upregulated during the natural aging process. Computational prediction analysis and overexpression studies identified SERPINH1, a specific chaperone of procollagens, as a potential miR-29a target. Corroborating to this, significantly downregulated SERPINH1 levels were found in skeletal muscle, heart, brain, kidney, and pancreas harvested from very old animals, therefore suggesting that aging process is associated to miR-29a-induced downregulation of SERPINH1 in multiple organs. In conclusion, our study indicates an adaptive increase of miR-29 during natural aging process with a potential therapeutic value by limiting the adverse matrix remodelling and aging-associated tissue fibrosis.

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Abstract N°27: Platelet dysfunction in HFpEF patients: altered platelet activation and thrombus formation under flow

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Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous syndrome resulting from a complex interplay of systemic comorbidities, including diabetes and hypertension. Chronic low-grade inflammation and microvascular dysfunction are considered to be key pathological factors. Recently, platelets emerged as important players in vascular inflammation and endothelial dysfunction, however, the role of platelets in HFpEF is still unclear. This study aims to investigate whether HFpEF patients present alterations in platelet activation and function. For this purpose, platelet integrin αIIbβ3 activation and platelet α-granule secretion were measured by flow cytometry using freshly isolated platelets from HFpEF patients (N=123) and controls (N=48), stimulated with different agonists. Microfluidics assays were performed with whole blood from HFpEF patients and controls to measure platelet adhesion, platelet activation markers (CD62P, fibrin(ogen), annexin A5), and thrombus growth under arterial flow conditions. Platelet-derived RNA was collected with the future purpose of performing RNA sequencing to compare transcriptome profiles of HFpEF patients and controls. Our preliminary results show platelets from HFpEF patients with a decreased integrin αIIbβ3 activation and α-granule secretion upon stimulation with collagen-related peptide and TRAP-6, and increased platelet integrin activation upon stimulation with ADP. Under coagulating conditions, HFpEF patients show an overall reduction in thrombus contraction, density, and fibrin formation under flow. In sum, HFpEF patients are characterized by general platelet dysfunction and these alterations may suggest a possible contribution of platelets in the complex pathophysiology of HFpEF.

Abstract N°6: A signature of mechanically-regulated long non-coding RNAs establish a gene regulatory network for pro-fibrotic cardiac fibroblasts programming

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Obesity and the metabolic syndrome associate with anxiety disorders in adult humans1,2, rodents3,4 and fish5,6, indicating evolutionary conservation of the link. However, the developmental and molecular mechanisms of this association are largely unknown. We examined transcriptomic profiles from two validated zebrafish larval models, one of caffeine-induced anxiety and the other of high fat diet (HFD)-induced obesity. RNA-seq revealed 231 differentially expressed (DE) genes (padj<0.05) common to both the obesity and anxiety models. Among these intersecting genes, 142 were upregulated in anxiety (>1.5 fold), and seventy-five percent of these upregulated genes were also downregulated (<0.67 fold) in obesity, demonstrating inverse regulation. This inverse regulation was related to immune reactions according to the overrepresented Panther immune system-related pathways, “interleukin signaling pathway”, “CCKR signaling map pathway” and “inflammation mediated by chemokine and cytokine signaling pathway” found among these DE genes. However, this apparent link between anxiety and obesity in zebrafish larvae only exists on a transcriptome level, as larvae fed a HFD did not develop anxiety-like behavior, and caffeine-induced anxious larvae did not acquire abdominal obesity. This contrasts with obese adult zebrafish, which do develop anxiety-like behavior 5,6. Thus, it was surprising that analyses of RNA-seq datasets of young adult zebrafish brains, one of inherently anxious versus less anxious strains, and the other of HFD- versus standard diet-fed fish, also demonstrated inverse regulation of transcript expression in anxiety and obesity. Among the 107 DE genes (padj<0.05) common to anxious and HFD-fed adult zebrafish, seventy-four percent of those upregulated in anxious adults (>1.5 fold), were also downregulated (<0.67 fold) in HFD-fed adults. In adults, as in larvae, immune system processes were overrepresented among the DE genes in anxiety and obesity, but the genes and processes were different, e.g., “T cell activation”, “leukocyte cell-cell adhesion”, “antigen processing and presentation”.

These findings imply that adaptive immune reactions may take part in the anxiety-obesity link in adult zebrafish, since larvae only have an innate immune system, whereas adult zebrafish have both innate and adaptive immune systems7. Moreover, the developmental change in immune gene regulation, occurring from larvae to adults, may imply an antagonistic pleiotropic pattern. Lastly, a high fat diet may normalize the upregulation of immune-system related genes in anxiety, in agreement with previously reported protective roles of HFD in anxiety and Alzheimer’s disease models in rodents8–11.

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Abstract Nº3: Combinatorial clinical data–miRNA biomarker system for early mortality prognostication in hospitalized COVID-19 patients

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Introduction: The prevention of adverse outcomes in COVID-19 is still challenging, especially for elderly, immunocompromised and comorbid patients. Heterogeneity in progression rates of hospitalized COVID-19 patients makes crucial the development of early biomarkers to improve clinical decision-making. In this regard, microRNA (miRNA) profiling has emerged as a potential tool not only to be implemented in prognostic strategies but also to improve biological characterization of COVID-19.

Objectives: First, to identify a miRNA profile associated with all-cause in-hospital mortality in elderly COVID-19 patients. Second, to identify biological processes and mechanistic pathways associated with fatal outcome of this condition.

Methods: We used samples from the COVIDPONENT cohort composed of hospitalized patients with COVID-19 admitted to Arnau de Vilanova and Santa Maria University Hospitals (Lleida, Spain) from June 2020 to May 2021. Patients older than 65 years with a plasma sample collected within the first 72 hours upon hospital admission were included in current investigation (n=178). miRNA profiling was analyzed using RT-qPCR. First, a screening was performed to identify potential miRNA candidates (50 patients, 179 miRNAs). Then, candidate miRNAs were validated in the whole cohort. Multivariable models including miRNA levels and clinical variables, previously proposed as predictors of fatal outcomes, were constructed using machine learning approaches (Random Forest). Functional enrichment analyzes were conducted to identify mechanistic pathways associated with adverse outcomes.

Results: In-hospital mortality occurred in the 20.8% of the subcohort. Median age was 78 years and 41.6% of subjects were female. In the screening phase, nineteen candidates were selected as predictors of all-cause in-hospital mortality (Fold-change >1.3, p-value <0.2). In the validation phase, seventeen candidates showed similar results than in the screening phase in terms of the size effect and direction of the association; and thus, were selected for multivariable analyses. Feature selection process based on Random Forest included 26 variables: 10 clinical predictors and 16 miRNAs. Neutrophile-lymphocyte ratio, miR-342-3p, temperature, neutrophile-count and miR-151a-3p were among the top-5 in variable importance. Bioinformatic analyzes provided evidence of molecular pathways and biological processes implicated in viral infection and host response (immune response, inflammation, cell death, hypoxia, vascularization, coagulation and fibrosis).

Conclusions: miRNA profiling could be a clinically useful tool for the management of the hospitalized patient with COVID-19 both for prognosis strategies and characterization of fatal outcomes. Subsequent validation is warranted.

Abstract N°11: An alternative splicing signature of genetic and ischemic dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is characterized by left ventricular enlargement and systolic dysfunction, representing the most frequent indication for heart transplantation. Genetic DCM represents 30-40% of cases and variants in more than 50 genes have been identified. A DCM phenotype can also be caused by ischemic injury (ICM), mainly secondary to coronary artery disease. Changes in cardiac alternative splicing (AS) have been found in association with heart dysfunction, but whether mis-splicing contributes as a molecular driver for heart failure remains unknown. Here, we performed a global cardiac transcriptomic analysis of left ventricular (LV) heart biopsies of control individuals (n=6) and end-stage heart failure patients with genetic (n=10) and ischemic cardiomyopathy (n=11), collected at the time of heart transplantation. Splicing analysis revealed that most of the AS events identified are common between DCM and ICM samples but differ from controls. In addition, several splicing factors show a tendency to be downregulated in the disease samples, with PCBP3, RBM47 and HNRNPF being differentially expressed.

AS events between disease and controls are enriched for cardiomyocyte-related functions, with the 452 disease-specific exon skipping events being distributed on 346 genes, including ones involved in cardiomyocyte sarcomeric structure, contractility, and metabolism. We analysed by RT and qRT-PCR a subset of splicing events in six genes associated with cardiomyocyte physiology and function. Disease-specific AS changes were validated for CAMK2D, EYA4, ESSRG, MYL6, PDLIM3 and SORBS1 in left ventricular samples of controls (n=23), DCM (n=54) and ICM (n=45) patients. To further understand if these AS events are specific to the LV, heart samples collected from the right ventricle (RV) and interventricular septum (IVS) were also analysed by qRT-PCR. Although we were able to validate the AS events in CAMK2D and PDLIM3 in the RV and IVS of both disease groups, for EYA4, ESSRG, MYL6 and SORBS1, changes in AS were detected only for DCM samples. This suggests that this disease-specific pattern of AS may be progressively activated in heart failure. Ongoing work aims to perform a global analysis to understand if this AS pattern corresponds to a reversion to fetal-like AS in heart failure. In this work, we identified an alternative splicing signature associated with dilated cardiomyopathy and heart failure. The finding that splicing is similarly dysregulated in cardiomyopathies with distinctive aetiologies suggests that mis-splicing represents a secondary disease effect. Future studies are needed to determine whether interventions aiming to restore normal splicing could help preventing heart failure.

Abstract N°19: MicroRNA editing in primary myocardial fibrosis

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Accumulation of extracellular matrix in the myocardium results in increased ventricular stiffness and disrupts the normal electrical cell-to-cell coupling and impulse conduction predisposing to arrhythmias and sudden cardiac death (SCD). MicroRNAs play a role in a variety of biological processes in the heart, including development of myocardial fibrosis. In the current study, our aim was to investigate the role of miRNA editing in myocardial fibrosis.

We have collected a consecutive series of SCD victims undergoing medico-legal autopsy in the FinGesture study since 1998 until now. In this population, we have observed primary myocardial fibrosis (PMF) without any known etiology to be a relatively common autopsy finding among the young SCD victims. In the current study, cardiac samples of age-matched control subjects (N=8) and SCD victims with PMF (N=8) were subjected to small RNA sequencing. We identified three editions in the seed sequence of the mature miRNAs: miR-126 and two distinct mutations in miR-29. Analyses of predicted targets in miRDB.org database revealed that canonical and edited miRNAs have distinct targetomes.

Human microvascular endothelial cells, human cardiac fibroblasts and neonatal rat cardiomyocytes are then used to investigate for biological function of edited and canonical miR-29 and miR-126. Cell proliferation will be analysed by EdU assay, collagen production by Sirius red/fast green assay, cytotoxicity/cell viability by Toxilight assay and Resazurin assay, angiogenesis by tube formation assay and protein synthesis by measuring leucine incorporation. mRNA targets of canonical and edited miR-29 and miR-126 are identified by RNA sequencing and confirmed by qPCR and Western blot analyses.

Our preliminary data shows that treatment of human cardiac fibroblasts with profibrotic TGF-β1 has no effect on expression of canonical miR-29 but represses the expression of edited miR-29. Overexpression of edited miR-29 attenuates both basal and TGF-β1 -induced collagen production in cardiac fibroblasts, whereas overexpression of canonical miR-29 promotes TGF-β1 -induced collagen production.

We conclude that myocardial fibrosis is associated with editing of specific miRNAs that regulate collagen production in human cardiac fibroblasts.

Abstract N°20: Identification of a potential microRNA as an important regulator in HHcy-related atherosclerosis

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Introduction: Increased serum levels of homocysteine (Hcy) are a risk factor for cardiovascular diseases, including atherosclerosis. However, the precise mechanisms by which Hcy contributes to this condition remain elusive. During the development of atherosclerosis, microRNAs influence the expression of several genes. Interestingly, miR-30d-5p was found to be up-regulated in plasma of patients with hyperhomocysteinemia (HHcy) and venous thrombosis. Moreover, TIMP3 gene was detected as a predicted target for miR-30d-5p. Thus, we sought to determine whether miR-30d-5p plays a role in the pathophysiology of HHcy-induced atherosclerosis through the regulation of TIMP3.

Methods: Mouse peritoneal macrophages (MPM) were obtained from C57BL/6J mice and treated with different concentrations of Hcy for 24h to evaluate gene expression. Transfection experiments and dual luciferase reporter assays were performed. The activity of MMP9 was evaluated by in situ zymography. The in vivo mouse model was developed by adding 0.9 g/L of DL-Hcy to the drinking water of ApoE-/- mice, and the aortic atherosclerotic lesion was analyzed by histological methods.

Results: We demonstrated that miR-30d-5p was up-regulated in MPM treated with 50μM of Hcy, whereas TIMP3 expression was significantly reduced. We identified TIMP3 as a direct target of miR-30d-5p by repressing the activity of the 3'UTR-TIMP3 reporter construct. The specific inhibition of endogenous miR-30d-5p in both human and mouse macrophages was found to up-regulate the expression of TIMP3. In contrast, the overexpression of miR-30d-5p activated the MMP9 activity. The in vivo mice models of HHcy-induced atherosclerosis showed higher aortic lesion size when compared with that of control mice.

Conclusion: These results provide evidence that miR-30d-5p downregulates TIMP3 and increases the activity of MMP9 in macrophages, representing a potential mechanism by which Hcy might influence atherogenicity.

Abstract N°10: Hidden cardiotoxicity of tyrosine kinas inhibitors is associated with a distinct expression pattern of long non-coding RNAs

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Background: Tyrosine kinase inhibitors (TKIs) may exert significant cardiovascular toxicity. This holds particularly true in the diseased heart, in which many targets of TKIs are upregulated and act compensatorily. Long non-coding RNAs (lncRNAs) are key regulators of cell viability and function and are highly cell-type specific. We here hypothesize that TKI therapy alters the expression of cardiac lncRNAs, which may contribute to TKI cardiotoxicity and could be targeted for cardioprotection.

Methods and Results: In H9c2 cells and neonatal rat ventricular myocytes, the TKI quizartinib dose-dependently decreased cell viability and increased apoptosis, which were both potentiated in the presence of oxidative stress (H2O2). Combination of quizartinib and H2O2 also enhanced phosphorylation of p38 MAP kinase, a known mediator of apoptosis and contractile dysfunction. Quizartinib alone did not alter cardiac morphology or function as assessed by echocardiography in healthy or sham-operated hearts. In contrast, compared to vehicle, quizartinib aggravated the maladaptive remodeling of infarcted hearts by enhancing left-ventricular dilatation both in diastole and systole and increasing apoptotic cell death at one-week post-infarct. Transcriptome analysis on total heart homogenates confirmed the lack of effect of quizartinib in the healthy myocardium, as no differences were found in gene expression between quizartinib- and vehicle-treated sham hearts. However, significant differences existed in infarcted hearts, with roughly twice as many genes altered above significance threshold in quizartinib- than in vehicle-treated hearts compared to respective sham.

Gene set enrichment analysis revealed an association of the double injury of quizartinib and infarct with down-regulated gene sets related to cardiac contraction and energy handling. Furthermore, RNA-sequencing uncovered 160 differentially expressed novel lncRNAs in quizartinib- compared to vehicle-treated infarcted hearts. Validation of the top 9 lncRNA candidates, which were chosen based on tissue specificity, enhancer locus, overall raw expression and existence of a human homologue, confirmed their regulation in quizartinib-treated infarcted hearts.

Conclusions: The TKI quizartinib exhibits a hidden cardiotoxicity that manifests in the ischemically injured heart and is associated with advanced maladaptive remodeling, downregulation of genes related to cardiac contractility and energy handling and distinct expression of lncRNAs. These latter provide a pool of potential targets to be explored for cardioprotection of the diseased heart under TKI therapy. Further ex vivo and in silico studies will be necessary to fully understand the transcriptomic regulatory network change by associating pathological features with epigenetic data, information on regulatory regions and functional candidate prediction in TKI-treated hearts.

Abstract N°30: Cardiovascular complications in Parkinson's disease patients: insights from Luxembourg Parkinson's Study

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Background: The cross talk between the brain and heart in disease states has been discussed for several years (1). Recent work has shown that Parkinson's disease (PD) sufferers have elevated risk of developing cardiovascular diseases (CVDs) due to shared pathogenesis and risk factors (2). However, the functional link between both conditions is understudied.

Aim-Our study aimed at determining the frequency of CVDs in PD patients in the Luxembourg Parkinson's Study (LuxPARK).

Methods: LuxPARK is a longitudinal-monocentric study comprising more than 1700 participants to date, recruited in Luxembourg and surrounding border regions. The participants were divided into two groups namely, PD (N=912) and Non-PD controls (N=826). The frequency of CVDs was investigated in PD patients vs the non-PD subset. Further, to study the risk of CVD in different forms of PD, the PD group was sub-divided into ten different parkinsonism types ('PD' (n=665), 'PD and Dementia' (n=87), 'Progressive supranuclear palsy' (PSP, n=55), 'Lewy Body Dementia' (n=32), 'Parkinsonism' (n=19), 'Secondary Parkinsonism' (n=18), 'Multisystem atrophy' (n=15), 'Cortico-Basal Syndrome' (n=13), 'Drug-induced PD' (n=7) and 'Frontotemporal dementia and parkinsonism (FTDP, n=1)).

Results: LuxPARK study representing the Luxembourgish PD population revealed higher prevalence of PD in males (PD - 66.2%, Non-PD -51.7%; $p<0.001$) as compared to females. The frequency of CVD occurrence was significantly higher in PD (51%) as compared to non-PD (33%) controls ($p<0.001$). The risk of developing CVD was also higher in the PD group (odds ratio; OR =1.983, 95% confidence interval (CI) = 1.635 – 2.405, p-value <0.001) which remained similar after adjusting for age and sex (OR = 1.252, 95% CI = 1.009 – 1.554, p = 0.041). In different parkinsonism types, the age and sex adjusted risk of CVD was significantly elevated for PD patient groups with PSP (OR=1.834, 95% CI = 1,001 - 3,360; p-value 0.05) and secondary parkinsonism (OR=3.608, 95% CI = 1,113 – 11,691; p-value = 0.032).

Conclusion: Our study sheds a light on the significance of brain and heart associations in the context of PD and CVDs. Our results show that men are at greater risk of developing PD than women. Additionally, PD patients in Luxembourg have a higher risk of developing CVDs compared to the non-PD controls. This study strengthens the link between various forms of PD and CVD occurrence and the dire need to study the two diseases in coherence.

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Abstract N°18: Increased plasma levels of miR-142-3p, miR-223-3p and mitochondrial DNA could predict unfavorable outcomes in patients after acute myocardial infarction

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Abstract: Myocardial infarction is one of the world leading causes of death, despite numerous efforts to find efficient prognostic biomarkers and treatment targets. In the present study, we aimed to identify new epigenetic prognostic tools for the occurrence of unfavorable outcomes after acute ST-segment elevation myocardial infarction (STEMI), such as major adverse cardiovascular events (MACE). For this purpose, we analyzed in the plasma six microRNAs known to be associated with cardiovascular diseases, along with cell-free DNA (cfDNA) and mitochondrial DNA (mtDNA). Fifty STEMI patients were enrolled and monitored for a period of 6 months after myocardial infarction for the occurrence of MACE. Blood was collected at 3 time-points: upon admission to the hospital (T0), at the discharge from the hospital (T1) and 6 months post-STEMI (T6). The levels of miR-223-3p, miR-142-3p, miR-155-5p, miR-486-5p, miR-125a-5p and miR-146a-5p, as well as those of cfDNA and mtDNA, were measured in the patients' plasma by TaqMan assays.

Results showed that all the assessed miRNAs, as well as cfDNA and mtDNA, were most increased at T1, as compared to the other two time-points. Hence, we used the values of all measured parameters at T1 for further statistical analysis. The results showed that increased levels of miRNAs, cfDNA and mtDNA at T1 in the plasma of STEMI patients with MACE compared to those without MACE were determined. We employed specific statistical models for the prediction of MACE in STEMI patients and we demonstrated that the increase of all six miRNAs and cfDNA plus mtDNA levels, respectively, could be associated with MACE. The most relevant of these multivariate statistical models was the one combining miR-142-3p, miR-223-3p and mtDNA, as revealed by the ROC analysis (AUC=0.833, p=1.17 × 10–3). In conclusion, the increased plasma levels of miR-142-3p, miR-223-3p, and of mtDNA at hospital discharge could be used to predict unfavorable (MACE) outcomes in STEMI patients.

Abstract N°15: Epi-metabolic drug design and characterization to prevent or ameliorate fibrosis in human-diseased cellular systems

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Introduction: Fibrosis is a pathologic scarring process consisting of impaired organ functions in different tissues due to an excessive extracellular matrix (ECM) deposition. Myofibroblasts are the primary cells mainly involved in cardiac fibrosis, and, notably, it has recently emerged that fibroblasts' activation to myofibroblasts may be under epigenetic control. It is also well documented that chronic hypoxia directly contributes to fibrogenesis and is directly associated with increased expression of pro-fibrotic genes and miRNAs, such as α- smooth muscle actin (αSMA), collagen 1 (COL1), and miR-200 family.
The project considers the role of DNA methylation in the processes of hypoxia and fibrosis and how they could be targeted by novel epigenetically active drugs controlling this mechanism - specifically TET protein - which represents a crucial target for novel therapeutic approaches.

Methods And Results: A library of ten TET2-selective inhibitor compounds (RL1-10) has been tested on human cardiac fibroblasts for their epigenetic and antifibrotic activity under chemical-induced hypoxic conditions. Among them, four compounds showed a significant accumulation of global DNA methylation. Furthermore, a panel of 84 fibrosis-related genes were analysed through real-time qPCR; it was possible to appreciate higher differential expression of ACTA2, COL1A2, COL3A1, CTGF, IL1A, TGFB2 genes, which resulted 2-3 folds more expressed in cells treated with CoCl2 compared to solvent. Remarkably, after the TET inhibitors treatment - their expression level was decreased and "restored" compared to normoxic condition. An opposite modulation is represented by Caveolin-1(CAV1) and Matrix metallopeptoidase-1 (MMP1), which resulted less expressed under hypoxic conditions and increased after the treatment with RL8 and RL9 compounds.

Conclusion: Cardiac fibroblasts, due to their characteristics, constitute a central focus in fibrosis research; in fact, inhibiting fibroblast-mediated ECM synthesis is the principal goal of antifibrotic therapeutic approaches. Our results present evidence about a new group of specific non-nucleoside analogs for DNA demethylation, which might become relevant tools to prevent/reduce cardiac fibrosis.

Abstract N°28: A systems approach to investigate the role of A-to-I RNA-editing in the dysregulation of diabetic endothelial cells

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Abstract: Cardiovascular disease is the leading cause of death in the diabetic population. In type-2 diabetic (T2D) patients, hyperglycaemia and insulin resistance drive a cascade of events leading to altered cardiac physiology. The mechanisms underlying dysregulated events in diabetic endothelial cells are still unclear. To advance our knowledge, we have investigated the transcriptome of the left ventricle myocardium (needle-biopsies) of diabetic patients with ischaemic heart disease undergoing cardiac surgery (controls; non-diabetic patients). Our pilot data show that T2D is associated with altered RNA-editing from Adenosine-to-Inosine (A-to-I, catalysed by adenosine deaminases family of enzymes) for genes that are critical regulators of key cardiac functions. To strengthen our observation, we have demonstrated that: (i) ADAR-2 (RNA-editing enzyme) is significantly up-regulated in db/db (a mouse model of diabetes). (ii) dysregulation of key RNA-editing enzymes in HUVEC cells altered their viability and proliferation. Moreover, (iii) there is a significant correlation between the number of RNA-editing events and the stability of RNA secondary structure. We propose to target dysregulated RNA-editing events that drive diabetes cardiomyopathy. In this project, we will generate RNA-sequencing and proteomic data and perform various function characterization on endothelial cells. The laboratory data and bioinformatic analyses will be integrated to prioritize targets for therapeutic interventions.