46th EWGCCE Meeting

Annual Meeting
The Annual Meeting of the ESC Working Group on Cardiac Cellular Electrophysiology

4 - 6 June 2022
Toledo, Spain
46th meeting. Toledo, Spain, 4-6 June 2022.

Dear Colleagues, after a long period during which scientific conferences have been impacted by the coronavirus pandemic, we are enthusiastic to welcome you in-person once again, in the imperial city of Toledo, Spain.

Organising committee

Ana M Gómez - Chair

Carol Ann Remme - Past Chair  Jordi Heijman - Chair Elect

Members

Dan Johnson, United Kingdom of Great Britain and Northern Ireland
Elisa Passini, United Kingdom of Great Britain and Northern Ireland
Marketa Bebarova, Czech Republic
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### SCIENTIFIC PROGRAMME

**Saturday, 4th JUNE 2022**

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| 15:30 – 17:00 | **SESSION 1: Ultrastructural basis of cardiomyocyte function**
|               | *Chairpersons: Morten Thomsen & Leonardo Sacconi*                                       |
| 15:30 – 16:00 | Junctophilin proteins in cellular function - Stephan Lehnart, Germany                    |
| 16:00 – 16:15 | **OR01.** Tortuous cardiac intercalated discs modulate ephaptic coupling: insights from a finite element model. Ena Ivanovic, University of Bern, Switzerland |
| 16:15 – 16:30 | **OR02.** Epicardial Adipose Tissue Facilitates Reentrant Arrhythmias by Remodeling of Myocardial Ion Channels. Auriane Ernault. Amsterdam Umc, The Netherlands |
| 16:30 – 16:45 | **OR03.** Mechanisms of postprandial cardiac growth in pythons. Claudia Crocini. Max Delbrück Center, Germany |
| 16:45 – 17:00 | **OR04.** Assessment of proarrhythmogenic risk for cannabidiol using dog and rabbit cardiac preparations: The electrophysiological effects on action potential and transmembrane potassium currents. Muhammad, Naveed. University Of Szeged, Hungary |

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<td>09:00 – 10:30</td>
<td>SESSION 2: Physiologic variation on channel expression</td>
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<td>09:30 – 09:45</td>
<td>OR09. NOD-1 activation increases the spontaneous activity and the I(f) current of sinoatrial node murine cells. Annalisa Bucchi, Dept. Of Biosciences, Università Degli Studi Di Milano, Italy</td>
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<td>09:45 – 10:00</td>
<td>OR10. On the biological rhythmicity of cardiac L-type Ca2+ channel expression Estelle Personnic. Signalling and Cardiovascular Pathophysiology, Umr-s180, Inserm, Université Paris-Saclay, France</td>
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<td>10:15 - 10:30</td>
<td>OR12. Succinate and exposure to hypoxia-reoxygenation cause similar pro-arrhythmic modifications in left atrial electrophysiology. Molly O’Reilly. University Of Amsterdam, The Netherlands</td>
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<td>10:30 – 10:45</td>
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<td>15:00 – 16:30</td>
<td>SESSION 3: Ion-channel regulation and dysfunction</td>
<td>CaMKII regulation of ionic channels. Senka Ljubojovic-Holzer, Austria</td>
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<td>15:45 – 16:00</td>
<td><strong>OR06</strong>. Unravelling the arrhythmogenic mechanisms of short QT syndrome type 3 in an animal model of Kir2.1 (^{M30TK}) gain-of-function mutation. <em>Ana Isabel Moreno Manuel. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Spain</em></td>
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<td>16:00 – 16:15</td>
<td><strong>OR07</strong>. Genetic ablation of G protein-gated inwardly rectifying K(^+) channels improves heart rate in Angiotensin II-induced sinus bradycardia mice model. <em>Eleonora Torre. Institut de Génomique Fonctionnelle, CNRS, Montpellier, France</em></td>
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<td>16:15 - 16:30</td>
<td><strong>OR08</strong>. Unravelling the signaling pathway downstream of the neurokinin receptor in atrium: discovering novel anti-arrhythmic targets for treatment of atrial fibrillation. <em>Mathilde Rivaud. Amsterdam University Medical Center, The Netherlands</em></td>
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<td>16:45 – 17:45</td>
<td><strong>SESSION 4: CCW lecture</strong> <em>Chairpersons: Ana Maria Gómez</em></td>
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<td>Love at first sight: exploring arrhythmogenic mechanisms is a great adventure. <em>Elisabetta Cerbai, Italy</em></td>
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*Elisabetta Cerbai (PhD, FESC) is Full Professor of Pharmacology at the University of Florence and Director of the European Laboratory for Non-linear Spectroscopy (LENS). She joined the WG in 1985 and chaired it in 2010-2012; she also co-organized two meetings in Ferrara (1992) and Florence (2006). Her research interests include biophysics of cardiac excitation-contraction coupling mechanisms and pharmacotherapy in cardiovascular diseases. She has been working on the arrhythmogenic mechanisms in atrial and ventricular cardiomyocytes from human biopsies for over 25 years. Today, her team joins a large, multidisciplinary*
network in Florence with experienced and young scientists, whose competence embraces clinical cardiomyopathies and channelopathies, physiology, optogenetics and biophotonics, pharmacology, stem cell biology and engineered heart tissue.

21:00  CONFERENCE DINNER (Venta de Aires restaurant)

Monday, 6th JUNE 2022

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<td>09:00 – 10:30</td>
<td>SESSION 5: Prohypertrophic and proarrhythmic effects of intracellular calcium</td>
<td>Dan Johnson &amp; Jean-Pierre Benitah</td>
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<td>09:00 – 09:30</td>
<td>Cardiac Ryanodine Receptor Mutations: What Determines Their Clinical Phenotype?</td>
<td>Héctor H Valdivia, USA</td>
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<td>09:30 – 09:45</td>
<td>OR13. Functional crosstalk between IP3R and RyR Ca^{2+} channels contributes to arrhythmic activity in failing human hearts.</td>
<td>Llewelyn Roderick, Kuleuven, Belgium</td>
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<td>09:45 – 10:00</td>
<td>OR14. STIM2 protein regulates Orai1-mediated store-operated Ca^{2+} entry in cardiomyocytes.</td>
<td>Rui Luo, Inserm Umr-s 1180, France</td>
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<td>10:00 – 10:15</td>
<td>OR15. Specific SERCA stimulation is more arrhythmogenic than β-adrenergic stimulation in cardiomyocytes harboring a CPVT mutation.</td>
<td>Rubén López Dicuru, Department of Physiology, University of Bern, Switzerland</td>
<td>15 min</td>
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<td>10:15 – 10:30</td>
<td>OR16. PLN-R14del mutation: discovering the pathophysiological role in a novel heterozygous mouse model of dilated cardiomyopathy.</td>
<td>Claudia Maniezzi, University of Milano-bicocca, Italy</td>
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<td>10:30 – 11:00</td>
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<td>11:00 – 12:30</td>
<td>SESSION 6: Intercellular channel communication</td>
<td>Cristina Molina &amp; José Jalife</td>
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<td>11:00 – 11:30</td>
<td>Epicardial origin of cardiac arrhythmias - Stephane Hatem, France</td>
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<td>11:30 – 11:45</td>
<td><strong>OR17.</strong> Drug-induced arrhythmias in zebrafish larvae: correlated changes in calcium, contraction, and hemodynamics. Jussep Salgado-Almario. Universidad De Castilla-La Mancha, Spain</td>
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<td>11:45 – 12:00</td>
<td><strong>OR18.</strong> Striatin knock out induces a gain of function of $I_{Na}$ current in mESC-derived cardiomyocytes. Alessandro Cospito. Dept. of Biosciences, Università Degli Studi Di Milano, Italy</td>
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<td>12:00 – 12:15</td>
<td><strong>OR19.</strong> Transition between L-type calcium channel isoforms prolongs action potential duration after acute myocardial infarction/reperfusion injury, Ehsan Amin. Institute of Neural and Sensory Physiology, Medical Faculty and University Hospital Düsseldorf, Germany.</td>
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<td>12:15 – 12:30</td>
<td><strong>OR20.</strong> Cardial tattoos lines: A new method to treat arrhythmias Constanze Schmidt, University Hospital Heidelberg, Germany.</td>
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<td>General Assembly and awards ceremony</td>
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Tortuous cardiac intercalated discs modulate ephaptic coupling: insights from a finite element model

Ms Ena Ivanovic\textsuperscript{1}, Prof. Jan P. Kucera\textsuperscript{1}

\textsuperscript{1}University of Bern, Bern, Switzerland

Background: Ephaptic coupling, a mechanism mediated by negative electric potentials occurring in the narrow intercellular clefts of intercalated discs, can influence action potential propagation by modulating the sodium current. We have previously shown that intercalated disc nanodomains known as perinexi, where sodium channels tend to cluster, are privileged sites for ephaptic coupling. However, intercalated discs are highly tortuous due to the mingling of plicate and interplicate regions, and how the tortuosity of the intercalated disc influences ephaptic coupling is not well understood.

Aim: Our aim was to investigate how the convoluted structure of the intercalated disc influences ephaptic effects.

Methods: We refined our previously developed finite element model of an intercalated disc shared by two cardiomyocytes. We tested different predefined folded intercalated disc geometries. These geometries were parametrized by the orientation of folds (concentric vs. radial) and by the amplitude and number of folds. A voltage clamp protocol was applied to both cells.

Results: Ephaptic interactions (assessed by the minimum cleft potential and by the amplitude of the sodium currents) were reinforced by concentric folds with increasing amplitude and number of folds. This reinforcement was explained by the longer average path from a point in the cleft to the bulk extracellular space, which increased the resistance of the cleft and led to a more negative cleft potential during the sodium current transient. In contrast, radial folds attenuated ephaptic interactions, although this effect was smaller. This attenuation was explained by more possible paths from a point in the cleft to the bulk extracellular space, which decreased cleft resistance.

Conclusion: Our results indicate that the folding pattern of the intercalated disc, in addition to the clustering of sodium channels in perinexi, plays an important role in cardiac conduction. These findings contribute to a comprehensive understanding of cardiac excitation at the nanoscale level.
Epicardial Adipose Tissue Facilitates Reentrant Arrhythmias by Remodeling of Myocardial Ion Channels

**OR 02**

**Epicardial Adipose Tissue Facilitates Reentrant Arrhythmias by Remodeling of Myocardial Ion Channels**

*Mrs Auriane Ernault*, 1 Arie O. Verkerk, 1 2 Jason D. Bayer, 3 4 Kedar Aras, 5 Pablo Montañés-Agudo, 1 Rajiv A. Mohan, 7 Marieke Veldkamp, 1 Mathilde Rivaud, 1 Makiri Kawasaki, 1 Shirley C. M. van Amersfoorth, 1 Eva R. Meulendijks, 1 Antoine H.G. Driessen, 1 Igor R. Efimov, 5 Joris R. de Groot, 1 Ruben Coronel

1 Department of Clinical and Experimental Cardiology, Amsterdam Umc, Amsterdam, Netherlands, 2 Department of Medical Biology, Amsterdam Umc, Amsterdam, Netherlands, 3 IHU-LIRYC, Electrophysiology and Heart Modeling Institute, Bordeaux University Foundation, Pessac, France, 4 Centre National De La Recherche Scientifique, Institut de Mathématiques de Bordeaux, UMR5251, Bordeaux, France, 5 Department of Biomedical Engineering, George Washington University, Washington, USA

Background: Epicardial Adipose Tissue (EAT) accumulation is associated with sustained arrhythmias, suggesting that EAT contributes to the development of a pro-arrhythmic substrate. We hypothesize that adipokines secreted by EAT exert a pro-arrhythmic effect on the neighboring myocardium.

Aim: Compare the effects of EAT and subcutaneous adipose tissue (SAT) secretomes incubation on cardiomyocytes electrophysiology.

Material and Methods: EAT and subcutaneous adipose tissue (SAT) were collected from 25 patients with persistent atrial fibrillation undergoing video-assisted thoracoscopic pulmonary vein isolation, and incubated for 24H in culture medium. The secretome of adipose tissue was harvested. Monolayers of neonatal rat ventricular myocytes (NRVMs) were cultured with the EAT or SAT secretome for 72H. Gene expression, action potentials and conduction velocity were measured. Lastly, we implemented the electrophysiological changes observed in cardiomyocytes after EAT secretome incubation into an in-silico model of human left atrium and tested arrhythmia inducibility.

Results: Incubation with EAT secretome decreased cardiomyocyte gene expression of the potassium channel subunit Kcnj2 by 26% and correspondingly reduced the inward rectifier K+ current (IK1) by 35%, resulting in a depolarized resting membrane of cardiomyocytes and a reduced action potential upstroke velocity in comparison to the control. EAT secretome also decreased expression of gap junction protein connexin43 (29% mRNA and 46% protein). Incubation of cardiomyocytes with SAT secretome did not lead to significant changes of ion channels expression or action potential characteristics. Finally, NRVMs incubated with the secretome of EAT showed reduced conduction velocity and increased conduction heterogeneity compared to SAT secretome and control. Computer modeling of human left atrium revealed that the electrophysiological changes induced by EAT secretome promote sustained reentrant arrhythmias when EAT partially covers the myocardium.

Conclusion: The EAT secretome slows conduction, depolarizes the resting membrane of cardiomyocytes, alters electrical coupling and facilitates reentrant arrhythmias. The EAT is a potential target to prevent arrhythmias.
Mechanisms of postprandial cardiac growth in pythons

Dr Claudia Crocini1,2,3, Dr KC Woulfe4, Dr Stefano Perni4, Christopher Ozeroff2, Dr Joe Cardiello2, Dr Mary Allen2, Prof. Dr. Leslie Leinwand2

1Max Delbrück Center, Berlin, Germany, 2University of Colorado at Boulder, Boulder, U.S.A., 3German Center for Cardiovascular Research (DZHK) Partner Site Berlin, Berlin, Germany, 4University of Colorado Anschutz Medical Campus, Denver, U.S.A.

Background – Pythons are infrequent feeders that can ingest meals equal to their own body mass. The extreme metabolic response required to digest such large meals is associated with a dramatic increase in metabolism and the mass of most organs, including the heart. The functional effects of feeding have not been reported in python cardiomyocytes or myofibrils, limiting our understanding of the mechanisms responsible for the extreme post-prandial cardiac adaptation in reptiles.

Aim – Here, we aimed at studying postprandial cardiac adaptation in fasted and 24h post-fed ball pythons (Python regius) using a functional, molecular, and structural approach.

Methods – We combined functional studies on isolated cardiomyocytes and myofibrils with transcriptomics, proteomics, and metabolomics, as well as structural assessment using electron microscopy.

Results – After feeding, python hearts showed a ~28% increase in mass and a ~31% decreased in stiffness. Unexpectedly, electron microscopy revealed reduced cardiac myofibril area and RNAseq analysis showed downregulation of sarcomere-related gene transcripts. Conversely, maximal force generation was increased in myofibrils from 24h post-fed pythons as compared to pythons fasted for 28 days. Myofibrils also showed prolonged relaxation time and reduced passive tension. Proteomic analysis of myofibrils and titin separating gels indicated that myosin and titin isoforms were not different between 24h post-fed and fasted ball pythons, but post-translational modifications were different between fasted and post-fed hearts. Ca2+ transients of isolated cardiomyocytes from 24h post-fed pythons were prolonged, with increased time-to-peak and slower Ca2+ decay. Myofibril Ca2+ sensitivity and peripheral coupling area identified by electron microscopy were not different between 24h post-fed and fasted ball pythons. Compared to fasted, cardiomyocytes isolated from 24h post-fed pythons produced more ATP via oxidative phosphorylation accompanied by activation of AMP-dependent kinase and increased expression of fatty acid oxidation genes. Additionally, metabolomics analysis indicated increased aminoacyl-tRNA biosynthesis and amino acid metabolism in 24h post-fed python hearts.

Conclusions – These results show that feeding promotes positive inotropy in python hearts by means of prolonged Ca2+ transients and increased myofilament force generation supported by increased energy production.
Assessment of proarrhythmogenic risk for cannabidiol using dog and rabbit cardiac preparations: The electrophysiological effects on action potential and transmembrane potassium currents

Mr Muhammad Naveed, Dr Leila Topal, Dr Janos Prorok, Dr Bence Paszti, Dr Dezso Csupor, Dr Istvan Baczko, Dr Laszlo Virag, Dr Norbert Jost, Dr Andras Varro

Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary,
2Department of Pharmacognosy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary

Background:
Cannabidiol (CBD), a major active phytogenic cannabinoid, is one of the main constituents of cannabis. Cannabis has been widely used as recreational drug over the decades and its use is constantly increasing as hallucinogenic and/or medicinal agent. However, significant cardiovascular side effects can accompany its use ranging from arrhythmia to sudden cardiac death.

Purpose:
The aim of the present work was to investigate the possible cardiac adverse electrophysiological effects of cannabidiol (CBD) on action potentials and various transmembrane potassium currents, such as the rapid (IKr) and slow (IKs) delayed rectifier, the transient outward (Ito) and inward rectifier (IK1) potassium currents in rabbit and dog cardiac preparations to assess the cardiac safety profile and proarrhythmic risk.

Methods:
In the current study, conventional microelectrode and voltage clamp techniques were used to record the action potential and transmembrane ionic currents in dog and rabbit ventricular tissue preparations and enzymatically isolated myocytes, respectively.

Results:
The results show that CBD lengthens APD90 significantly at the concentration of 5 µM both in dog and rabbit ventricular tissues without changing other action potential parameters significantly. To further investigate the APD90 lengthening effect of CBD, transmembrane potassium currents (IKr, IKs, Ito and IK1) were investigated in dog and/or rabbit ventricular myocytes using voltage clamp technique. CBD significantly inhibited IKr and IKs currents in rabbit ventricular myocytes with an estimated EC50 values of 4.9 and 3.1 µM, respectively. The effect of CBD on rabbit’s Ito current was not significant while it was significant on dog’s Ito current with an estimated EC50 value of 5 µM. IK1 was not responsive to CBD even at high concentration.

Conclusion:
In conclusion, looking at the inhibitory effects of CBD on repolarizing potassium currents, despite of the fact that these calculated EC50 values are higher than pharmacokinetics based Cmax values of CBD recorded after smoking and oral intake, it can be speculated that in the presence of certain cardio active drugs or co-morbidity where CBD metabolism or cardiac repolarization reserve is impaired CBD can have an additive and proarrhythmic effect.
Stress-induced premature senescence in hiPSC-derived cardiomyocytes recapitulates aging-induced cardiac remodelling

Dr Martina Arici¹, Edoardo Lazzarini², Alessandra Maria Lodrini¹, Sara Bolis², Stefano Panella³, Azucena Rendon Angel², Tiziano Torre², Giuseppe Vassallij²,³, Claudia Altomare², Lucio Barile²,³,⁴, Marcella Rocchetti¹

¹University Of Milano-Bicocca, Milan, Italy, ²Cardiocentro Ticino Institute, Lugano, Switzerland, ³University of Svizzera Italiana, Lugano, Switzerland, ⁴Scuola Superiore Sant’Anna, Pisa, Italy

Aging of the heart involves adverse remodeling in cardiomyocytes (CMs), resulting in heart failure, which increases with age. This study exploits CMs differentiated from human induced pluripotent stem cells (iPSC) as a tool to reproduce and characterize mechanisms involved in aging. A stress–induced premature senescence was induced by short exposure to doxorubicin (Dox) at the sub-lethal concentration of 0.2 µM (Sen-CMs). We explored phenotypic and functional properties of Sen-CMs in comparison to untreated CMs, correlating them with the results obtained in mouse CMs (mCMs) isolated from young (7 weeks) and old (18 months) C57BL/6 mice. Dox treatment induced expression of cyclin-dependent kinase inhibitors p21 and p16, and increased positivity to senescence-associated beta-galactosidase (SA-β-gal), typical markers of cellular senescence. Moreover, Sen-CMs showed increased oxidative stress and a depolarized mitochondrial membrane potential, which resulted in decreased ATP production. Functionally, Sen-CMs showed altered electrical activity in terms of prolonged multicellular QTc interval and action potential duration (APD), together with increased incidence of delayed after-depolarizations (DADs). APD prolongation was ascribable to increased late sodium current (INaL) and reduced rapid delayed rectifier potassium current (IKr). Pretreatment with resveratrol prevented Dox-induced multicellular QT prolongation. In parallel, old mCMs in comparison to young mCMs, showed APD prolongation and INaL enhancement, thus reproducing Dox-induced electrical abnormalities in human CMs. Moreover, in both Sen-CMs and old mCMs, pCAMKII level was increased in comparison to untreated CMs and young mCMs respectively.

Overall, Sen-CMs largely recapitulate the phenotype of aged primary CMs and thus they can be considered a novel in vitro platform to study aging mechanisms and to envision cardiac specific anti-aging approach in humans.
Unravelling the arrhythmogenic mechanisms of short QT syndrome type 3 in an animal model of Kir2.1[\sup]M301K[/sup] gain-of-function mutation

**Ms Ana I Moreno Manuel**, Alvaro Macías, Francisco M Cruz Uréndez, Lilian K Gutiérrez, Isabel Martínez Carrascoso, Francisco J Bermúdez Jiménez, María L Vera Pedrosa, Patricia Sánchez Pérez, José Jalife

1Centro Nacional De Investigaciones Cardiovasculares (CNIC), Madrid, Spain, 2Hospital Universitario Virgen de las Nieves, Granada, Spain

Introduction: Short QT Syndrome Type 3 (SQTS3) is an extremely rare, highly arrhythmogenic disease caused by gain-of-function mutations in the KCNJ2 gene coding the inward rectifier potassium channel Kir2.1.

Objective: To investigate mechanisms of life-threatening arrhythmias produced by Kir2.1-M301K mutation. We tested the hypothesis that, in addition to abolishing IK1 rectification, Kir2.1-M301K produces functional defects in Kir2.1 partner proteins, predisposing patients to an arrhythmogenic phenotype.

Methods: We used intravenous cardiac-specific adeno-associated virus-mediated gene transfer to generate mice expressing wild-type (WT) and M301K mutant channel. We characterized the mice using molecular and cellular biology techniques, electrocardiograms (ECGs), intracardiac stimulation and patch-clamping.

Results: We confirmed Kir2.1-WT or Kir2.1-M301K gene expression specifically in the mouse heart. On ECG, the corrected QT (QTc) interval of Kir2.1-M301K mice was significantly shorter than Kir2.1-WT mice (p<0.0001), and the QRS complex was prolonged (N=20). On intracardiac stimulation, 7 out of 8 Kir2.1-M301K mice had inducible ventricular tachycardia/fibrillation of >500ms duration, while none of 10 Kir2.1-WT mice were inducible (p=0.0009). Compared with Kir2.1-WT, the current/voltage relation of Kir2.1-M301K cardiomyocytes showed increased outward IK1 at voltages positive to -80 mV (p<0.0001) with no inward-going rectification. However, inward IK1 was reduced at voltages negative to -80 mV (p=0.006). Unexpectedly, Kir2.1-M301K cardiomyocytes had a reduced INa density (p<0.0001) with voltage shifted activation and inactivation. Membrane fractionation and western blot analysis revealed that, while 100% of NaV1.5 channels reached the membrane in Kir2.1-WT cardiomyocytes, only 77% did in Kir2.1-M301K cardiomyocytes.

Conclusions: The Kir2.1-M301K mouse recapitulates the arrhythmogenic phenotype of the SQTS3 patient. Kir2.1-M301K mutation produces both IK1 and INa dysfunction, confirming the reciprocal modulation between these channels in macromolecular complexes. This is the first demonstration that a KCNJ2 gain-of-function mutation modifies NaV1.5 current, thus producing severe arrhythmias by both shortening QT interval and reducing ventricular excitability.
Genetic ablation of G protein-gated inwardly rectifying K+ channels improves heart rate in Angiotensin II-induced sinus bradycardia mice model

Eleonora Torre¹,², Isabelle Bidaud¹,², MatteoElia Mangoni¹,², Pietro Mesirca¹,²

¹Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS, INSERM, Montpellier, France, ²LabEx Ion Channels Science and Therapeutics (ICST), Montpellier, France

Background: Secondary sinus bradycardia is associated with heart failure (HF) and is responsible for sudden death in hospital. We recently reported that genetic ablation of G protein gated K⁺ (IKACH) channels (Girk4⁻/⁻) prevents sinus bradycardia induced by intensive exercise training in mice.

Aim: To test if genetic ablation of IKACH prevents bradycardia in Angiotensin II (AngII) -induced HF mouse model.

Methods: Control wild-type (WT) and Girk4⁻/⁻ mice were assigned to NaCl- or AngII- treated groups. In vivo ECG, echocardiographic recordings and patch clamp experiments in isolated sinus-atrial node (SAN) cells were performed at week 5 after the beginning of treatments.

Results: AngII-treated WT mice presented a significantly lower heart rate in comparison to the other groups. AngII-treated WT mice presented a significantly lower ejection fraction (EF, -30%), cardiac output (CO, -28%) and fractional shortening (FS, -38%) in comparison to the NaCl- counterpart group. In contrast AngII-treated Girk4⁻/⁻ mice did not show any significant difference compared NaCl- counterpart group. The “funny” current (If) density was significantly decreased (37% at -135mV) only in SAN cells derived from AngII-treated WT mice. L-type Ca2+ peak current (ICaL) was reduced by 37% in AngII-treated WT group and by 17% in AngII-treated Girk4⁻/⁻ group compared to NaCl-treated counterparties. Similarly, T-type Ca2⁺ peak current (ICaT) was diminished by 38% in AngII-treated WT group and by 14% in AngII-treated Girk4⁻/⁻ group compared to NaCl-treated counterparties. Conclusions: Genetic ablation of cardiac IKACH prevents AngII-induced in vivo alterations of EF, CO and FS. Moreover, genetic ablation of cardiac IKACH prevents AngII-induced sinus bradycardia by restoring If and partially rescuing ICaL and ICaT.
Unravelling the signaling pathway downstream of the neurokinin receptor in atrium: discovering novel anti-arrhythmic targets for treatment of atrial fibrillation

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Background: We have previously shown that stimulation of neurokinin receptor (NKR) 3 bears anti-fibrillatory properties by prolonging the action potential duration (APD) of atrial cardiomyocytes via inhibition of a background potassium current. The NKR is a Gq/11 protein-coupled receptor, but the downstream intracellular pathway and the nature of the end-effector K\(^+\)-channel in atrium are unknown. We aimed at unravelling the components of this pathway in atrial cardiomyocytes.

Materials and Methods: We recorded action potentials using patch-clamp methodology. We performed RNA sequencing on rabbit and human left atrial cardiomyocytes and human immortalized atrial cardiomyocytes (HiAMs).

Results: In rabbit atrial cardiomyocytes, stimulation of NKR3 prolonged APD by ~50%. Upon inhibition of phospholipase C (PLC) or phosphokinase C (PKC), APD increase by NKR3 stimulation was reduced by five- and fourfold respectively. RNA sequencing analysis of human atrial tissue and HiAMs demonstrated the expression of these intracellular signaling molecules. Accordingly, direct PLC activation in HiAMs prolonged APD by 34%. RNA sequencing analysis further revealed small conductance potassium (SK) channels (KCNN2/KCNN3), the TASK-1 channel (KCNK3), and (constitutively active) KACh channels (KCNJ3/KCNJ5) as potential end-targets of the NKR3 pathway in rabbit. Inhibition of SK or TASK-1 channels (prolonging APD by ~15%) did not abolish the APD prolongation effect by subsequent NKR3 stimulation (~50%), indicating that these K\(^+\)-channels do not represent the end-effector background K\(^+\)-channel inhibited by NKR3 stimulation. Inhibition of constitutive KACh channels had little effect on APD at baseline. Nevertheless, APD shortening by agonist-induced KACh channel activity could be completely reversed by NKR3 stimulation, indicating that increased outward KACh current can be fully counterbalanced by the reduction in K\(^+\) background current.

Conclusions: PLC and PKC are intracellular downstream components of NKR signaling pathway in atrial cardiomyocytes. Stimulation of this NKR receptor pathway may have an anti-arrhythmic potential in patients with (vagally-mediated) atrial fibrillation.

Funding sources: The collaboration project is co-funded by the PPP Allowance made available by Health-Holland, Top Sector Life Sciences & Health, to stimulate public-private partnerships.
NOD-1 activation increases the spontaneous activity and the I(f) current of sinoatrial node murine cells

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Background: The nucleotide-binding oligomerization domain (NOD1) receptors are subfamily members of innate immunity proteins that recognize infectious and non-infectious factors and play a key role in the activation of the inflammatory response. Recent data indicate that in ventricular myocytes the activation of NOD1 causes cardiac dysfunction and pro-arrhythmogenic events mainly by regulating intracellular Ca²⁺ handling processes. However, it is unclear whether the selective activation of NOD-1 can regulate the sinus rhythm and the funny current (I_f).

Aim: The aim of this study is to investigate whether NOD1 activation affects the electrical activity of murine sinoatrial (SAN) cells.

Methods: Patch-clamp experiments in whole-cell configuration were performed in single SAN cells isolated from 2-3 month-old mice. Prior to cell isolation, SAN tissues were exposed for 48hrs to: 1) vehicle, 2) C12-iE-DAP (NOD-1 activator; 20 μg/ml), 3) iE-Lys (negative control for NOD-1 activation; 20 μg/ml).

Results: Prolonged exposure to C12-iE-DAP causes an increase of both spontaneous action potential rate (+30%) and the I_f current density (~65% at full activation). In addition, C12-iE-DAP positively shifts the I_f activation curve by ~5mV. These effects were not observed when NOD1 was not activated (iE-Lys exposure). Interestingly, preliminary evidence shows that C12-iE-DAP also induces a reduction of the β-adrenergic-induced modulation of cell chronotropism.

Conclusion: These findings suggest that NOD-1-induced alteration of SAN electrical activity may contribute to the clinically observed changes in heart rate and the associated heart rate variability observed during septic conditions.
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On the biological rhythmicity of cardiac L-type Ca2+ channel expression

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Background: Diurnal variations in the ion channels that govern the excitation dynamics of cardiac cells may provide the crucial link between circadian clock and cardiac arrhythmias, a leading cause of cardiovascular death which are more likely to occur in the morning after waking. This has been mainly related to K+ channels, but little is known about the L-type Ca2+ channels, whose pore is formed by the Cav1.2 subunit and are the main mediators of Ca2+ influx of ventricular cardiomyocytes participating to cardiac electrical activity, contraction and gene expression.

Aim: To study the temporal dynamic of cardiac Cav1.2 expression.

Methods: Protein, mRNA expression and promoter activity of ventricles and isolated ventricular cardiomyocytes from wild type and transgenic mice expressing luciferase under the control of the cardiac Cav1.2 promoter (PCaLuc mice) were analyzed.

Results: Under normal light:dark cycle, we observed a circadian rhythm of Cav1.2 protein expression in ventricle, which peak at Zeitgeber time (ZT) 13.5. This is associated with a coordinated 18 h-oscillation of Cav1.2 mRNA level and its regulatory subunits with peak at ZT12, as well as the core molecular clock machinery and major repolarizing K+ channels. In vivo bioluminescence (BLI) revealed also a significant oscillation in the Cav1.2 promoter activity with a ~1.4-fold increase at ZT9 and 18. Remarkably, similar results were observed in vitro suggesting participation of endogenous cardiac clock. Surprisingly, over 3 month BLI monitoring of PCaLuc mice (recorded twice a week at ZT1) revealed a ~2-fold variations of the Cav1.2 cardiac promoter activity, with a ~16-day period.

Conclusion: Our findings show on top of circadian rhythm, an infradian regulation of cardiac Cav1.2 transcriptional control that might have an impact in cardiac physiopathology.
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Genotype-differences in the extent of mechano-induced electrical QT-changes in transgenic long-QT, wildtype, and transgenic short-QT syndrome rabbits

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Background: Electro-mechanical (EMC) and mechano-electrical coupling (MEC) are essential for normal cardiac function; alterations in these, however, can result in arrhythmia formation. In ‘electrical’ cardiac diseases, long-QT (LQTS) and short-QT syndrome (SQTS), mechanical function is altered via EMC; but to which extent MEC further impacts on the electrical phenotype is unclear.

Aim: In this study, we aimed to investigate how acute changes in mechanics may impact on electrical function (MEC) in these diseases.

Methods: To determine how acute changes in preload impact on QT-duration, adult rabbits of both sexes were given NaCl boluses IV and 12-lead ECGs were assessed in wildtype (WT), transgenic LQT2, SQ1T1 rabbits.

Results: At baseline, LQT2 (n=3) and SQ1T1 rabbits (n=11) demonstrated prolonged (p<0.0001) or shortened (p=0.012) QTc-duration compared to WT (n=12). Moreover, in LQT2, QT-dispersion - a marker for regional heterogeneity of repolarization - was also increased (QTMax-Min [ms], LQT2 25.8±10.6 vs. WT 15.1±5.2; p<0.05).

Increased preload acutely prolonged QTc-duration in all groups: in LQT2 [ms] 240.1±19.9 to 276.5±22.3 (p<0.01), in WT [ms] 174.5±15.8 to 202.8±17.9 (p<0.0001) and SQ1T1 158± 12.6 to 180.5± 10.3 (p<0.0001). The extent of MEC differed among genotypes: in LQT2 there was a trend towards more pronounced QTc-prolongation as in WT (ΔQTc [ms], LQT2 36.4±2.5 vs. WT 28.4±8.1; p=0.12), while it was reduced in SQ1T1 (ΔQTc [ms], SQ1T1 22.6± 8.8 vs. WT; p=0.11).

Of note, similar mechano-induced QT-changes were observed upon autonomic blockade, indicating that mechano-induced electrical changes are (mainly) driven by cardiac-intrinsic mechanisms and not by autonomic reflexes.

Conclusion: Acute changes in mechanical function result in electrical changes via MEC in LQT2, WT, and SQ1T1 rabbits. The extent of these changes, however, depends on the underlying QTc-duration, with the most pronounced QTc-prolongation in LQT2 rabbits and the least pronounced QTc-changes in SQ1T1. Mechano-induced QTc-changes might therefore additionally contribute to LQTS-related arrhythmogenesis.
Succinate and exposure to hypoxia-reoxygenation cause similar pro-arrhythmic modifications in left atrial electrophysiology

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Background: Exposure to hypoxia-reoxygenation greatly increases the risk of atrial arrhythmias. Hypoxia-reoxygenation is common in patients with sleep disordered breathing, ischemic heart disease and post-myocardial infarction. The mechanisms underpinning atrial electrical remodelling after hypoxia-reoxygenation are unknown, but could be dependent on elevated mitochondrial succinate metabolism.

Aim: Assess changes in left atrial cardiac electrophysiology caused by exposure to hypoxia-reoxygenation and succinate.

Methods: Hearts were isolated under terminal anaesthesia (4-5% isoflurane in O2, 3 L/min) from adult mice of either sex (hypoxia-reoxygenation studies, n=11; succinate studies, n=22). For optical mapping, Langendorff-perfused hearts were loaded with Di-4-ANEPPS (5 µM). Isolated intact left atria were pinned to the recording chamber, perfused with Krebs-Henseleit solution containing blebbistatin (35 µM), and exposed to acute hypoxia for 10 minutes (superfusate PO₂ approximately 76 mmHg), followed by reoxygenation for 20 minutes (PO₂ approximately 706 mmHg). In separate experiments, left atria were exposed to the cell permeable diethyl succinate (succinate, 5 mM) for 15 minutes. Peak sodium currents (INa) were measured from isolated left atrial cardiomyocytes in the presence and absence of 5 mM succinate using patch clamp.

Results: Acute hypoxia-reoxygenation caused significant left atrial conduction slowing, a reduction in optical action potential (OAP) amplitude, OAP duration prolongation and increased intra-atrial OAP heterogeneity. Exposure to succinate caused a similar decrease in conduction velocity and OAP amplitude as well as prolongation of OAP duration. Succinate also induced a rapid and partially reversible decrease (~30%) in peak INa, in addition to a 9 mV left-shift in the steady-state INa inactivation V50, and slowing of INa recovery.

Conclusions: Succinate and hypoxia-reoxygenation produce pro-arrhythmic alterations in left atrial electrophysiology, likely driven by succinate-mediated reduced peak INa and altered INa kinetics. These findings indicate that preserving INa may help protect against hypoxia-reoxygenation induced arrhythmias.

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Functional crosstalk between IP$_3$R and RyR Ca$^{2+}$ channels contributes to arrhythmic activity in failing human hearts

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Background,
Remodelling of Ca$^{2+}$ handling is central to the increased arrhythmic activity and diminished function of cardiomyocytes (CM) in heart failure (HF). Ca$^{2+}$ release via IP3R channels is reported to elicit spontaneous Ca$^{2+}$ release events and arrhythmic activity in rodent models of cardiac hypertrophy. Here we tested the hypothesis that altered IP3 signalling contributed to increased arrhythmic activity and altered function of failing human heart.

Material and Methods,
Human heart tissue and CM were from unsuitable donor or explanted failing hearts procured under a study protocol which conformed to the Helsinki declaration approved by the ethical committee of UZ Leuven (S58824). Ca$^{2+}$ dynamics and electrophysiological recordings were in acutely isolated CM. 50 µM IP3 was introduced via the pipette. Tissue recordings were in acutely isolated wedges perfused with isoproterenol, AngII with and without 2-APB. Localisation of IP3Rs and RyRs was by STED microscopy.

Results
Ca$^{2+}$ transients (CaT) were similar between NF and HF CM but were decreased in HF by IP3. IP3 increased RyR dependent Ca$^{2+}$ sparks and SR Ca$^{2+}$ leak in HF CM. In IP3 stimulated HF CM, Ca$^{2+}$ sparks were enriched at RyRs localised to electrically non-coupled dyads. STED microscopy showed increased IP3R colocalization with RyRs in HF CM supporting the potential for IP3R-RyR crosstalk. In HF CM, IP3 induced Ca$^{2+}$ release rescued delayed Ca$^{2+}$ release at non coupled sites during the CaT. IP3 augmented Ca$^{2+}$ waves and triggered activity in HF CM, which was mediated by NCX activity. In isoproterenol perfused tissue wedges, AngII increased arrhythmic activity in HF that was suppressed following IP3R inhibition with 2-APB.

Conclusions
Increased IP3R signalling to proximal RyR in HF sensitises them to Ca$^{2+}$ release, resulting in increased spontaneous Ca$^{2+}$ release activity that in turn acts via NCX to induce arrhythmias.

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STIM2 protein regulates Orai1-mediated store-operated Ca2+ entry in cardiomyocytes

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Background: Stromal interaction molecules, STIM1 and STIM2, are ER Ca2+ sensors that initiate store-operated Ca2+ entry (SOCE). Recently, it has been identified two STIM2 splice variants: STIM2.1 as SOCE inhibitor and STIM2.2 as SOCE activator. The role of STIM1-mediated SOCE has been highlighted in cardiac pathobiology but the one of STIM2.1 and STIM2.2 is unknown. Aim: To assess whether and how STIM2.1 and STIM2.2 regulate SOCE in neonatal rat ventricular cardiomyocytes (NRVMs). Methods: To analyze the function of STIM2.1 and STIM2.2 variants, the NRVMs were transfected with STIM2.2 or STIM2.1 in combination or not with dn-Orai1106A mutant or with STIM2 siRNAs. Results: The two variants were expressed in NRVMs with predominance for STIM2.2. At rest, native STIM2 and exogenous STIM2.1 or STIM2.2 immunostaining presented a reticular organization. Live cell confocal imaging showed that STIM2.1 and STIM2.2 were aggregated and translocated towards the sarcolemma following the SR Ca2+ store depletion. By co-immunoprecipitation, we found that STIM2, STIM1 and Orai1 interacted together. With Ca2+ imaging, we showed that silencing of endogenous STIM2 increased SOCE. In addition, we found that STIM2.2 overexpression significantly increased SOCE. The STIM2.2-enhanced SOCE is suppressed by functional Orai1 inhibition, either by co-expressing STIM2.2 with the dn-Orai1106A mutant or with the selective Orai1 inhibitor, JPIII. In addition, the JPIII-sensitive Isoc current was also higher in STIM2.2 overexpressing cells. By contrast, STIM2.1 overexpression suppressed Orai1-mediated SOCE and Isoc. Functionally, STIM2.2 overexpression resulted in elevated diastolic Ca2+ level and in decreased Ca2+ cycling. While STIM2.1 overexpression had no impact on the diastolic Ca2+ level and Ca2+ cycling, it strongly decreased the mitochondrial Ca2+ uptake. Conclusion: We demonstrated that exogenous STIM2.2, as activator of Orai1-mediated SOCE, regulated the diastolic Ca2+ level while STIM2.1 regulated the mitochondrial Ca2+. In endogenous system, STIM2.1 constitutes the predominant functional variant that negatively regulates SOCE in NRVMs.
Specific SERCA stimulation is more arrhythmogenic than β-adrenergic stimulation in cardiomyocytes harboring a CPVT mutation

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Background/Introduction: Mutations of the cardiac ryanodine receptor (RyR2) can predispose to life threatening catecholaminergic polymorphic ventricular tachycardias (CPVTs) during exercise or stress, however the underlying mechanisms are complex, most likely mutation-dependent and not yet fully understood.

Aim: Here we examined two potential sources of arrhythmogenic events namely: 1) phosphorylation of the mutated RyR2s and 2) stimulation of the SR Ca2+ pump (SERCA), which could increase SR Ca2+ loading.

Methods: Potentially arrhythmogenic Ca2+ signals, such as Ca2+ waves, were recorded and analyzed from WT and RyR2R420Q+/- mouse cardiomyocytes with confocal microscopy after field stimulation at 1Hz. Wave velocity from raw traces, as well as amplitude and decay time constant (τ) analyzed in de-skewed traces were comparable in both cell types. To obtain further insight into the role of the SERCA we selectively stimulated SERCA in permeabilized myocytes using Fab fragments of a PLB antibody (2D12).

Results: In RyR2R420Q+/- cardiomyocytes we found a higher occurrence and frequency of Ca2+ waves, particularly upon β-AR stimulation with isoproterenol. This was accompanied by a shorter latency to the first spontaneous wave. Surprisingly, this selective SERCA stimulation resulted in considerably higher wave frequencies than when mimicking β-AR stimulation with cAMP, particularly in RyR2R420Q+/- cardiomyocytes. Furthermore, we found that the SR Ca2+ level was significantly higher under selective SERCA stimulation with Fab. This may be a consequence of some protective SR Ca2+ unloading resulting from the SR Ca2+ leak via phosphorylated RyR2s in cAMP. Spark-to-spark recovery analysis suggested a remarkably higher Ca2+ release sensitivity in RyR2R420Q+/- cells, both in control and upon β-AR stimulation.

Conclusions: Together these findings suggest that the fine balance between SR Ca2+ loading via SERCA and the Ca2+ leak via mutated and phosphorylated RyR2s are important determinants for the overall cellular arrhythmogenicity prevailing in the RyR2R420Q+/- myocytes.
PLN-R14del mutation: discovering the pathophysiological role in a novel heterozygous mouse model of dilated cardiomyopathy

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The sarco/endoplasmic Ca\(^{2+}\) ATPase 2a (SERCA2a) and its inhibitor phospholamban (PLN) play a key role in cardiac excitation-contraction coupling. The heterozygous human PLN-R14del mutation is associated with an arrhythmogenic dilated cardiomyopathy (DCM). It has been previously shown that overexpression of PLN-R14del leads to a decrease in SERCA2a affinity for Ca\(^{2+}\), thus proposing that a “superinhibitory” effect of mutant PLN may account for the DCM phenotype. This theory is not consistently supported by recent experimental evidence, suggesting alternative pathogenetic mechanisms, including defects in energy metabolism.

The aim is to investigate the pathophysiological mechanisms of PLN-R14del DCM by exploiting a heterozygous (PLN-R14Δ+/+) transgenic model (Mut) that recapitulates the clinical human phenotype. Intracellular Ca\(^{2+}\) dynamics have been optically measured (Fluo-4AM) at 36° during field stimulation in cardiomyocytes (CMs) isolated from 8-12 weeks old Mut and WT mice. At this age the mutation has no overt cardiac phenotype, thus ruling out remodeling effects. To compare mutation effect with pharmacological SERCA2a modulation, CMs were perfused with a pure SERCA2a activator (PST-3093). To test the involvement of metabolic alterations, oxidative phosphorylation, glycolysis, and ROS production have been investigated.

In Mut CMs (vs WT CMs) CaT amplitude was increased and CaT decay time constant (\(t_{\text{decay}}\)) was reduced. In WT CMs, PST-3093 increased \(d\text{Ca}/dt_{\text{MAX}}\) (but not CaT amplitude) and decreased \(t_{\text{decay}}\) to approach the value found in Mut cells. In Mut CMs, PST-3093 had no effect on CaT parameters. In Mut CMs O2 consumption and glycolytic acidification were reduced and ROS production was increased. These results indicate enhancement of SR Ca\(^{2+}\) uptake rate in Mut CMs, which is compatible with diminished PLN inhibitory function (i.e., opposite to SERCA2a superinhibition). Future work will be directed to establish if the abnormality in energy metabolism may be related to altered Ca\(^{2+}\) dynamics, or be an independent mechanism for PLN-R14del DCM.
Drug-induced arrhythmias in zebrafish larvae: correlated changes in calcium, contraction, and hemodynamics

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Background: Dysregulation of Ca2+ fluxes is at the core of inherited and acquired arrhythmias. Zebrafish is an attractive vertebrate model to study cardiotoxicity and heart diseases. We reported that the transgenic zebrafish line Tg(myl7:Twitch-4) expressing the ratiometric Ca2+ indicator Twitch-4 in the heart, allows imaging Ca2+ transients (CaT) simultaneously with heart contractions, thus maintaining the physiological mechano-electrical feedback.

Aim: To characterize disturbances in Ca2+ levels, contractility, and hemodynamics induced by arrhythmogenic drugs in zebrafish larvae.

Methods: We used Tg(myl7:Twitch-4) larvae and a custom software to acquire and analyze fluorescence emission ratio images of the heart. We determined the effects of the IKr inhibitor dofetilide and the ICaT inhibitor ML 218 on the systolic and diastolic Ca2+, and the CaT amplitude, in the atrium and ventricle. As the ventricular diameter was assessed in the same fluorescence images, we correlated Ca2+ with contraction. The systolic and diastolic areas were used to estimate hemodynamic parameters.

Results: Dofetilide caused 2:1 atrioventricular (AV) block with an increase in the ventricular CaT amplitude. The end-diastolic volume, ejection fraction, and stroke volume increased, but the cardiac output decreased due to the low ventricular heart rate. Blocking of T-type Ca2+ channels induced bradycardia and alterations in AV conduction with dose-dependent severity. Thus, 0.3 mM ML 218 increased the delay between atrial and ventricular Ca2+ rise (AV delay) and, in 7 out of 30 larvae, progressively prolonged the AV delay in successive beats until a ventricular contraction failed, a phenomenon reminiscent of a Mobitz type I second degree AV block in humans. In addition, 1 mM ML 218 triggered a 2:1 AV block.

Conclusion: The zebrafish line Tg(myl7:Twitch-4) can be used to investigate pathophysiological mechanisms and arrhythmias in great detail, allowing to correlate Ca2+ changes, contraction, and hemodynamics.

Striatin knock out induces a gain of function of INa current in mESC-derived cardiomyocytes.

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Introduction. Striatin (STRN) is a scaffolding protein that in cardiomyocytes is expressed in caveolae, t-tubules, and intercalated discs. Patients with dilated cardiomyopathy show a loss of STRN from the intercalated discs.

Aim. To understand the role of STRN in cardiomyocytes, by comparing functional alterations in the properties of cardiomyocytes derived from a STRN KO mouse embryonic stem cell (mESC) to its parental (WT) lines.

Methods. mESC were differentiated into embryoid bodies by the hanging drop method. Cardiomyocytes were dissociated at d10-12 of differentiation. We used patch-clamp, motion video tracking and immunofluorescence analysis to investigate the effect of STRN-KO.

Results and discussion. STRN-KO showed a higher action potential (AP) rate (5.2±1.9 Hz) than WT cells (3.9±1.2 Hz) and a higher dV/dt max. The other AP parameters did not vary. STRN-KO cells showed a significantly larger INa current than WT (2.72 nS/pF and 1.38 nS/pF, respectively) with unchanged kinetics. ICaL, If and IKr currents were similar. Motion video tracking analysis and immunofluorescence staining highlighted that STRN-KO cells exhibited altered contraction pattern and dysregulated sarcomere organization. Since in HEK cells downregulation of STRN was reported to destabilize microtubules and increase INa, incubation of mESC cardiomyocytes with Taxol, a microtubule stabilizer, induced in STRN-KO cells a functional rescue with a reduction of INa conductance (from 2.68 nS/pF to 0.81 nS/pF). Immunofluorescence analysis confirmed the recovery of microtubules and sarcomeres structure in Taxol-treated STRN-KO cells.

Conclusion. Loss of STRN alters cardiomyocytes electrical profile and affect cardiac functionality by a disarrangement of multi-protein complexes leading to impairment of microtubules structure and trafficking of sodium channels.
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Transition between L-type calcium channel isoforms prolongs action potential duration after acute myocardial infarction/reperfusion injury

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Myocardial ischemia bears a high risk of sudden cardiac death due to malignant ventricular arrhythmias. Complex changes in ionic currents favor the development of life-threatening cardiac arrhythmias. A large body of research has attempted to unravel the underlying mechanisms of cardiac arrhythmias in acute ischemia, but further elucidation is needed after ischemia-reperfusion (I/R) injury. We have developed an optical mapping-plus approach, visualizing spatio-temporal signal propagation through adult mouse ventricular heart slices and employed TTC staining and in situ hybridization to directly relate electrical information with structural alterations and ion channel regulations. Mathematical modeling was used to bridge the experimental observations and provide a causal link between them. Within the epicardial vicinity of I/R injury, early repolarization of the action potential was delayed and AP alternans evolved, both of which were accompanied by local de novo expression of the L-type calcium channel (LTCC) CaV1.3 gene in cardiomyocytes. Remarkably, our transmural AP analysis reveals an inversion in endo-epi cardial AP duration (APD) gradients. Mathematical modeling of the experimentally observed transition from CaV1.2 to CaV1.3 channels predicted a similar prolongation of AP duration (APD), eventually inducing arrhythmogenic early afterdepolarizations (EAD) and even repolarization failure. In addition, pharmacological mimicry of LTCC isoform remodeling by a LTCC agonist confirmed early APD prolongation with the emergence of EAD and repolarization failure in control heart slices. By pharmacological LTCC inhibition, APD could be reduced, as was the spatial dispersion of AP repolarization. In summary, we utilize a multi-modal approach to study the molecular mechanisms of electrical disturbances in cardiopathological conditions. Employing this approach after I/R injury, we identified adult re-expression of CaV1.3 to yield early repolarization dysfunction of the cardiac AP, rendering LTCC remodeling a putative therapeutic target in cardiac ischemia.
Cardial tattoos lines: A new method to treat arrhythmias

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Introduction: Atrial cardiomyopathy is a highly complex and heterogeneous disease that occurs in the context of various clinical backgrounds, which explains the heterogenous success of established atrial ablation strategies such as pulmonary vein isolation. Here we developed a new interventional method for the treatment of atrial arrhythmias using a tattooing procedure of silver nanowires in the atria to create circular lines of increased conductivity to prevent irregular electrical propagation.

Purpose: In a translational study, we tested a newly developed interventional method to terminate arrhythmias by injecting lines of conductive material to increase tissue conductivity.

Methods: The developed tattoo method was tested in vivo using an established large animal model of the domestic pig. Pigs (n = 10) with atrial arrhythmias were either treated with atrial tattoo lines using an injection procedure of silver nanowires as lines in the atrium or got a sham treatment with saline injections. Over 21 days, atrial arrhythmias was induced and monitored by an intracardiac dual-chamber device with a biofeedback induction algorithm. Initially and before final termination, conventional electrophysiological investigation and atrial 3D mapping, echocardiography and epicardial multi electrode array (MEA) measurements were performed. Following the 21 days observation period, the heart was extracted and freshly isolated atrial cardiomyocytes were subjected to cellular electrophysiological, molecular and histochemical characterization. Electrophysiological data was used for in silico modelling of arrhythmia termination and wave propagation. The study protocol was approved by the animal ethics committee.

Results: The burden of atrial arrhythmias was significantly reduced (< 10 %) in the group treated with the new atrial tattoo line method compared to sham treated pigs. The bi-atrial diameters, quantified by echocardiography, were significantly smaller in the treatment group. Atrial refractory period was significantly shorter in the sham treated pigs. A significant increase of connexins was observed in the injection area of silver nanowires in the myocardium. Measurements with MEA demonstrated increased conduction velocity by a factor of 1.5–2.0 in the areas of silver nanowire injections. Additionally, in silico modelling showed the termination of atrial arrhythmias via the created conduction lines.

Conclusion: The newly developed interventional method of the creation of atrial lines with increased tissue conductivity using conductive material could successfully terminate atrial arrhythmias in pigs. This new
myocardial tattooing technique may be a promising treatment strategy for patients with complex atrial cardiomyopathies to terminate atrial arrhythmias.
Severe remodeling processes may occur in the heart due to both genetic and non-genetic diseases. Structural remodeling, such as collagen deposition (fibrosis) and cellular misalignment, can affect electrical conduction at different orders of magnitude and, eventually, lead to arrhythmias. In this scenario, arrhythmogenic cardiomyopathy (ACM) is an inherited heart disease that involves ventricular dysfunction, arrhythmias, and localized replacement of contractile fibers with fibrofatty scar tissue. Unfortunately, nowadays, predicting the impact of fine structural alterations on the electrical dysfunction in entire organs is challenging, due to the inefficacy of standard imaging methods in performing high-resolution three-dimensional reconstructions in massive tissues.

In this work, we developed a new full-optical correlative approach to quantify and integrate the electrical dysfunctions with three-dimensional structural reconstructions of entire hearts, both in controls and in a mouse model of ACM. We combined optical mapping of the action potential propagation (APP) with advances in tissue clearing and light-sheet microscopy techniques. First, we employed an optical platform to map and analyze the APP in Langendorff-perfused hearts. Then, we optimized the SHIELD procedure for the clearing of cardiac tissue, thus converting the previously electrically characterized samples into well-preserved and fully-transparent specimens. A high-throughput light-sheet microscope has been developed following the mesoSPIM project: the conceived microscope allows the reconstruction of the whole mouse heart with a micrometric resolution allowing fine quantification of myocytes alignment and fibrosis deposition across the organ. Finally, we developed a software pipeline that employs high-resolution 3D images to analyze and co-register APP maps with the 3D anatomy, contractile fibers disarray, and fibrosis deposition on each heart.

We believe that this promising methodological framework will allow clarifying the involvement of fine structural alterations in the electrical dysfunctions, thus enabling a unified investigation of the structural causes that lead to electrical and mechanical alterations after the tissue remodeling.
Three-dimensional virtual histology of cardiovascular diseases

-X-ray phase contrast tomography as a tool for (pre-)clinical research

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Introduction
Myocardial disorders alter the intricate molecular and cellular three-dimensional (3d) architecture of the complex, dense and co-dependent cellular network within the heart with a crucial impact on signal conduction and contractility. Up to now, physiologic and disease-related tissue alterations of the 3d structure are mainly investigated by optical microscopy or electron microscopy of slices. However, sectioning of tissue makes it very difficult to access the multi-scale 3d structure from sarcomere to the entire organ.

Aim
We aim to investigate unlabelled cardiac tissue at sub-cellular resolution in three dimensions and describe pathological alterations using structural biomarkers.

Methods
We explore X-ray phase-contrast tomography to depict the weakly absorbing, soft tissue of the heart. The samples were scanned at a laboratory microfocus X-ray source based on a liquid metal jet anode and with higher data quality and resolution at high-brilliance synchrotron X-ray sources.

Based on the reconstructed 3d heart structure, we determined multiple structural biomarkers such as myocardial mass, stroke volume, local wall thickness local myocyte orientation and degree of alignment of individual muscle strands. Furthermore, we rebuild the vasculature network down to the capillary level. We use this approach to describe structural changes of the 3d cardiac architecture in different mouse models such as myocardial infarction, hypertrophy and Duchenne muscle disease.

In addition, we determine 3d pathological alterations of the cardiac tissue structure caused by Covid-19, coxachie myocarditis and H1N1 influenza.

Results
The laboratory setup enabled an isotropic resolution <10 µm and the synchrotron investigations allowed a voxel size of 650 nm. Thereby, we could identify significant structural alterations between the different pathologies. For the murine models, differences in myocardial mass, stroke volume wall thickness were observed. The cardiac tissue of patients who succumbed from Covid-19 showed a higher variance in structural alignment and a higher degree of vasculature splitting, which is probably caused by intussusceptive angiogenesis. In order to support this hypothesis, we acquired regions-of-interest tomograms with an effective voxel size of less than 200 nm and demonstrated the presence of intralaminar pillars in the cardiac capillary system of Covid-19 samples.
Conclusion
The multi-scale X-ray imaging approach allows analysis of the heart structure from the scale of entire intact murine hearts to subcellular features displaying the vasculature at the capillary level, the arrangement of cardiac muscle cells and to describe pathological alterations in three dimensions. Future progress of this method is directed to cell type specific 3d imaging, the derivation of functional parameters and the correlation to functional analysis, especially the cardiac electrophysiology.
Ca2+ handling remodelling in a porcine model of right ventricular dysfunction

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Background: Increased afterload is the main pathophysiologic mechanism for right ventricular failure (RVF) which is the most important determinant of survival in patients with pulmonary hypertension (PH). The cellular and molecular processes involved in the RV remodelling, including Ca2+ handling as instrumental actor in the heart, remain incompletely understood. Aim: We gain insights into the cellular Ca2+ signaling in a large animal model of RV dysfunction, the chronic thrombo-embolic pulmonary hypertension (CTEPH) piglet model, in order to develop specific therapy targeting RVF. Methods: CTEPH piglet model consisted of a primary left pulmonary artery ligation followed by weekly embolization in the right lower lobe leading to PH and progressive RV hypertrophy and failure. Results: After 6 weeks, the piglet model replicated all the features of human CTEPH: increased pulmonary vascular resistance and mean pulmonary arterial pressure (mPAP) associated with RV dysfunction as assessed by the reduced tricuspid annular plane systolic excursion (TAPSE) and TAPSE/mPAP ratio. RV histological analyses revealed hypertrophied cardiomyocytes and aberrant fibrosis. At cellular level, hypertrophied RV myocytes from CTEPH piglets presented longer action potential duration, lower and slower [Ca2+]i transients, decreased sarcoplasmic reticulum (SR) Ca2+ content and cell shortening compared to myocytes from sham piglets. This was related to reduced Sarco/Endoplasmic Reticulum Ca2+-ATPase isof orm 2a (SERCA2a) protein expression. We also found that Ca2+ sparks are larger and longer in RV myocytes from CTEPH piglets, whereas their frequencies and amplitude were unchanged compared to sham. This was associated with a profound disorganization of T tubules, as well as ryanodine receptors clustering. Conclusion: Our data highlight a cellular Ca2+ cycling remodelling that participates to the pathogenesis of RVF in CTEPH piglets.
Active force generation is involved in the response to stretch and in the complexity of spatiotemporal beating variability patterns in spontaneously active murine cardiomyocyte cultures

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Background: Monolayer cultures of cardiac cells exhibit spontaneous electrical activity and represent an in vitro model of a natural cardiac pacemaker. Upon electrical excitation and contraction, tissue deformation and mechanical forces feedback on the electrical properties of the cardiomyocytes (mechano-electrical feedback). In such preparations, it has been shown that beat rate varies continuously, whereby beat rate variability (BRV) recapitulates the power-law behavior of heart rate variability in vivo. However, it is not known how mechano-electrical feedback affects BRV in such preparations.

Aim: Our aim was to assess the effects of the myosin inhibitor blebbistatin and the stretch-activated channel (SAC) blocker streptomycin on BRV and on the response of murine ventricular myocyte cultures to mechanical deformation.

Methods: The preparations were grown on custom stretchable microelectrode arrays. Spontaneous electrical activity was recorded either without deformation or upon predefined stretch protocols (5% uniaxial and 2% biaxial strain, applied repeatedly for 1 min every 3 min).

Results: In experiments without stretch, spontaneous activity originated from the edge of the preparations and the site of origin switched frequently. Blebbistatin did not change the mean beat rate, but it decreased the spatial complexity of beating variability. In contrast, streptomycin did not exert any manifest effects on spontaneous activity. In experiments with stretch, beat rate increased upon both stretch and release. However, the acceleration of beat rate was transient. Blebbistatin attenuated the response to stretch, while this response was not affected by streptomycin.

Conclusions: Our results thus indicate that any change of shape transiently increases beat rate. Importantly, active force generation, rather than SACs, appears to be involved in this response. Moreover, active force generation is involved in determining the complexity of spatiotemporal patterns of BRV. Our study thus contributes to understand how mechano-electric feedback may influence heart rate variability.
Manipulation of cardiac electrical dynamics using sub-threshold optogenetic illumination: exploring the role of cardiac alternans in terminating rapid rhythms

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Cardiac action potential (AP) shape and propagation are regulated by several key dynamic factors such as ion channel recovery and intracellular Ca2+-cycling. Experimental methods for manipulating AP electrical dynamics commonly use ion channel inhibitors that lack spatial and temporal specificity. In this work, we propose an approach based on optogenetics to manipulate cardiac electrical activity employing a light-modulated depolarizing current with intensities that are too low to elicit APs (sub-threshold illumination) but are sufficient to fine-tune AP electrical dynamics. We investigated the effects of sub-threshold illumination in isolated cardiomyocytes and whole hearts by using transgenic mice constitutively expressing a light-gated ion channel (channelrhodopsin-2, ChR2). We find that ChR2-mediated depolarizing current prolongs APs and reduces conduction velocity (CV) in a space-selective and reversible manner. Sub-threshold manipulation also affects the dynamics of cardiac electrical activity, increasing the magnitude of cardiac alternans. We used an optical system that uses real-time feedback control to generate re-entrant circuits with user-defined cycle lengths to explored the role of cardiac alternans in spontaneous termination of ventricular tachycardias (VTs). We demonstrate that VT stability significantly decreases during sub-threshold illumination primarily due to an increase in the amplitude of electrical oscillations, which implies that cardiac alternans may be beneficial in the context of self-termination of VT.
Contribution of ionic remodelling to arrhythmic risk during ischemia in hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease and a leading cause of sudden cardiac death (SCD) in the young. Existing SCD risk stratification is limited because SCD causal mechanisms are incompletely understood, leading to suboptimal implantable defibrillator allocation. Myocardial ischemia on perfusion scans is very common in HCM and identifies a subgroup of HCM patients at higher risk of SCD, but the assessment of ischemia is not yet fully incorporated into clinical guidelines for HCM.

The present study aimed to investigate the electrophysiological mechanisms by which myocardial ischemia could cause lethal arrhythmias in HCM, which is expected to improve SCD risk stratification or yield novel therapies for these high-risk patients.

Using biophysically detailed computational models of cardiac electrophysiology, phase 1A acute myocardial ischemia was simulated in human HCM ventricles, which are affected by ionic remodelling (characterised by significant action potential prolongation) in regions of hypertrophy. The hypothesis was that hypertrophic ionic remodelling would increase arrhythmic risk through enhanced refractoriness gradients, which are the precursor to ischemia-induced re-entries. Arrhythmic risk was measured using S1-S2 protocols for the cases in which (i) myocardial ischemia coincides with the ionic remodelling, and (ii) myocardial ischemia occurs with control electrophysiology.

Simulated ventricles affected by HCM ionic remodelling had significantly greater arrhythmic risk at a milder extent of ischemia than in cases with purely control electrophysiology. This was because prolonged refractoriness was already present in non-ischemic regions affected by ionic remodelling, whereas in controls a threshold extracellular K+ accumulation was required. Instead, conduction impairment and stark reductions in refractory period promoted increasingly sustained re-entries in HCM. This may be relevant in the context of exercise management in HCM, because even though the extent of exercise perfusion defects and ischemia may be self-limiting through dyspnea and angina, a greater arrhythmic risk is possible.
A Case Study: High Resolution Optical Mapping Of A 19 Year Old Male Victim Of Sudden Cardiac Death During A Marathon

50000 athletes suffer sudden cardiac death every year world-wide. In young adults, the origins of sudden cardiac death often go unexplained due to the absence of detectable structural or electrical disease. The His-Purkinje component of the cardiac conduction system is essential for normal heart function, however, its role in lethal electrical disruption remains unclear.

The objective is to investigate the electrophysiological properties of the Purkinje network in a 19 year old male after suffering sudden cardiac arrest during a marathon event.

The perfused left ventricle was imaged using high resolution optical mapping of epicardial and endocardial surfaces using a voltage-sensitive dye, Di-4-ANEPPS. Activation time (AT), repolarization time and action potential duration (APD) were assessed during paced activity through the His bundle and endocardial and epicardial surfaces. Conduction system activation origins were identified as distant endocardial origins during His bundle pacing. Short-coupled pacing was used to determine the effective refractory period (ERP). The influence of isoprenaline (ISO) at 100nM, 500nM and 1µM was investigated to mimic physiological stress conditions.

No changes in total AT are observed during myocardial pacing cycle lengths of 667ms in control (63ms) and under ISO (64ms). In contrast, His-ventricle delay during conduction system pacing was shortened from 61ms in control to 42ms, 44ms and 16ms under 100nM, 500nM and 1µM of ISO respectively. Under His pacing, local APD gradients are observed near Purkinje-muscle junctions (3ms/mm, 3 ms/mm, 40ms/mm and 41ms/mm, respectively). ERP under His pacing was 55ms shorter than endocardial pacing. 82% of the ectopy origins observed have less than 4mm distance from the PMJs. Repeated focal activations were observed during VF and 76% of them had less than 4mm distance from PMJs.

In conclusion, conduction system abnormalities likely played a critical role in arrhythmogenicity and maintenance of VF in this patient during sudden cardiac death.
CaMKIIδ-oxidation is not a critical factor for the development of early-reperfusion arrhythmias in Langendorff-perfused hearts

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Background: Ventricular arrhythmias are a common complication following an ischemic event, particularly during the early reperfusion phase. Indirect evidence suggests reactive oxygen species (ROS) contribute to the development of these arrhythmias. One proposed mechanism is ROS-dependent activation of the Ca2+/calmodulin-dependent protein kinase II δ (CaMKIIδ) through oxidation of two methionines at positions 281/282 within the regulatory domain. However, the importance of oxidized-CaMKIIδ (Ox-CaMKIIδ) in early reperfusion ventricular arrhythmias remains unresolved.

Methods: We evaluated the propensity for early reperfusion arrhythmias in Langendorff-perfused hearts from mice with CaMKIIδ resistant to oxidation of methionines 281 and 282 (homozygous CaMKIIδ M281/282V mice) and wild type control mice (WT). The effect of beta-adrenoceptor stimulation, indirect inhibition of CaMKII via calmodulin antagonism, and antioxidant therapy, was evaluated by the addition of isoprenaline, KN93 and N-acetylcysteine (NAC), respectively. Diastolic Ca2+ release events were recorded by confocal line scan imaging of isolated cardiomyocytes exposed to simulated ischemia-reperfusion, in the presence or absence of isoprenaline and NAC. Effects on phosphorylation of Ca2+ handling proteins were quantified by immunoblotting.

Results: KN93 and NAC reduced the incidence of ventricular arrhythmias when tested in WT hearts during beta-adrenoceptor stimulation. NAC treated WT cardiomyocytes had a lower spark frequency during reperfusion, compared to untreated controls. Langendorff-perfused hearts from CaMKIIδ M281/282V mice showed no significant difference in ventricular arrhythmia prevalence compared to WT during early reperfusion, also during beta-adrenoceptor stimulation. Immunoblotting revealed no significant differences in the abundance or phosphorylation levels of Ca2+-handling proteins in Langendorff-hearts. Ca2+ imaging experiments showed no significant differences between CaMKIIδ M281/282V and WT cardiomyocytes for spontaneous transients, Ca2+ waves or Ca2+ sparks.

Conclusions: Genetic ablation of CaMKIIδ oxidation at methionines 281 and 282 did not protect against early reperfusion arrhythmias in Langendorff-hearts, indicating that oxidation at these sites is not a critical factor in the development of such arrhythmias.
Cardiac Alternans Instigate Tachyarrhythmias in Chronic Myocardial Infarction Ovine Hearts

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**Background:** Chronic myocardial infarction (MI) is a known substrate for life threatening ventricular tachyarrhythmias (VT/VF). T-wave alternans (TWA) has been associated with an increased propensity to VT/VF.

**Aim:** Investigate the utility of cardiac alternans as a predictor of VT/VF in a chronic MI ovine model using in-vivo telemetry and high resolution ex-vivo optical mapping.

**Methods:** A novel chronic MI sheep model was developed by deployment of a coil device in a target coronary artery. 24-hour telemetry was performed in an ambulatory animal for 6 weeks post occlusion to monitor the progression of microvolt TWA with chronic MI. At 6 weeks, ex-vivo optical mapping experiments were performed on the endocardium of the left ventricle (LV) to assess the spatiotemporal evolution of alternans in sham (n=5) and chronic MI hearts (n=8). Langendorff perfused wedge preparations were paced at increasing rates (1Hz-5Hz) until loss of capture or VT/VF induction. 2D action potential duration (APD) and amplitude (APA) maps were analyzed to gauge the effect of MI on the LV.

**Results:** Chronic MI led to significant electrophysiological changes in the cardiac substrate. Significant increase in TWA was observed post occlusion and a steady rise in alternans was seen with progression of MI. Chronic MI hearts demonstrated significant presence of localized APA alternans compared to sham hearts. Onset of APA and APD alternans was observed at mean frequencies of 2.2±0.5Hz and 3.3±0.1Hz, respectively, and preceded VT/VF episodes. Conduction in scar tissue was irregular and out of phase with healthy myocardium prior to onset of VT/VF. APA alternans were more prominent and spatially evolved with increase in pacing frequency, with mean area of the LV covered by APA alternans increasing from 14.15±6.5% at 1Hz to 32.97±7.3% at 5Hz.

**Conclusion:** Cardiac alternans underlie arrhythmogenesis in chronic MI hearts and can serve as a biomarker of disease progression.
Dual Dysfunction of Kir2.1 Underlies Cardiac Arrhythmias in a Mouse Model of Andersen-Tawil Syndrome Type 1

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**Background:** Andersen-Tawil syndrome type 1 (ATS1), caused by trafficking deficient mutations in the gene KCNJ2 coding the inward rectifier K+ channel Kir2.1, is associated with life-threatening arrhythmias, which in some patients resemble catecholaminergic polymorphic ventricular tachycardia (CPVT), but the mechanisms are poorly understood. We tested the hypothesis that dysfunction of two different populations of mutant Kir2.1 channels, one at the sarcolemma, and the other at the sarcoplasmic reticulum (SR) membrane, directly alters conduction and intracellular calcium dynamics, respectively, to promote the ATS1 phenotype and CPVT-type arrhythmias.

**Material and Methods:** We generated a new mouse model of ATS1 by a single i.v. injection of cardiac specific adeno-associated viral (AAV) transduction with the mutant Kir2.1Δ314-315 protein. We characterized and investigated the arrhythmogenic mechanisms of the mouse model using in-vivo, cellular, structural and functional analyses of the model were carried out by electrocardiogram (ECG), magnetic resonance imaging (MRI), intracardiac stimulation, patch-clamping, membrane fractionation, western blot, immunolocalization and live calcium imaging.

**Results:** The mouse model recapitulated the ATS1 phenotype without changes in ventricular function. On ECG, Kir2.1Δ314-315 mice had prolonged PR, QRS and QT intervals and occasional U waves. They showed significantly slower conduction velocities than wildtype mice in response to flecainide, additional QT prolongation in response to isoproterenol, and increased vulnerability to cardiac fibrillation. Cardiomyocytes from Kir2.1Δ314-315 mice had significantly reduced inward rectifier K+ and Na+ inward currents, depolarized resting membrane potential and prolonged action potential duration. Immunolocalization in wildtype cardiomyocytes and skeletal muscle cells revealed a novel SR microdomain of functional Kir2.1 channels contributing to intracellular Ca²⁺ homeostasis. Kir2.1Δ314-315 cardiomyocytes showed defects in SR Kir2.1 localization and function, which contributed to abnormal spontaneous Ca²⁺ release events.

**Conclusions:** Cardiac-specific AAV transduction with Kir2.1Δ314-315 in mice recapitulates the ATS1 phenotype by disrupting localization and function of Kir2.1 channels at the SR, and the Kir2.1-NaV1.5 channelosome at the sarcolemma. These results reveal a novel dual mechanism of arrhythmogenesis in ATS1 involving defects in Kir2.1 channel trafficking and function at two different microdomains. They also provide the first demonstration at the molecular level of the mechanism underlying the overlap between ATS1 and CPVT associated with defects in intracellular calcium homeostasis.
Altered biophysical properties of atrial sodium channels increase flecainide effectiveness

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**Background:** Sodium channel blockade is a common treatment for atrial fibrillation. A feared side effect of inhibitors of the cardiac voltage-gated sodium current (INa), such as flecainide, is ventricular arrhythmia. We investigated the biophysical reasons for the relative safety of sodium blockers.

**Materials and Methods:** Whole cell patch clamping was performed to measure INa and action potentials (APs). Optical mapping of the LA and LV was performed in the intact mouse heart using voltage dye Di-4-ANEPPS. LA and LV INa data were used to model changes in AP morphology. Expression of NaV1.5, NaVβ2, and NaVβ4 was measured by Western blotting and SCN5A, SCN4B, and SCN2B by RNAseq analysis in matched mouse and non-failing human LA and LV.

**Results:** At physiological holding potentials (-75mV), LA peak INa current density was reduced compared to LV. Western blotting data demonstrate that this is not due to reduced atrial NaV1.5/SCN5A expression in either mouse or human. AP recordings revealed LA cells exhibit reduced upstroke velocity (238.6±27.7 vs 304.2±27.9 mV/ms, p=0.0018) compared to the LV (n=23-40/5 cells/mice). Modelling studies confirmed these findings. At all holding potentials, 1µM flecainide inhibited INa to a larger extent in the LA compared to LV cells. Furthermore, flecainide significantly decreased AP upstroke velocity more in the LA (47.9±18.8% vs LV cells (18.6±9.8%, p=0.04, n=4 mice). Optical mapping demonstrated reduced conduction velocity in the LA (24.4±2.7 cm/s) compared to LV (36.1±6.2 cm/s, p=0.03, n=5). Flecainide significantly decreased conduction velocity in the LA (-40.4±7.2%, p<0.05 vs time control), but not LV.

**Conclusions** Significant differences exist in the biophysical properties of sodium channels in the LA and LV, and their response to flecainide. The reduced effectiveness of flecainide in the LV can explain the relative safety of sodium channel blocker therapy.

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Localized conduction abnormalities due to a SCN5A variant associated with Brugada Syndrome: a human heart ex vivo study

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Background: Brugada syndrome (BrS) is a significant cause of arrhythmic sudden cardiac death (SCD) in the young, but the underlying mechanisms, including the genotype-phenotype relationship, remain poorly understood.

Aim: To complete this knowledge gap by providing detailed ex vivo functional studies in human hearts from BrS patients.

Methods: A human heart was obtained through our organ donation program from a 15-year-old male subject (normal ECG and echocardiography) who suffered SCD. Post-mortem gene sequencing was performed on NexSeq500 using the Sophia Genetics technology. Ex vivo optical mapping of the right ventricle (RV) was carried out and micro-structure was assessed by histology and magnetic resonance imaging (MRI 9.4T). Cardiac conduction related-proteins expression levels were studied using immunoblotting. Results were compared to human hearts from donors with no cardiac disease. Clinical examinations were performed on first-degree relatives (12-lead ECG, ajmaline challenge, genotyping).

Results: The donor carried a SCN5A mutation (p.D356N) previously associated with BrS in databases. His mother carries the same mutation and had a positive ajmaline test, supporting a BrS-related SCD diagnosis for the donor. A variant of uncertain significance in NKX2-5 was also found in the donor and his father, who displays a normal phenotype. Optical mapping highlighted a localized epicardial region of impaired conduction near the RV outflow tract in the absence of repolarization alterations, consistent with previous reports of the SCN5A variant associated with a loss of function of NaV1.5. Increasing pacing frequency resulted in the enlargement of the area with impaired conduction and led to conduction blocks and figure-of-8 patterns. The microstructure was normal in these regions. Immunoblotting suggested decreased expressions of desmoglein-2, N-cadherin, Connexin-43 and NaV1.5.

Conclusion: In early-stage BrS, mutations in the cardiac sodium channel can cause localized functional conduction disorders, which may be responsible for SCD even in the absence of structural defects.
Imaging Ca2+ in the beating heart with the ratiometric biosensor Twitch-1 in zebrafish larvae

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**Background:** Ca2+ has a vital function in cardiac contraction and alterations in its homeostasis lead to diseases like heart failure and arrhythmia. Zebrafish has become a useful model for cardiac physiology due to its electrophysiological properties similar to those of humans, early development of the heart and transparency of the larvae. Single-wavelength genetically encoded calcium indicators (GECI) are widely used to measure Ca2+ in vivo in zebrafish larvae. However, to avoid motion artefacts, they require stopping the heart with an antisense morpholino against tntt2a (MO-tntt2) or the myosin inhibitor para-amino blebbistatin (PAB).

**Twitch-1**, a GECI based on fluorescent resonance energy transfer (FRET), is a ratiometric indicator: it has two emissions that can be separated and ratioed to cancel out motion artefacts in imaging experiments. Therefore, there is no need to stop the heart.

**AIM:** the purpose of this research is to measure Ca2+ in moving hearts of zebrafish larvae in physiological and pathological conditions.

**Methods:** We generated a transgenic line, Tg(myl7:Twitch1), expressing Twitch-1 in the heart under the control of the myl7 promoter. Ca2+ transients were imaged in vivo in the beating heart of 3 days post-fertilization larvae.

**Results:** Our data proved that heart beating did not affect the measurements by comparing with a control line, Tg(myl7:ECFP-16aa-EYFP), which does not respond to Ca2+. Furthermore, Ca2+ levels were correlated with ventricular shortening. Interestingly, Ca2+ levels and heart rate changed in Tg(myl7:Twitch1) larvae treated with MO-tntt2 or PAB compared to control larvae. Finally, the biosensor reported changes in Ca2+ transients during an atrioventricular block induced by the treatment with astemizole, an antihistaminic drug associated with arrhythmic events in humans.

**Conclusions:** the Tg(myl7:Twitch1) line is a novel tool to study cardiac Ca2+ levels in the beating hearts of zebrafish. This line could also be used in drug screening and to study pathophysiological mechanisms in genetic models.
Sex differences and sex hormone effects on atrial electrical features

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Background: Pronounced sex differences are known in the incidence and recurrence rates of atrial fibrillation (AF). In general, men carry a higher risk for AF - particularly at younger age - during which women are protected.

Aim: We aimed to investigate atrial electrophysiological properties that may underlie sex differences in AF development in the younger population.

Methods: We assessed sex differences in P-wave morphology in 12-lead ECG in healthy volunteers and wildtype (WT) rabbits. We investigated sex differences in atrial action potential (AP) in WT rabbits using sharp electrode technique. Using the patch-clamp technique, we evaluated sex differences and acute sex hormones effect on IK1 at 37°C; to evaluate the chronic effect of sex hormones on IK1, we cultured isolated atrial cardiomyocytes (CMs) for 24h with 17β-estradiol (EST) or dihydrotestosterone (DHT).

Results: In ECG (Fig.1), we observed a prolonged P-wave duration and an increased P-wave duration in men and in male rabbits as compared to women or female rabbits, which may theoretically be due to a slower conduction velocity or longer AP duration (APD) in males. Intracellular AP recordings (Fig.2) showed a hyperpolarized resting membrane potential and prolonged APD in males compared to females. In the IK1-IV curve (Fig.3), we observed a smaller IK1 in male atrial CMs, which could be due to DHT presence. Indeed, both acute and chronic administration of DHT in CMs from females was able to reduce IK1.

Conclusions: Sex and sex hormones impact on atrial electrophysiology, resulting in sex differences in P-wave morphology, AP parameters and ionic current densities. DHT administration was able to reduce IK1 in females CMs, mimicking the sex differences observed. To further elucidate our findings, we are currently evaluating sex differences in IK1/Kir2.1 on the protein level and potential sex differences in atrial fibrosis content.
Comprehensive electrophysiological analysis of cardiomyocytes

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Fundamental insights into the electrical and mechanical dynamics of the human heart can uncover the complexity of cardiac physiology and disease. Uniting automated patch clamp (APC), impedance, contractile force, and electrical MEA-like recording readouts from cardiac cells, such as hiPSCs cardiomyocytes, gives valuable insight into cellular phenotypes. This holistic approach establishes a more predictive model for cardiovascular risk assessment and opens the way for better screening of new drug candidates.

We investigated cardiac ion channels in cell models, including hiPSCs, using APC systems in current-, voltage- and dynamic clamp-mode then validated the data on 2D cell monolayers in cell monitoring systems. Additionally, we developed a label-free, pro-maturation physiological environment by recording hiPSCs on flexible substrates, with successful mechanical stimulation.

We show the evaluation of ion currents, action potentials, maturation, and contractile force after compound or mechanical stimulation. Ion channel currents and myocyte repolarization data were investigated using 20 reference compounds such as “high risk” hERG blockers dofetilide and E-4031 and compounds acting on sodium and calcium channels. Data demonstrate that high-risk compounds prolong the FPD (field potential duration), intermediate risk compounds induced arrhythmia in almost all cases at the highest dose, and low-risk compounds either decreased FPD or had no impact. By developing flexible substrate technology, we show that classical ionotropic compounds (S-Bay-K8644, isoproterenol) induce mature, positive inotropic responses without additional external stimulation. Application of different levels of static pressure potentiating membrane stretching resulted in higher contraction amplitudes. This finding confirmed the reproducibility of the Frank-Starling mechanism. In addition, cyclic stretching resulted in a significant increase in beat amplitude (2.7±0.2 a.u. vs. 1.4±0.3 a.u) and rate (87.7±1.2 bpm vs. 70.2±3 bpm) compared to non-stimulated cells.

In summary, we show a comprehensive overview of data obtained using three complementary electrophysiology technologies, enabling recordings of cardiac channels and contractility of hiPSCs.
Reliable and straightforward cardiac safety liability and proarrhythmic assessment using automated patch clamp

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Background: Automated patch clamp (APC) devices have become important, higher throughput alternatives to manual patch clamp for cardiac safety testing and for studying ion channel mutations and pharmacology. As hiPSC-CMs are shown to well recapitulate cardiac physiology, there is growing interest to use them on those platforms, triggering the development of optimized tools and assays to enable action potential (AP) recordings in addition to voltage clamp recordings. Here, we developed a range of APC assays for commercially available hiPSC-CM lines, aiming to establish routine recordings in APC.

Materials and methods: Recordings were performed in voltage clamp or current clamp mode combined with dynamic clamp to obtain reliable AP pharmacology recordings on APC. We studied the effects of calcium, sodium, late sodium and hERG channel modulators on AP parameters. Experiments were performed at room temperature and at 37°C.

Results: Class 1/C blocker flecainide effectively inhibited the sodium current and accordingly reduced the AP amplitude (APA) of hiPSC-CMs in a concentration-dependent manner; Class 1/B blocker mexiletine also showed the expected concentration-response curve (IC50: 5.6 µM). The late sodium channel inhibitor ranolazine significantly reduced the APA (14%), upstroke velocity (24%) and AP duration (APD90) at high concentrations. Increasing the pacing rate from 0.5 Hz to 3Hz resulted in more pronounced effects on APA, as expected. Selective hERG blocker dofetilide prolonged the APD90 and increased the short-term variability of the APs. L-type calcium channel showed sensitivity to blockers (nifedipine and diltiazem), while channel activator BayK 8644 prolonged APD90 in a concentration-dependent manner, which could be reversed by nifedipine.

Conclusion: Our data demonstrate that cardiac ion channel pharmacology can be successfully recorded using hiPSC-CMs on APC, providing a reliable tool for cardiac safety screening and the study of cardiac ion channel diseases in a model system closer to in vivo physiology than heterologous expression systems.
Pro-arrhythmic mechanisms of beta-adrenergic response in hypertrophic cardiomyopathy

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**Background**

Hypertrophic cardiomyopathy (HCM) patients often present an enhanced arrhythmogenicity that can lead to lethal arrhythmias, especially during exercise. Recent studies have indicated an abnormal response of HCM cardiomyocytes to β-adrenergic receptor stimulation (β-ARS), with prolongation of their action potential rather than shortening.

**Aims**

This work aims to investigate the key ionic mechanisms underlying the HCM abnormal response to β-ARS and its possible proarrhythmic role using human-based experimental and computational methodologies.

**Material and Methods**

The latest models of human ventricular electrophysiology and β-ARS were integrated and calibrated using experimental measurements of human adult cardiomyocytes from control and HCM patients. A population of models approach was used to isolate the main changes of cellular response in HCM under β-ARS and the principal ionic mechanisms underlying them. Organ level simulations were also performed to study how these changes are reflected in the electrocardiogram and the evolution of the dispersion of repolarization.

**Results**

The developed in silico models of β-ARS capture the behaviour observed in the experimental data, including the aberrant response of HCM cardiomyocytes to β-ARS. A reduced increase of potassium currents under β-ARS was identified as the main mechanism of action potential prolongation in HCM. Dispersion of repolarisation between healthy and HCM tissue was increased upon β-ARS, while transmural dispersion in HCM tissue was reduced.

**Conclusions**

Our results identify causal relationships between the HCM phenotype and its arrhythmogenic response to β-ARS through the downregulation of potassium currents. The developed model offers new insights into the mode of function of β-blockers in HCM and on the ionic mechanisms underlying proarrhythmic events in the disease.
Acute effects of cardiomyopathic phospholamban R9C mutation on calcium and cardiac contraction in a zebrafish model

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Background: The phospholamban mutation Arg 9 to Cys (R9C) was first identified in an American family with ventricular dilatation and premature death. Phospholamban, a transmembrane protein located in the sarcoplasmic reticulum, regulates SERCA2a pump activity by reducing its calcium affinity in the heart. Under β-adrenergic stimulation, protein kinase A phosphorylates phospholamban attenuating its inhibitory effect on SERCA2a. Emerging evidence suggests that phospholamban R9C is a loss-of-function mutation with dominant negative effect on SERCA2a activity. We explored calcium and cardiac function simultaneously in 3 and 9 days-post-fecundation (dpf) zebrafish larvae expressing plnbR9C in the heart.

Aim: This study aim to unveil the early pathological pathway that triggers the disease.

Methods: We generated transgenic zebrafish lines overexpressing phospholamban wild-type (TgPLNwt) and phospholamban R9C (TgPLNR9C) in the heart of zebrafish. To measure calcium and cardiac function in 3 and 9 dpf larvae, TgPLNwt and TgPLNR9C fish were outcrossed with a transgenic zebrafish line expressing the fluorescent ratiometric calcium biosensor mCyRFP1-GCaMP6f.

Results: We found that plnbR9C induces calcium dysregulation and positive inotropy and lusitropy in 3 dpf larvae. Isoproterenol treatment blunted β-adrenergic response in 3 dpf TgPLNR9C larvae. As plnbR9C exposure persisted in the heart, 9 dpf larvae exhibited ventricular dilatation, systolic disfunction and negative lusitropy. Importantly, N-acetylcysteine (a ROS scavenger) rescued the deleterious phenotype observed in plnbR9C larvae.

Conclusions: We propose that phospholamban R9C mutation impairs regulation of SERCA2a and induces calcium dysregulation, leading to enhanced contractility and blunted response to β-agonists. The heart lacks the regulatory axis of the β-adrenergic signaling to respond to physiological stress, resulting in chronic inotropic stimulation. Increased energy demand exacerbates oxidative stress that reinforces oxidative modification of the R9C mutated protein. Intensified ROS production contributes to the pathological pathway resulting in ventricular dilatation and systolic dysfunction.
Retinoic acid concentration is crucial to recapitulate repolarization pattern in atrial hiPSC-CM like in human atrium

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Background
Inclusion of retinoic acid (RA) in protocols for the differentiation of human induced pluripotent stem cells to cardiomyocytes (hiPSC-CM) induces an atrial phenotype. However, so far action potentials (AP) of atrial hiPSC-CM did not fully recapitulate key findings of human atrium and showed variability.

Aim
We suspected methodological issues in RA treatment and therefore prospectively investigated the concentration-dependent effects of RA on AP and amplitudes of atrial selective potassium currents (IKur, IK,ACh).

Material and Methods
Human iPSC from a healthy control line were differentiated into CM using standard protocols complemented with different RA concentrations: 0, 0.01, 0.1 and 1 µM. The resulting hiPSC-CMs were cultured as engineered heart tissue (EHT). IKur currents were measured in hiPSC-CM dissociated from EHTs. The contribution of the IKur and of the G-protein-gated potassium current (IK,ACh) to repolarization was estimated from AP recorded by sharp microelectrodes in intact EHT.

Results
RA treatment increased the amplitude of IKur in a concentration-dependent manner. Even a very low concentration (0.01 µM) induced a substantial IKur (4-AP sensitive peak current @+50 mV; 0.01 µM: 3.8±1.1 pA/pF, n=14; 0.1 µM: 5.8±2.5 pA/pF, n=15; 1 µM: 12.2±4.3, n=16) compared to the absence of RA (1.1±0.54). However, only EHT from atrial hiPSC-CM differentiated with 1 µM RA exhibited the typical strong early repolarization leading to a short APD20 (9.7±1.5 ms, n=6) and a plateau below 0 mV (VPlateau: -16±2.7 mV, n=6). Like in human atrium, low concentrations of 4-aminopyridine (4-AP) increased APD20 (from 10±1.3 to 31±2.2 ms, n=6; p<0.05, paired t-test), but shortened APD90 (from 140.2±10.4 to 114.4±3.6, n=6; p<0.05, paired t-test). Each atrial hiPSC-CM treated with 1 µM RA showed a robust increase in inward rectifier IK,ACh upon carbachol (2 µM) exposure (from 7.8±1.4 to 19±2.7 pA/pF, n=30; p<0.05, paired t-test). Retrospective analysis of RA stock solutions revealed substantial loss by sterile filtration.

Conclusions
RA concentrations are prone to methodological issues and have a profound impact on atrial differentiation. A concentration of 1 µM appears necessary and sufficient to induce a full atrial AP shape in hiPSC-CM in EHT format.
UNDERSTANDING THE CONTRIBUTION OF STRETCH-ACTIVATED ION CHANNELS TO CARDIAC ARRHYTHMOGENESIS USING COMPUTATIONAL MODELLING

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Background
Cardiac electrophysiology and mechanics are strongly interconnected. Their interaction is mediated by cardiac mechano-electric feedback through, among others, stretch-activated ion channels (SACs). These channels may contribute to the development of arrhythmias, but the precise proarrhythmic mechanisms remain incompletely understood.

Aim: To elucidate the contribution of SACs to cellular proarrhythmic effects using a novel computational model of cardiac electromechanics.

Methods
We implemented potassium-selective SACs and non-selective SACs, conducting both sodium and potassium in the O’Hara-Rudy model of human ventricular electrophysiology. The model was calibrated based on experimental data of isolated cardiomyocytes undergoing stretch and partially replaced the original background potassium current. The calibration also considered inter-species differences and disease-related stretch remodelling. Subsequently, to investigate the effects of stretch on action potential (AP), we varied stretch amplitude, duration, and timing.

Results
Afterdepolarization events were observed with short stretch stimuli (10ms), and their amplitude was modulated by stretch amplitude and time of application. The events were not triggered during AP phase 1-3 and with stretch<15%, while stretch>35% during phase 4 elicited triggered activities. Furthermore, stretch could shorten subsequent AP duration (APD) or prevent AP generation. Particularly, this happened in a time window during AP phase 4 with stretch>30% because of stretch-induced inactivation of sodium and L-type calcium channels. These effects, including AP repolarization failure with prolonged stretch stimuli (1s), were more pronounced with disease-related stretch remodelling due to increased stretch sensitivity of older and diseased hearts.

Conclusions
Using a novel human electromechanical computational model, we quantified the contribution of SACs to cardiac AP changes. We showed that SACs may induce afterdepolarizations, triggered activities, can shorten the subsequent APD, and can prevent a beat to be triggered. These effects can be modulated by both disease-related SAC remodelling and variations of amplitude, timing, and duration of cardiomyocyte stretch and may contribute to arrhythmias generation.
Fibrinogen-associated risk for arrhythmias: direct electrophysiological effects in human ventricle

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Background: Increased fibrinogen plasma concentrations are an independent indicator for cardiovascular death and, in few studies, associated with the risk for ventricular arrhythmias. We found earlier that fibrinogen reduced peak force and slowed relaxation in engineered heart tissue (EHT) based on human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CM). The mechanism is unclear.

Aim: We investigated whether fibrinogen has direct electrophysiological effects on human heart tissue.

Methods: Action potentials were measured in EHT in modified Tyrode’s solution at 37 °C and 1 Hz pacing rate. Na+ and Ca2+ currents were measured in human embryonic kidney cells expressing SCN5A, and isolated hiPSC-CM, respectively.

Results: Fibrinogen (10 mg/ml) increased action potential duration at 20% and 90% of repolarization: APD20 from 145±10 to 308±20 ms (n = 9, p<0.05) and APD90 from 270±30 ms to 525±30 ms (n = 37, p<0.05). To test whether fibrinogen prolongs APD by IKr blocker, we repeated the experiments in the presence of a high concentration of E-4031 (1 µM) and found that fibrinogen prolonged APD90 even stronger (from 600±50 ms to 1000±50 ms; n = 4, p<0.05). In patch-clamp experiments, fibrinogen decreased Ca2+ currents, but increased Na+ peak current. Furthermore, deactivation of Na+ currents was slowed, leading to a substantial late current. Prolongation of APD90 in EHT by fibrinogen could not be prevented by TTX (10 µM). While maximum upstroke velocity decreased with TTX from 150±25 to 75±25 V/s; (n = 8, p<0.05), the fibrinogen-induced prolongation of APD was preserved. The relevance of the findings for adult human heart electrophysiology were evaluated by measuring action potentials in human ventricular tissue. Prolongation of APD90 by fibrinogen was smaller than in EHT, but still significant from to 325±20 to 400±35 ms; (n = 3).

Conclusion: Fibrinogen has direct electrophysiological effects that may explain the increased risk for arrhythmias.
A SUR2A mutation increases IKATP channel conductance by decreasing the interaction between Kir6.2 and Ankyrin B.

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Background: ATP-sensitive potassium (KATP) channels composed of Kir6.x and sulfonylurea receptor (SURs) subunits couple cellular metabolism to electrical activity. Cantú syndrome (CS) is a rare disease characterized by a range of anatomical and functional defects. CS is caused by mutations in the genes encoding Kir6.1 (KCNJ8) and SUR2A (ABCC9) that produce KATP channel hyperactivity as a consequence of a reduced block of the channels by physiological ATP concentrations.

Aim: To functionally characterized the p.S1054Y SUR2A mutation identified in CS carriers, who exhibited a mild phenotype although the mutation was predicted as highly pathogenic.

Material and Methods: We recorded macroscopic, inside-out and single-channel currents using the patch-clamp technique in CHO and HEK-293 cells.

Results: The mutation significantly (P<0.05, n≥5) increased macroscopic basal current density and, at the single channel level, opening frequency (13.4±2.3 vs. 28.5±5.0 Hz), slope conductance (γ=62.4±3.9 vs. 98.0±4.0 pS), and opening probability-Po (0.027±0.004 vs. 0.065±0.01). Furthermore, it led to the appearance of multiple conductance levels, with the most frequently observed levels being those with the highest amplitudes, open dwell-times and Po, indicating that p.S1054Y SUR2A stabilized the KATP channel in its open state. Biotinylation assays demonstrated that the mutation also reduced Kir6.2 and SUR2A expression specifically at the membrane. Gating and expression defects were prevented when the cytoskeletal adapter protein Ankyrin B (AnkB) was overexpressed. Furthermore, inside-out experiments demonstrated that overexpression of AnkB restored the ATP sensitivity of the mutated channels decreasing the 50% inhibitory concentration from 3.4 to 0.4 μM.

Conclusions: Our results provide a novel mechanism by which CS mutations can reduce ATP block and may help to explain the mild phenotype associated to this mutation. Furthermore, this is the first demonstration of a CS mutation whose functional consequences involve the disruption of AnkB effects on KATP channels.
PO 025

The p.L889V variant produces a marked dominant negative effect on Nav1.5 native channels

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Background. A novel mutation in Nav1.5 channels (p.L889V) was found in two Spanish families with cardiac conduction block that in some individuals was associated with dilated cardiomyopathy.

Aim. To functionally analyze p.L889V Nav1.5 channels to elucidate whether this mutation could underlie the phenotype of the carriers.

Methods. INa was recorded in CHO cells transiently expressing human native (WT) and (0.5:0.5 ratio)/or p.L889V Nav1.5 channels together with Navβ1 proteins using patch-clamp.

Results. The variant produced a strong dominant-negative effect since the peak INa generated by p.L889V channels, either alone (-69.4±9.0 pA/pF) or in combination with WT (-62.2±14.6 pA/pF), was significantly (n=17, P<0.05) reduced compared to that generated by WT channels alone (-199.1±44.1 pA/pF). p.L889V channels, either alone or in combination with WT, were activated (Vhact=-38.5±2.6 vs. -26.5±1.6 mV) and inactivated (Vhinact=-80.8±2.3 vs. -70.2±2.3 mV) at more positive potentials compared to WT (n=21, P<0.05). As a consequence, the window current was reduced, and its peak shifted to more depolarized potentials. Recovery from fast inactivation of p.L889V channels, either alone or in combination with WT, was significantly faster than that of WT (10.6±1.9 vs 5.8±0.7 ms, n=10, P<0.05). The p.L889V variant slowed the induction kinetics of slow inactivation (t=296±142 vs 526±168 ms, n=7, P<0.05), decreased the fraction of channels entering into the slow inactivated state (0.55±0.06 vs 0.40±0.02, n=7, P<0.05), and accelerated recovery kinetics from slow inactivation (35.6±8.4 vs 10.4±3.2 ms, n=4, P<0.05). Conversely, the density of the late component of INa generated by WT and p.L889V channels was not different. Biotinylation assays demonstrated that the sarcolemma expression was significantly reduced in cells expressing the variant.

Conclusion. The p.L889V variant produces a marked dominant-negative effect decreasing INa by reducing the Nav1.5 channel expression and altering the gating of the channels. These loss-of-function effects could account for the phenotype of the carriers.
Empagliflozin and Dapagliflozin augmented INa and prolonged action potential duration in human cardiomyocytes

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Background. Empagliflozin (EMPA) and dapagliflozin (DAPA) are sodium-glucose cotransporter-2 inhibitors (SGLT2i) used for the treatment of type 2 diabetes (T2DM) that reduce mortality in heart failure (HF) patients irrespective of the presence of T2DM. The sodium current (INa), generated by Nav1.5 channels, is responsible for cardiac action potential (AP) depolarization, excitability, and conduction velocity. HF reduces Nav1.5 expression at the cardiomyocyte sarcolemma enhancing arrhythmic risk.

Aims. To determine EMPA and DAPA effects on human cardiac INa and AP characteristics.

Methods. INa and ventricular-like APs were recorded in human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CM) using patch-clamp. INa was also recorded in CHO cells transiently expressing human Nav1.5+Navβ1 channels. Cells were incubated for 24 h with EMPA or DAPA (1 µM).

Results. hiPSC-CMs exhibited automatic activity and incubation with EMPA or DAPA did not modify the spontaneous beating frequency. In cells driven at 1 Hz, none of the drugs modified resting membrane potential but significantly increased AP amplitude from 98.6±3.6 to 105±2.2 (DAPA) and 107±2.3 mV (EMPA) (P<0.05, n≥13). EMPA significantly lengthened the AP duration (APD) measured at 20%, 50%, and 90% of repolarization, while DAPA only prolonged APD at 20% of repolarization. EMPA increased maximum INa density by 64% (from -156.0±28.0 to -256.4±28.1 pA/pF, P<0.05, n≥7) and shifted the midpoint of the inactivation curve from -97.3±4.5 to -108.6±4.4 mV (P<0.05, n≥7). DAPA significantly increased the peak INa density by 24% (to -193.8±26.6 pA/pF) and shifted the activation curve to more negative potentials (from -47.2±1.6 to -55.5±2.8 mV, P<0.05). In CHO cells, effects produced by EMPA and DAPA on INa were identical to those observed on hiPSC-CM.

Conclusions. EMPA and DAPA increase INa and AP amplitude in human cardiomyocytes. EMPA also prolonged AP duration. EMPA and DAPA exhibit a unique mechanism that could increase cardiac excitability and conduction velocity.
The naturally occurring HCN domain p.V240M variant increases Hcn4 channel conductance and open probability

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Background: The hyperpolarization-activated cyclic nucleotide-gated type 4 (HCN4) channels generate the funny current (If) in human sinoatrial node cells. The N-terminal HCN domain (HCND) stabilizes the HCN4 pore in the closed state. In a Spanish family with inappropriate sinus tachycardia (IST), we identified a novel mutation (p.V240M) within the HCND.

Aim: To functionally analyze the characteristics of the p.V240M HCN4 variant.

Methods: Macroscopic (IHCN4) and single-channel (iHCN4) currents were recorded using patch-clamp techniques in CHO and HEK293 cells transiently expressing human native (WT) and/or p.V240M HCN4 channels.

Results: At a wide range of membrane potentials IHCN4 generated by p.V240M channels either alone or in combination with WT (0.5:0.5 ratio) was significantly greater than that generated by WT channels alone (P<0.05, n=19). The midpoint activation voltage of p.V240M channels was ≈20 mV depolarized compared to that of WT (-111.7±2.6 vs -131.3±2.5 mV, P<0.05, n≥19). Interestingly, an identical shift was observed in HEK293 cells in the presence of saturating concentrations of cAMP in the pipette solution (10 μM). Mutated channels deactivation was significantly slower than that of WT (0.9±0.2 vs 2.1±0.4 ms, n≥7, P<0.05), while activation was significantly accelerated.

At the single channel-level, opening frequency (fo) and probability (Po) of p.V240M channels were significantly greater than that of WT channels, while mean open time was significantly lower (17.1±3.5 vs 36.2±9.8 ms, P<0.05, n=5). Interestingly, single-channel conductance of p.V240M channels was doubled than that of WT channels (γ=39.8±4.2 vs 16.3±2.7 pS, P<0.05, n=5). Biotinylation assays demonstrated that membrane expression of WT and p.V240M HCN4 channels was not different.

Conclusion(s): The presence of the p.V240M variant disrupts the closed-state stabilization produced by the HCND and increases the HCN4 channel conductance without modifying its cAMP sensitivity. These effects increase If during diastole which can account for the IST of all the variant carriers.
Computational modeling identifies the cellular electromechanical effects of disrupted intracellular calcium handling in arrhythmogenic cardiomyopathy patients

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**Background:** Arrhythmogenic cardiomyopathy (ACM) patients mostly remain asymptomatic until life-threatening arrhythmias occur. Disturbed calcium (Ca2+) handling may translate to arrhythmogenesis and mechanical dysfunction, but the underlying mechanisms remain unknown.

**Aim:** Characterize disturbed intracellular Ca2+ in ACM patients and predict its effects on cellular electromechanics in left and right ventricles (LV, RV) using a computer model.

**Methods:** Changes in LV and RV protein levels in 5 ACM patients and 5 controls were measured and implemented in our human electromechanical cardiomyocyte computer model (Lyon et al. AJPHeart 2020). Calcium transient (CaT), action potential (AP) and tension traces were simulated.

**Results:** Exemplary results for one patient are described below. In the LV, AP duration was shorter than control (221ms vs. 255ms), CaT peak and diastolic Ca2+ were increased (0.52µM vs. 0.39µM, 0.26µM vs. 0.060µM). Relaxation was impaired, with longer CaT and tension (965ms vs. 640ms) and increased diastolic tension (10mN vs. 4.8mN). In the RV, AP duration was shortened, and CaT and tension peak were lower than in the LV (0.37µM, 13.6mN). Diastolic levels were elevated compared to control, and CaT and tension development were prolonged. These effects related to the measured Ca2+ changes: in the LV, a lower sodium-calcium exchanger (NCX) and SERCA pump combined with increased ryanodine receptor (RyR) may impair Ca2+ extrusion, leading to increased diastolic Ca2+. In the RV, milder NCX and RyR changes may explain the larger Ca2+ extrusion, lower CaT peak and diastolic levels.

**Conclusion:** By integrating protein data from ACM patients into an electromechanical computational model, we showed that patient-specific calcium handling changes (specifically lower NCX and SERCA and increased RyR) may lead to longer CaT and tension, increased diastolic levels, and impaired relaxation. This aligned with clinical data reporting increased ventricular stiffness. Future whole-heart extensions of this modeling approach may help understand ACM proarrhythmic mechanisms.
A new medium-sized animal model for human short-QT and early-repolarization syndromes?

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Background:
For a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied.

Methods:
Ten lions were anesthetized (in mg/kg: ketamine 3; medetomidine 0.03; midazolam 0.2; butorphanol 0.1) in three different Danish zoos for reasons not relating to this study. We opportunistically obtained a 6-lead ECG in the Einthoven configuration and a 3-lead orthogonal ECG via needle electrodes. In addition, 12 lions from the Kgalagadi Transfrontier Park (South Africa) were anesthetized and heart-rate loggers (11.8 g, DST milli-HRT from Star-Oddi, Iceland) were implanted subcutaneously on the left side of the thorax. The loggers continuously recorded heart rate and a 3-sec ECG every 8 min and the loggers were recovered after two weeks.

Results:
Einthoven ECG showed normal PQRST sequence with a large R wave, negligible Q and S waves and a positive, symmetrical T wave in lead II. RR interval was 1063±173 ms (mean±SD); PR interval was 183±21 ms. The QRS and QT intervals were surprisingly short at 61±10 and 294±12 ms, respectively. Eight of the 10 ECGs showed terminal QRS slurring or notching comparable to an early repolarization pattern. The orthogonal leads showed a cardiac electrical axis in the cranial-to-caudal direction. The loggers recorded clear 24-h diurnal rhythms in RR, PR, QRS and QT intervals. RR and PR intervals were longest around midday when the lions are least active. Interestingly, the QT diurnal rhythm peaked in the morning hours, not synchronous with the RR-interval cycle.

Conclusions:
The chronobiology of QT intervals in lions is not in phase with heart rate, as it is in mice and man. The anesthetized lion shows an ECG reminiscent of human short-QT and early-repolarization syndromes. Despite some obvious impracticalities, the repolarization of lion heart needs further clarification.
High performance automated patch-clamp of mammalian atrial cardiomyocytes

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Background: Atrial fibrillation (AF) is the most commonly reported cardiac arrhythmia. Current AF therapeutics lack efficacy, and mechanistic models to examine ion channel remodelling in AF are limited by a lack of atrial specificity in expression systems or low throughput methodologies. Much progress has recently been made in the development of automated patch-clamp (APC) systems that allow for high throughput electrophysiological measurements. APC could therefore be a useful tool for increasing the throughput of experiments involving complex AF-induced ionic remodelling events.

Methods: We describe the application of a newly-designed high throughput APC device (Nanion Syncropatch 384) to investigate key currents and action potentials (AP) in human atrial-specific iPSC-derived cardiomyocytes (iPSC-CM), and in atrial cardiomyocytes directly isolated from native swine myocardium.

Results: For the first time, we describe successful current and AP acquisition from native mammalian cardiomyocytes using APC (total: 203 atrial and 218 ventricular recordings from a single animal). We observed typical subtype-specific electrophysiological characteristics including a shorter AP and smaller L-type calcium current (ICa,L) in atrial preparations compared with ventricular in both iPSC-CM and native cardiomyocytes. In addition, Ba\textsuperscript{2+}-sensitive inward rectifier potassium current (IK1) was smaller in atrial cells. Finally, activation of the atrial specific acetylcholine-activated inward rectifier potassium current (IK\textsubscript{ACh}) was seen in atrial but not in ventricular cells following application of the M-receptor agonist carbachol (2 \textmu M).

Conclusion: The successful application of a high throughput APC-system for the recording of atrial APs and ionic currents in freshly isolated mammalian cardiomyocytes implies that APC constitutes a crucial tool for increasing the throughput of electrophysiological measurements of mammalian cardiac tissue. This will facilitate robust studies of AF mechanisms and substantially impact AF-related drug development programs.
Establishing a new cardiac toxicity screening method using pig myocardial slices

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Introduction
Cardiotoxicity screening is crucial in drug development to reduce risks to patients and economic losses. Different approaches have been developed, but they are still lacking normal tissue composition and physiological load.

Aim
Herein, we explore myocardial slices from healthy pigs together with biomimetic cultivation setups (BMCS) as a new cardiotoxicity screening approach.

Methods
Pig left-ventricular samples were cut into 300 µm thick slices which were mounted into BMCS. Quasi-isometric force was recorded during electrical stimulation to determine the force-frequency relationship (FFR), the frequency dependence of contraction duration, the effective refractory period (ERP), and pacing threshold. First, we explored which cardiac ion channels influence which analysis parameter by applying specific blockers. Next, we blindly evaluated five drug candidates selected from the CiPA list as well as acetylsalicylic acid, PBS and DMSO as controls.

Results
Slices generated 2.4±1.4 mN twitch force (1 Hz), showed a positive FFR, and a shortening of contraction duration with increased pacing rates. Addition of 300 µM lidocaine, a selective blocker of Na+ channels, increased the pacing threshold by ~300%. The hERG K+[sup]+/[/sup] channel blocker dofetilide (3 nM) prolonged the ERP and contraction duration to 195±9.1% and 188±4.1% (n=3), respectively. Additionally, we observed that late sodium currents were provoked by 100 nM dofetilide after 12h treatment, but not by 100 µM moxifloxacin, as identified by the inhibitory action of 10 µM ranolazine. Within the CiPA drug list, the Na+[sup]+/[/sup] channel blocker disopyramide increased the pacing threshold by ~200%. ERP and contraction duration were prolonged after applying three IKr blockers. The L-type Ca+[sup]2+/[/sup] channel blocker bepridil decreased the contraction force while also increasing the diastolic force, presumably by blocking NCX.

Conclusion
We established a new approach for cardiotoxicity assessment. Importantly, this approach can be upscaled to medium throughput screening and could thus improve the current preclinical cardiotoxicity screening.
Red-light modulation of the function of cardiomyocytes derived from human induced pluripotent stem cells

**PO 032**

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**Background:** Human induced-pluripotent stem cells derived cardiomyocytes (hiPSC-CMs) are widely recognized as a valuable experimental model to study mechanisms of cardiovascular disease and for the development of targeted therapies. In recent years, light-modulation by conjugate polymer has been reported as a novel approach to modulate cellular activity with high spatial and temporal resolution, permitting lower invasiveness and high selectivity. Furthermore, photo-biomodulation with red and NIR is a promising tool to control cell activity. The potential of photo-stimulation by a red-light absorbing conjugated polymer to modify the hiPSC-CMs functionality could be extremely relevant for their potential use as treatment in cardiovascular disorders. This study aims to investigate conjugated polymer optical modulation of Ca²⁺ homeostasis and electrical activity in hiPSC-CMs.

**Methods:** Cells were plated on a red light-absorbing conjugated polymer substrate (PCPDTBT). Uncoated glass coverslips (GLASS) were used as reference. Ca²⁺ dynamics and electrical activity were assessed with FLUO4AM and with patch-clamp, respectively. Pharmacological measurements were carried out to investigate the role of SERCA and Na⁺/Ca²⁺ exchanger.

**Results:** Polymer photoexcitation results in faster Ca²⁺ dynamics in a power density dependency manner in PCPDTBT and GLASS groups; however, the red-light effect was larger in hiPSC-CMs plated on polymer. Furthermore, red-light photo-stimulation of PCPDTBT induced a statistically significant enhancement of the hiPSC-CMs with regular beating and an increment of spontaneous beating frequency. Under red-light stimulation, PCPDTBT reduced the inhibitory effect of SERCA2a and NCX blockade on Ca²⁺ reuptake. The optical stimulation resulted in a shortening of the AP in both groups; however, the percentage of light-induced shortening of the AP duration was higher in PCPDTBT compared to GLASS.

**Conclusion:** This work demonstrated that photomodulation mediated by PCDPTBT: i) accelerates the Ca²⁺ transient decay of hiPSC-CMs through an indirect modulation of SERCA and NCX; ii) induces an APD shortening; iii) enhances the spontaneous beating frequency.
Involvement of the Exchange Protein Activated by cAMP (EPAC) in atrial electrophysiology and atrial fibrillation mechanisms.

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Background Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and is associated with increased mortality and morbidity. The Exchange Protein directly Activated by cAMP (EPAC), has been identified as potentially implicated in pro-arrhythmic signalling pathways in the atria, but the precise mechanisms remain unknown.

Objective To investigate the involvement of EPAC1 and 2 isoforms in the genesis of AF.

Methods Transesophageal stimulation (TS) was used to characterize the induction of AF in vivo in wild type mice using EPAC activator (8-CPT; 10µM; N=11), EPAC1 (AM-001; 20µM; N=10) and EPAC2 (ESI-05; 25µM; N=10) inhibitors, then in EPAC1 and EPAC2 knockout mice. Optical mapping experiments on isolated atria were performed to study EPAC pathways during 8-CPT (N=7), and AM-001 (N=6) or ESI-05 (N=12) followed by 8-CPT, to assess action potential durations at 80% of repolarization (APD80) and conduction velocities (CV).

Results TS showed AF induction success in 44% in WT mice, 25% in KO EPAC1 mice and 40% in KO EPAC2 mice suggesting a more prominent role for EPAC1 in AF vulnerability. AF induction was increased by 8-CPT, whereas AM-001 blunted it and ESI-05 was similar to 8-CPT. APD80 increased after 8-CPT perfusion (33.8 vs 42.6ms p=0.02). AM-001 also increased APD80 and even in the presence of 8-CPT (33.2 vs 38.4 vs 44.6ms p=0.04). Similarly, ESI-05 alone and with 8-CPT had no effect on APD80 increase (32.8 vs 35.3 vs 39.5ms; p=0.03). 8-CPT decreased CV (17 vs 10cm/sec p=0.04) while only AM-001 blocked 8-CPT effects (20 vs 21 vs 21cm/sec) and not ESI-05 (20 vs 16 vs 14cm/sec p=0.04).

Conclusion These results suggest the EPAC1 signalling pathway involvement in atrial electrophysiology and AF mechanisms. Disturbance of CV by EPAC1 activation could play a major role in the genesis of AF which can be prevented by an EPAC1 inhibit, AM-001.
PO 034

Slack: a novel player in cardiac electrophysiology

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Title: "Slack: a novel player in cardiac electrophysiology"

Introduction:
The sodium-activated potassium channel Slack is encoded by the KCNT1 gene. It has a well-recognized physiological role in the neuronal function. In fact, variants of the KCNT1 gene have been implicated in a spectrum of severe and intractable neurological disorders associated with cognitive and behavioural deficits. At cardiac level, a large K+ conductance activated by elevated intracellular Na+ was originally observed by Kameyama in 1984, but its molecular determinant is not defined yet. Moreover, the Slack variant p.Arg1106Gln was reported in a Brugada Syndrome patient.

Aim:
This work aimed at deepen the knowledge on the expression and function of Slack in cardiomyocytes in human and animal models and to elucidate the mechanism of the p.Arg1106Gln KCNT1 variant within a cardiac context.

Methods/Results:
qRT-PCR assays and western blot analysis performed on both ventricles and right atrium samples from adult human cardiac tissue confirmed the presence of KCNT1 products (more abundantly in the ventricles compared to the atrium). Immunofluorescence analysis on ventricular tissue sections revealed a preferential localization of the protein at the level of the intercalated discs and, to a lesser extent, in the T tubules. Similar results were obtained in rat ventricular cardiomyocytes in which patch-clamp measurements indirectly suggested the presence of the IKNa. Overexpression of the p.R1106Q variant in tsA-201 cells revealed a gain-of-function, compared to wild-type, observed at a higher-than-basal intracellular sodium concentration (188,1±42,3 pA/pF and 86,6±12,9 pA/pF respectively, in 20 mM [Na+]).

Conclusion:
In conclusion, the data presented suggest that Slack is expressed in rat and human cardiomyocytes with a preferential and specific localization and that it may be responsible for the IKNa. Mutations in KCNT1 gene may be pro-arrhythmogenic. Thus, we suggest to consider this gene as a novel player in cardiac electrophysiology.
PO 035

Functional analysis of a novel SCN5A mutation found in a patient affected by Brugada Syndrome.

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Introduction:
Brugada syndrome (BrS) is an inherited cardiac arrhythmogenic disorder that is characterized by structurally normal heart with a typical electrocardiographic pattern and an increased risk of sudden cardiac death. Syncope and cardiac arrest are the most common clinical manifestations and are often associated with an enhancement of the parasympathetic nervous system. Mutations in the SCN5A gene are the most recurrent causes of the pathology. In this study, the proband is a young female patient affected by BrS. She was diagnosed with the novel missense mutation c. 1030G>T in the SCN5A gene (p.A344S in Nav1.5) localized in a very conserved region within the S5-S6 extracellular loop of the first domain of the protein. Further genetic analysis on proband’s relatives revealed the presence of the same mutation.

Aim:
The aim of the project is to characterize the pathophysiological role of A344S mutation.

Methods:
Transient transfections of SCN5A, together with the accessory β-subunit SCN1B were performed in Human Embryonic Kidney (HEK293) cells and the patch-clamp was used to record the INa currents 48 hours after transfection.

Results:
A344S mutation in homomeric (Homo) and heteromeric (Hetero) conditions reduce the INa current density (measured at -20 mV) by 71% and 42%, respectively (WT -134.5±14.7 pA/pF, n=17; Hetero -77.8±9.5 pA/pF, n=20; Homo -38.7±5.6 pA/pF, n=12; P< 0.05). The voltage dependence of inactivation curve was significantly positive shifted in Homo compared to WT (V½: WT -82.4±0.5 mV, n=17; Hetero -82.5±0.7mV, n=20; Homo -77.6±0.6 mV, n=12, P<0.05) as well as the voltage dependence of activation (V½: WT -33.7±0.5 mV, n=17; Hetero -29.3±0.6 mV, n=20 Homo -25.9±0.8 mV, n=12).

Conclusion:
All together, these effects produced by the A344S mutation result in an overall reduction of the INa current, suggesting a loss-of-function nature of the mutation.
HiPSC-Derived Cardiomyocyte to Model Brugada Syndrome: Both Asymptomatic and Symptomatic Mutation Carriers Reveal Increased Arrhythmogenicity

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Brugada syndrome is an inherited cardiac arrhythmia disorder that is mainly associated with mutations of the cardiac voltage-gated sodium channel alpha subunit 5 (SCN5A) gene. The clinical symptoms include ventricular fibrillation and an increased risk of sudden cardiac death. Human-induced pluripotent stem cell (hiPSC) lines were derived from symptomatic and asymptomatic individuals carrying the R1913C mutation in the SCN5A gene. The present work aimed to observe the phenotype-specific differences in hiPSC-derived cardiomyocytes (CMs) obtained from symptomatic and asymptomatic mutation carriers. In this study, CM electrophysiological properties, beating abilities and calcium parameters were measured. Mutant CMs exhibited higher average sodium current densities than healthy CMs, but the differences were not statistically significant. Action potential durations were significantly shorter in CMs from the symptomatic individual, and a spike-and-dome morphology of action potential was exclusively observed in CMs from the symptomatic individual. More arrhythmias occurred in mutant CMs at single cell and cell aggregate levels compared with those observed in wild-type CMs. Moreover, there were no major differences in ionic currents or calcium parameters between the CMs of asymptomatic and symptomatic individuals after the administration of adrenaline and flecainide. In conclusion, mutant CMs were more prone to arrhythmia than healthy CMs. This cellular model reproduced the Brugada phenotype but did not explain why only one of the mutation carriers was symptomatic.
The effect of antiepileptic drugs on Nav1.5 channels stably expressed in HEK 293 cells

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Background: Sudden cardiac arrest (SCA) generally results from cardiac arrhythmia secondary to disruptions in cardiac electrophysiology. Anti-epileptic drugs (AEDs) were found to be associated with increased risk of SCA in our previous studies. Levetiracetam, pregabalin, gabapentin, valproic acid and lamotrigine are the most commonly used AEDs. Some AEDs derive their anti-epileptic effects from block of neuronal sodium channels, which have great homology with cardiac sodium channels. SCN5A-encoded Nav1.5 is the most predominant sodium channel in the heart and responsible for the rapid upstroke of cardiac action potential. Malfunction of Nav1.5 channels underlies cardiac arrhythmia in various conditions. We speculated that some AEDs block cardiac sodium channels, thereby exhibiting an association with increased risk of SCA.

Objective: To test the hypothesis that AEDs block Nav1.5 channels.

Methods: The effects of AEDs (levetiracetam, pregabalin, gabapentin, valproic acid, lamotrigine) on human cardiac Nav1.5 channels stably expressed in HEK 293 cells with different concentrations (from 0 to 3000μM) were studied in whole-cell patch-clamp studies.

Results: Lamotrigine and valproic acid showed inhibitory effects on Nav1.5 current in a concentration-dependent manner, while pregabalin, gabapentin and levetiracetam showed no effect. The IC50 of lamotrigine and valproic acid were 142.2μM and 2022μM, respectively. Lamotrigine also blocked Nav1.5 current in a use-dependent manner, and it significantly induced a hyperpolarizing shift of steady-state Nav1.5 current activation and inactivation. Lamotrigine also markedly slowed the recovery from Nav1.5 current inactivation. Valproic acid blocked Nav1.5 current in voltage-dependent manner. Valproic acid induced a hyperpolarizing shift of steady-state inactivation of Na v1.5 current. Valproic acid-treated Nav1.5 current blockage was not use-dependent, and exhibited no slowed recovery from inactivation of Nav1.5 current.

Conclusion: Lamotrigine and valproic acid are blockers of Nav1.5 channels stably expressed in HEK 293 cells. Their effects as cardiac sodium channel blockers may contribute to the observed association with increased risk of SCA.
Blebbistatin inhibits actin-myosin interaction, preventing contractile activity of cardiomyocytes, despite electrical excitation of action potentials. We collected intracellular microelectrode recordings of pacemaker cells of the zebrafish heart at room temperature and during acute warming to investigate the effects of blebbistatin on pacemaker cell electrophysiology, evaluating 16 action potential variables.

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Introduction
Blebbistatin potently inhibits actin-myosin interaction, preventing contractile activity of excitable cells including cardiac myocytes, despite electrical excitation of an action potential (AP). Despite its extensive use in cardiac research, its effects remain largely unexplored, notably in pacemaker cells.

Aim
The aim of this study was to determine the effect of blebbistatin on the pacemaker action potential at ecologically relevant temperatures.

Methods
We collected intracellular microelectrode recordings of pacemaker cells located in the sinoatrial region (SAR) of the zebrafish heart at room temperature and during acute warming to investigate whether or not blebbistatin inhibition of contraction significantly alters pacemaker cell electrophysiology. Changes were evaluated based on a comprehensive 16 variable analysis of the AP waveform.

Conclusion
None of these AP variables nor the spontaneous heart rate were significantly modified with the application of 10 μM blebbistatin when recordings were made at room temperature. Compared with the control group, the blebbistatin-treated group showed minor changes in the rate of spontaneous diastolic depolarization (P = 0.027) and the 50% and 80% repolarization (P = 0.008 and 0.010, respectively) in the 26°C–29°C temperature bin, but not at higher temperatures.

Conclusion
These findings suggest that blebbistatin is an effective excitation-contraction uncoupler that does not appreciably affect APs generated in pacemaking cells of the SAR and can, therefore, be used in zebrafish cardiac studies.
PO 039

Functional characterisation of a novel KCNH2 variant of unknown significance, identified in patients treated in multiple Dutch medical centres

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Introduction; Variants in KCNH2, encoding the hERG channel and responsible for the rapid component of the cardiac delayed rectifier K+ current (IKr), have been linked to Long QT Syndrome type 2 (LQTS2). We identified seven index patients with a new variant of unknown significance (VUS) KCNH2-p.S906L. To date, functional assessment of this variant is lacking, hampering clinical follow up of these patients and their relatives.

Aim; We aimed to elucidate the biophysiological effect of the KCNH2-p.S906L variant, in order to enable proper classification and consequent clinical decision making.

Materials & Methods; All data of the patients and available relatives were used to generate a genotype-phenotype overview. The biophysiological effects were assessed by manual whole-cell patch-clamp using HEK293a cells expressing: (i) the wild type (WT) KCNH2, (ii) KCNH2-p.S906L alone (homozygous, Hm) or KCNH2-p.S906L in combination with WT (1:1) (heterozygous, Hz).

Results; Incomplete penetrance of the LQTS2 in KCNH2-p.S906L carriers was observed. In addition to KCNH2-p.S906L, some index patients were heterozygous for other VUS’ in CACNA1C, PKP2, RYR2, and AKAP9. The phenotype of carriers of KCNH2-p.S906L ranged from asymptomatic to life-threatening arrhythmic events. Whole-cell patch-clamp showed a reduced current density in both the homozygous and heterozygous models, compared to KCNH2-WT. Current density was reduced by 69.8%, and 60.4% in KCNH2-p.S906L-Hm and KCNH2-p.S906L-Hz, respectively. Furthermore, the time constant of activation was increased in KCNH2-p.S906L-Hm compared to KCNH2-WT. While the time constant of the deactivation and inactivation kinetics were unaffected.

Conclusion; The reduced current density in the KCNH2-p.S906L-Hz indicates a moderate loss-of-function of hERG. These observed functional changes, combined with the reduced penetrance indicate that this variant in KCNH2 is a risk factor for LQTS2. We hypothesize that in combination with other genetic substrates, or known risk factors LQTS2 carriers with this variant can experience cardiac events.
PO 040

Evaluation of angiogenesis biomarkers dynamics after Cardiac shock wave therapy in patients with stable angina. Sub-study results from the prospective, randomized, triple-blind, sham procedure-controlled trial

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**Background:** Cardiac shock-wave therapy (CSWT) is a non-invasive treatment based on low-frequency ultrasound waves that stimulate angiogenesis. Current data about the effects of revascularization procedures on angiogenesis biomarkers is limited. An association of endocan and catestatin with coronary collateral development was shown in several trials.

In this study, we aimed to evaluate the effects of the CSWT on biomarkers concentration and investigate their correlation with parameters of exercise capacity.

**Material and Methods:** Prospective, randomized, triple-blind, sham procedure-controlled study enrolled 72 adult subjects who complied with defined inclusion criteria (NCT02339454). We measured serum levels of biomarkers in 33 patients with stable angina (optimal medical treatment (OMT)+CSWT group -17 patients, OMT+sham-procedure (SP) group - 16 patients) who entered a biomarker sub-study. The blood samples were collected at baseline and after the last treatment procedure (9th treatment week) to evaluate biomarkers concentration and stored at -80°C until analysis. Serum endocan and catestatin levels were determined by commercially available ELISA kits.

**Results:** There were no significant clinical and laboratory differences between groups at baseline. Serum endocan concentration significantly increased in OMT+CSWT group (98.6±6.4 to 103.4±8.2 pg/ml, p=0.005), but not in OMT+SP group (98.9±5.6 to 102.4±8.1 pg/ml, p=0.185). Contrary, catestatin concentration increased in OMT+SP group (1069.1±123.1 to 1134.3±134.4 pg/ml, p=0.03) at follow up but not in the intervention group (1144.3±91.9 to 1131.3±98.4 pg/ml, p=0.630).

Interestingly, a significant negative correlation was observed between exercise duration and endocan levels in the OMT+CSWT group (r=-0.51, p=0.036), and exercise duration and catestatin levels OMT+SP group (r=-0.58, p=0.025). This indicates that catestatin may have inhibitory effect on angiogenesis.

**Conclusions:** This study showed a statistically significant association of angiogenesis biomarkers levels with CSWT at the 9th week of follow-up. Whereas endocan and catestatin exhibit cardioprotection after CSWT remains unclear, thus further studies are required.

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β-adrenergic receptor stimulation is essential for maintaining NCX-CaMKII signalling under increased workload

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Background: Cardiac remodelling encompasses changes at the molecular, cellular and transcriptional level following pathologic insult to the heart. Initially, it maintains cardiovascular homeostasis and allows patients to remain asymptomatic, but if untreated, eventually progresses to symptomatic heart failure. Excessive β-adrenergic stimulation and tachycardia are potent triggers of cardiac remodelling; however, the underlying mechanisms of their cellular effects remain elusive.

Aim: Study the individual and synergistic potency of β-adrenergic stimulation and tachycardia to modulate pathological gene expression profiles, as well as the efficacy of β-blockers (BB) in preventing these alterations.

Methods: Neonatal rat ventricular cardiac myocytes (NRVCMs) isolated from 1-day-old neonates, were cultured (3 days) and subsequently stimulated for 3h under basal (1Hz) and tachycardic (8Hz) conditions with/without isoprenaline (ISO; 10µM) and propranolol (ISO+BB; 1µM, 1h preincubation) to screen for the effects on hypertrophic mRNA marker gene expression via qPCR. Protein expression levels and their activation levels were assessed by immunocytochemistry (ICC).

Results: ICC revealed that tachycardia caused a significant upregulation of the sodium-calcium exchanger (NCX) followed by downstream activation of the calcium/calmodulin-dependent kinase II (CaMKII) in the nucleoplasm and nuclear envelope. Interestingly, ISO-treatment ameliorated NCX-upregulation and excessive nuclear CaMKII-activity, while preincubation with BB abolished these ISO-mediated effects. qPCR further showed that tachycardia caused transcriptional upregulation of regulator of calcineurin 1 (RCAN1) and interleukin-6 receptor (IL6R). Here, ISO-treatment partially prevented IL6R-upregulation in an NCX-dependent manner.

Conclusion: In summary, we show that β-adrenergic stimulation—in addition to the well-documented effect on protein kinase A-dependent signalling in cardiomyocytes—has a direct effect on NCX protein expression and downstream CaMKII subcellular activation/localisation. We propose a previously unidentified protective role of short-term ISO stimulation in inhibiting IL6R activation and NCX dysregulation. A better understanding of these processes may contribute to refinement of patient care with special regard to the use of β-blockers.
Use of hiPSC-derived cardiomyocytes to study LQTS-variant specific proarrhythmic effects of drugs.

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Background: Congenital Long QT Syndrome (LQTS) is a condition characterized by prolongation of the QT interval on the electrocardiogram and association with potentially life-threatening arrhythmias. The incomplete penetrance and phenotypic variability of LQTS complicates the classification of variants emerging from genetic screenings. Affected individuals are also susceptible to develop drug-induced LQTS (diLQTS) when exposed to drugs that block IKr. The functional characterization of variants emerging from genetic screenings is crucial for their classification, for the risk stratification of LQTS patients and for estimating the risk for silent carriers. Patient-specific human induced pluripotent stem cells (hiPSCs) may be key to characterize variants potentially predisposing patients to a higher susceptibility towards diLQTS and sudden cardiac death.

Material and Methods: We generated hiPSCs from a family composed by two asymptomatic LQTS parents, affected by different variants in hotspot coding regions of KCNQ1, and by the symptomatic compound heterozygote daughter affected by Jervell and Lange-Nielsen Syndrome (LQTS with congenital deafness). The asymptomatic individuals, as well as other six genotype-positive relatives, would not have been screened for variants in LQTS genes if the symptomatic proband had not been referred to our clinical attention. We aimed to: 1) reproduce in vitro the different clinical severity observed in asymptomatic and symptomatic subjects; 2) demonstrate that asymptomatic or symptomatic carriers show proportional responses to drugs, potentially providing variant-specific drug responses. hiPSC-derived cardiomyocytes (hiPSC-CMs) were studied with patch clamp and Multi-Electrode Arrays (MEAs).

Results and Conclusions: hiPSC-CMs developed variant-specific action potential and field potential prolongation and reproduced the differential severity of the individual variants and of their compound combination. In line with the pathogenicity of the variant, we reproduced a higher susceptibility to IKr blockers in hiPSC-CMs from the symptomatic patient. These findings validate the use of cohort-specific hiPSC-CMs for variant classification, safety pharmacology screenings and precision medicine purposes.
PO 043

Endothelin-1 enhances transmural heterogeneities in healthy porcine ventricular myocardium

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Background: Endothelin-1 (ET-1) is an autocrine/paracrine factor secreted by endothelial cells, cardiomyocytes and fibroblasts. In the heart, ET-1 is associated with physiological and pathophysiological processes, such as oxidative stress, apoptosis regulation and ventricular remodeling associated with heart failure and ischemic cardiomyopathy. ET-1 has been shown to modulate calcium and potassium currents and to contribute to arrhythmogenesis and sudden cardiac death.

Aims: To characterize the role of ET-1 in cardiac electrophysiology across ventricular regions from base to apex and from endocardium to epicardium in healthy pigs.

Methods: Domestic pigs (85-120 kg, n=4) were cardioplegically arrested under deep anesthesia and sacrificed. Transmural ventricular blocks of 1 cm² area were taken from six ventricular regions: A) at the base, close to the left anterior descending (LAD) artery; B) at the base, close to the left circumflex (LCx) artery; C) at the center, near the LAD; D) at the center, near the LCx; E) at the apex; and F) at the center, in the posterior wall. 350 µm-thick ventricular slices were produced from the subepicardium, mid-myocardium and subendocardium of each region. The slices were optically mapped and Action Potential Duration (APD) was measured at 80% repolarization while pacing at 1 Hz in the absence and presence of 100 nM ET-1. The notation n/N indicates n slices from N pigs.

Results: ET-1 prolonged APD in all ventricular regions, with a mean prolongation of 18% (n/N=57/4). APD prolongation in the subendocardium (30%, n/N=13/4) was remarkably larger than in the mid-myocardium (14%, n/N=27/4) and subepicardium (14%, n/N=17/4), with the increased prolongation in the subendocardium being consistent across all six tested regions. No significant apex-to-base differences were observed.

Conclusions: ET-1 induces strong APD prolongation in pig myocardium, with enhanced responses in the subendocardium. Transmural heterogeneities in response to ET-1 might play a role in arrhythmia vulnerability.
Optogenetic investigation of heterocellular electrotonic coupling between myocytes and non-myocytes in cardiac remodeling

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Introduction
The heart is composed of cardiomyocytes (CM) and non-myocytes (NM), the latter including interstitial fibroblasts (FB) and resident macrophages (MΦ). NM have long been suspected to electrically couple to CM in native myocardium. We are interested in how FB and MΦ electrotonically couple to CM and alter electrical activity during myocardial remodeling in response to injury.

Methods
Tcf21-MerCreMer and Cx3cr1-CreERT2 mouse lines were used to target channelrhodopsin-2 (ChR2) to FB and MΦ, respectively. We studied non-transmural scars in hearts following left ventricular (LV) cryo-ablation or ischemia reperfusion injury. We performed electrical pacing of isolated Langendorff-perfused hearts together with optical stimulation ChR2 in NM to evaluate the effect of NM depolarization on CM electrophysiology, assessing different locations, scar centre, border zone and remote myocardium. Additionally, we imaged reporter fluorescence in NM with confocal microscopy after optical tissue clearing. We reconstructed 3D models of FB and MΦ structure and assessed the morphology, distribution, interconnectivity, and surface area in the heart tissue.

Results and Conclusions
In healthy hearts, we found that FB networks consist of elongated, thin strands of interconnected cells, which appear to wrap around CM with finger-like nano-protrusions. Surface area of 3D FB are not significant difference between atrial and ventricular walls (e.g. average surface area in right atrium (RA) and LV were 2130±280 µm² and 1770±190 µm² [N=3 hearts], respectively). MΦ appear as solitary cells in intact ventricles and atrial with an average surface area of 1,160±80 µm² in LV and 1203±86 µm² in RA (N=3 hearts). Upon injury and scar formation, these cell populations undergo pronounced changes leading to larger NM numbers in the scar and scar border, but also altered structures of cellular networks, increasing the ratio of NM to CM, and thus altering their impact on cardiac physiology.
In silico mechanistic investigation into proarrhythmic triggers arising from human Purkinje electrophysiology following acute myocardial infarction remodelling

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Clinical and experimental studies on acute myocardial infarction (AMI) highlighted the role of the His-Purkinje system in the initiation and maintenance of ventricular arrhythmias. Biophysically-detailed mathematical models and simulations help in gathering insights into arrhythmia mechanisms.

This study aims to investigate in a population of human Purkinje virtual cells the mechanisms underlying proarrhythmic triggers following AMI-induced remodelling.

The Trovato2020 model was used to simulate human cardiac Purkinje electrophysiology. A population of 2000 models was generated by varying the main ionic currents' conductances and calibrated using healthy human Purkinje data. AMI-induced ionic remodelling was implemented as altering the conductance for Na⁺, Ca²⁺ and K⁺ currents as reported experimentally. Simulations were performed to quantify the impact of AMI-related ionic remodelling on action potential (AP) biomarkers (at pacing frequencies varying from 1 to 3 Hz), and to assess susceptibility to abnormal automaticity and early and delayed afterdepolarisations (EADs, DADs).

At 1Hz, simulated Purkinje cells affected by AMI displayed elevated diastolic potential, reduced AP amplitude and depolarisation velocity as in experiments due to reduced fast Na⁺ and inward K⁺ rectifier currents and enhancement of the funny current. Moreover, AMI remodelling lead to automaticity in the 3% of Purkinje, whereas no model exhibited automaticity in control conditions. DADs and triggered APs were observed at fast pacing in 10% of models following remodelling. AMI-induced reduction of L-type Ca²⁺ current increased propensity to automaticity in Purkinje cells by 100%, whereas reduction in T-type Ca²⁺ current abolished automaticity in all models. AMI-induced reduction of the rapid delayed repolarisation current induced EADs on 1% of models without affecting automaticity.

AMI remodelling in human Purkinje cells promotes distinct types of proarrhythmic triggers. Ca²⁺ and the funny currents were the major players underling Purkinje automaticity and could be targeted by new antiarrhythmic treatments.
PO 046

Effects of dofetilide on isolated hearts suggests a role for Kv11.1 in the mouse sinus node

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Background:
Dofetilide is a selective IKr channel blocker used to treat arrhythmia in humans. Although studies have shown the presence of mRNA encoding Kv.11.1 in mouse hearts, it is not known whether these channels play a physiological role in repolarization.

Aim:
Our goal with this study was to elucidate the role of Kv11.1 in mice hearts by investigating the effects of dofetilide on isolated perfused hearts.

Methods:
Hearts were excised from c57bl/6 wild-type mice and perfused retrogradely with Krebs-Henseleit buffer. Three ECG electrodes were placed on the heart to obtain a lead II and a ventricular lead recording. Pacing was conducted on the right atrium and left ventricle by two individual pacing electrodes. Dofetilide (1000 nmol/L) was infused mid-protocol via an external syringe pump.

Results:
Dofetilide significantly slowed heart rate mean±SD: 324±48 versus 296±59 BPM (n=13, p-value=0.0044). No changes were seen in QT interval during normal sinus rhythm: 66±3 versus 65±3 (n=11, p-value=0.31). To further confirm this, no significant change in QT was observed at 8Hz atrial pacing post-infusion: 65±6 versus 64±5 (n=8, p-value=0.18) nor did the ventricular effective refractory period change: 45±8 versus 45±10 (n=9, p-value=0.7). Sinus node recovery time did seem to decrease post infusion, -but not statistically significantly: 171±21 versus 221±65 (n=7, p-value=0.13).

No differences were seen in any of these parameters for vehicle controls.

Conclusion:
The decrease in heart rate suggests a possible role of Kv11.1 in the mouse sinus node. As neither QT nor ventricular effective refractory period changed after dofetilide treatment, the Kv11.1 does not seem to be important for murine ventricular repolarization. Further investigation into the role of Kv11.1 in the sinus node is needed.
L-type Cav1.3 calcium channels are required for beta-adrenergic triggered automaticity in dormant mouse sinoatrial pacemaker cells

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Background: Sinoatrial node cells (SANC) automaticity is generated by functional association between the activity of plasmalemmal ion channels and local diastolic intracellular Ca2+ release (LCRs) from ryanodine receptors. Strikingly, most of isolated SANC exhibit a “dormant” state, whereas only a fraction shows regular firing as the intact SAN. Recent studies showed that β-adrenergic activation can initiate spontaneous firing in dormant SANC though this mechanism is not entirely understood.

Methods: Here we investigated the role of L-type Cav1.3 Ca2+ channels in β-adrenergic activation of automaticity in dormant SANC. We used a knock-in mouse strain in which the sensitivity of L-type Cav1.2 α1 subunits to dihydropyridines (DHPs) was inactivated (Cav1.2DHP−/−), enabling selective pharmacological inhibition of Cav1.3 by DHPs.

Results: Firing SANCs expressed higher densities of Cav1.3-mediated L-type Ca2+ current (ICaL) and of the “funny” current (If), in comparison to dormant SANCs. In contrast, expression of Cav1.2 mediated ICaL was similar in dormant and firing SANCs. In dormant SANC, β-adrenergic stimulation with isoproterenol (ISO) induced spontaneous action potentials (AP) and Ca2+ transients, which were completely arrested with concomitant perfusion of the DHP Nifedipine. In spontaneously firing SANC at baseline, Cav1.3 inhibition completely reversed the effect of β-adrenergic stimulation on AP and frequency of Ca2+ transients. Imaging of intracellular Ca2+ dynamics in SANC showed that Cav1.3 is critical for β-adrenergic-induced synchronization of LCRs during triggering of SANC automaticity, or stimulation of firing.

Conclusions: Our study shows a novel role of Cav1.3 channels in initiating and maintaining automaticity in dormant SANC upon β-adrenergic stimulation.
PO 048

Magnesium ions moderate activation of calcium sparks

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In vitro and in situ experiments show the inhibitory effect of Mg2+ on cardiac ryanodine receptor (RyR2) activity. The impact of RyR modulation by Mg2+ on calcium release in cardiac myocytes is not well understood neither in experiments nor in theoretical models.

We have constructed a homotetrameric RyR gating model that describes the Mg2+/Ca2+ competitive binding to the RyR activation site, the binding of Mg2+ to the RyR inhibitory site, and allosteric regulation of RyR opening by Ca2+ and Mg2+ binding to the activation site. The model was validated on selected published data on RyR open probabilities, mean open times, and activation times. To inspect calcium release events (CREs) in silico, RyRs were placed into a model calcium release site (CRS) consisting of 20 realistically distributed RyRs. CREs were simulated at a range of Mg2+-binding parameters at near-physiological Mg2+ and ATP concentrations [1].

It was found that the characteristics of CREs can be described as a function of the effective coupling strength between RyRs, defined as a weighted product of RyR vicinity (the descriptor of RyR placement in a CRS), single-channel calcium current, and Mg-binding parameters. The Mg2+ unbinding rate from the RyR activation site and Mg2+ binding rate to the RyR inhibition site contributed substantially more, the Mg2+ binding rate to the RyR activation site and calcium current amplitude contributed similarly, and the Mg2+ unbinding rate from the RyR inhibition site and the allosteric coefficient of Mg2+ contributed less than the RyR vicinity to the effective coupling strength. These findings define the role of Mg2+ ions as a protector of the CRS from spontaneous activation in the absence of an external stimulus.

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PO 049

Generating a Novel Ex Vivo Model of Ischaemia Reperfusion in Porcine Myocardial Slices

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Porcine myocardial slices are a novel ex vivo cardiac model with retained viability, morphology, electrophysiology, and cell-cell interactions – advantages over some in vitro models. The slices are highly translational, as porcine hearts are closer to human in electrophysiology, size, cardiac output, and heart rate than commonly used small animal models. We aim to model ischaemia/reperfusion (IR) in porcine myocardial slices through increasing durations of hypoxia/reoxygenation (HR), measuring the effect on viability and contractile force.

1.3cm\(^2\) left ventricle blocks from unused porcine hearts (Pirbright Institute) were vibratome-cut into 300μm thick myocardial slices. Sub-endocardial slices were cultured in a standard 5% CO\(_2\) (normoxia) or 1% O\(_2\) (hypoxia, using N\(_2\)) incubator. Two HR durations were investigated – short (2-hour hypoxia, 1-hour reoxygenation) and long (16.5-hour hypoxia, 2.5-hour reoxygenation). Hypoxia-inducible factor 1α (HIF1α) was used as an endogenous hypoxia marker. Viability was assessed through lactate dehydrogenase (LDH) release in medium. Contractility was measured with a force transducer at 1Hz stimulation. Data was analysed using unpaired, two-tailed, student’s t-test with Welch’s correction.

Immunofluorescence showed cardiomyocytes and cells around blood vessels with HIF1α-positive nuclei, comparable to published human myocardial infarction biopsy immunohistochemistry. Viability (measured by LDH release) and maximal active force (mN/mm\(^2\)) significantly decreased after long (n=7 slices, N=3 hearts, p=0.0183; and n=5 slices, N=2 hearts, p=0.0159, respectively), but not short HR (n=6 slices, N=2 hearts, p=0.0745; and n=8 slices, N=3 hearts, p=0.2853, respectively) (Fig.1). Interestingly, long HR significantly increased LDH release during reoxygenation (n=7 slices, N=3 hearts, p=0.0183), but not hypoxia (n=7 slices, N=3 hearts, p=0.1471) (Fig.1B) – reflecting in vivo and improvement over some in vitro data. Significantly decreased contractility after long HR (Fig.1D) was advantageous over published cryoinjury models without effect on maximal force.

Our results suggest an advantageous IR translational model with hypoxic staining and duration-dependent effects on viability and contractility.
PO 050

Exercise training post MI is anti-arrhythmic through the modification of ventricular electrophysiology at high heart rates in non-infarct and infarct zones.

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Background: The aim of the study was to determine if exercise training alters the propensity to arrhythmias post-myocardial infarction and whether these changes are due to altered electrophysiology of the non-infarcted zone (NZ), the infarct zone (IZ) and border zone (BZ) in a rat model.

Materials and Methods: Rats were divided into: sedentary sham (SedS), sedentary MI (SedMI), and exercise training MI (ExMI). The impact of MI and exercise training on propensity to ventricular tachycardias (VTs) was evaluated by in vivo intra-cardiac S1-S5 pacing protocol before optical imaging on perfused hearts.

Results: None of the SedS group experienced sustained VTs where 7/8 MI SedMI and 6/10 ExMI groups experienced VTs, respectively. When VTs lasting for more than one second were considered, only the SedMI group had significantly more arrhythmias compared to sham. Optical mapping of LV NZ showed shortened diastolic interval (DI, p=0.006) and prolongation of action potential duration at 50% (APD50) at higher pacing rates (6.5-10Hz) in SedMI hearts (p<0.001). APD50 and APD90 showed spatial heterogeneity in SedMI hearts (p<0.01). Exercise training partly restored DI and APD heterogeneity at high heart rates. AP rise time became progressively slower in the BZ and IZ of both SedMI and ExMI groups and APs of comparable amplitude were seen in the IZs of both groups reflecting similar amounts of remnant myocardium. AP rise time was slower in the IZ of SedMI hearts compared to ExMI hearts (p=0.01).

Conclusion: The SedMI group were significantly more prone to arrhythmias compared to SedS. The exercise training intervention partly restored the electrophysiological status of the MI rats and this was associated with a reduced capacity to maintain arrhythmias. This anti-arrhythmic effect of exercise was associated with partly normalization of APD, DI and AP rise time of the NZ and the remnant myocardium within IZ.
mTORC1 is not involved in the AMPK/PI3K long-term regulation of HCN4

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The “funny current” (If) is a mixed Na+/K+ current expressed in cardiac pacemaker cells, inward and activated on hyperpolarization, that contributes essentially to generation of spontaneous activity and rate regulation. The funny current is carried by the hyperpolarization activated cyclic nucleotide gated channel 4 (HCN4), which is predominantly expressed in the natural pacemaker, the sinus atrial node (SAN). Whereas the short-term regulation of HCN4 channels by cAMP is a well-established mechanism, the long-term regulation that affects HCN4 membrane expression and the normal heart rate in ageing, prolonged training or chronic pathologies is still unclear. Recent studies demonstrated that PI3K and AMPK have opposite effects on If amplitude, indicating a role of these protein in the regulation of HCN4 membrane expression. Since AMPK shares intracellular pathways with protein kinases such as PKA, Akt and PI3K (e.g. mTORC1 pathway), we focused on the relation between the two kinases to understand if there is a common mechanism that underlies the regulation of If by the two kinases.

By whole-cell patch-clamp recording, we measured the amplitude of If and found that the pharmacological activation of AMPK by Metformin 5 µM or AICAR 1 mM downregulated the current in both isolated murine SAN cells and HEK293T cells transiently transfected with hHCN4. To assess the involvement of mTOR1 complex we repeated the experiments exposing cells to Rapamycin 10 µM for different time intervals; we did not find significant differences on the current amplitude between treated cells and controls. In addition, since AMPK activators and PI3K inhibitors have similar effects on If, we are testing the combined treatments. In conclusion, our preliminary data suggest that although both AMPK and PI3K modulate the If amplitude, mTORC1 does not appear to be involved as a common pathway shared by the two proteins in the regulation of HCN4 channels.
Electrophysiological remodelling in primary versus secondary cardiac hypertrophy: a study in human cardiomyocytes

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**Background**

Myocardial hypertrophy is an adaptive disease often involving the left ventricle (LV) and leading to an enhanced risk of arrhythmias by different mechanisms. LV hypertrophy may be caused by genetic abnormalities (hypertrophic cardiomyopathy (HCM)) or mediated by pathological conditions as the aortic stenosis. In this work, we will compare the cellular mechanisms of disease associated with primary (HCM) or secondary LV hypertrophy (aortic stenosis with hypertrophic septum: AoS-HT).

**Methods**

Human septal specimens from HCM, AoS-HT and non-failing non-hypertrophic control (CTRL) surgical patients were collected from the operating room, then processed to isolate single cardiac cells. We performed patch clamp experiments to measure action potential duration (APD) and calcium current (ICaL). To study intracellular Ca2+ handling, we performed fluorescence measurements on isolated cardiomyocytes using Ca2+ sensitive dye (CAL520).

**Results**

We evaluated APD, ICaL, and Ca2+ handling in the 3 groups: HCM, AoS-HT and CTRL cardiomyocytes. Both pathological groups show depolarized resting potential, prolonged APD, elevated diastolic [Ca2+] and slower Ca-transient (CaT) kinetics, as compared with CTRL cardiac cells. HCM cardiomyocytes show longer APDs, higher diastolic [Ca2+] and slower CaT kinetics when compared with AoS-HT cells. ICaL is comparable in CTRL and AoS-HT; however, the amplitude of ICaL is higher in HCM and the inactivation kinetics are slower, when compared with the other two groups.

**Discussion and Conclusion**

The main cellular functional alterations in primary and secondary LV hypertrophy are similar. Both show slower CaT kinetics and increase diastolic [Ca2+], that are notably associated with diastolic dysfunction and arrhythmic events. The abnormalities observed in the hypertrophic cells are not disease-specific responses but rather common compensatory mechanisms that go in parallel with the hypertrophic remodelling. Indeed, as the degree of hypertrophy is higher in HCM patients, most of cardiomyocytes functional changes are more severe in HCM rather than in secondary hypertrophy.
Type 2 phosphodiesterase regulates cardiac pacemaker activity

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Introduction: Elevated heart rate (HR) and lower HR variability predict cardiovascular morbi-mortality. HR is mainly controlled by the autonomic nervous system (ANS). During sympathetic stimulation, the activation of β-adrenergic receptors increases cAMP levels leading to positive chronotropic effect. cAMP is hydrolyzed by 6 different phosphodiesterases (PDE). One of these is PDE2 which has recently been reported to be beneficial for the failing heart. Indeed, its cardiac overexpression improves ventricular function (PDE2TG mice) and lowers heart rate1. However, whether this is due to a direct control sinoatrial node (SAN) function or an indirect effect via the ANS is unknown.

Objective: Define the role of PDE2 in regulating HR.

Methods PDE2 expression and cAMP-hydrolytic activity were measured in SAN by western blot and radioenzymatic assay, respectively. Spontaneous Ca²⁺ transients were measured in Fluo4-loaded-SAN tissue using confocal microscopy at baseline and following PDE2 inhibition with Bay 60-7550 (100 nM). ECG telemetry was recorded in conscious mice in basal conditions, after ANS inhibition with atropine (atro, 1 mg/kg, ip) + propranolol (propra, 2 mg/kg, ip), or after β-adrenergic stimulation with isoprenaline (Iso, 1.5 mg/kg, ip).

Results: PDE2 is expressed in WT SAN and overexpressed by ~6-fold in PDE2TG SAN. Total cAMP-hydrolyzing activity is increased ~3.5-fold in PDE2TG SAN. PDE2TG mice display lower basal HR, during day and night. This phenotype is maintained after atro+propra and after Iso. Ex vivo, basal beating rate of PDE2TG SAN tissue is lower than that of WT SAN. The difference between the two genotypes is lost after PDE2 inhibition. While no difference in Ca²⁺ transient amplitude and time-to-peak is observed between WT and PDE2TG SAN cells, the decay time (tau) is increased in PDE2TG SAN cells.

Relationship between ion currents and membrane capacitance in canine ventricular myocytes

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In describing transmembrane ion currents, current density, the membrane current value divided by membrane capacitance (Cm) is widely used. This convention assumes that Cm and ion current magnitudes are linearly related for any given ion current, however there is no data about this in cardiac muscle. Therefore, we retrospectively analyzed parameters of major cardiac ion currents and Cm statistically, including experimental data from conventional voltage clamp (CVC) as well as action potential voltage clamp (APVC) experiments. We performed normality tests with effect size calculations and tested if dividing the original current parameters with Cm had any effect on the parameter distribution or the coefficient of variance. Relationship between the measured parameters and Cm was tested with correlation analysis and linear regression.

Under CVC conditions correlations were high for IK1, moderate for IKr and ICa,L, while negligible for IKs. In case of Ito1, correlation between peak Ito1 amplitude and Cm was negligible when analyzing all cells together, however, the analysis showed high correlations when cells of subepicardial, subendocardial or midmyocardial origin were analyzed separately.

In APVC experiments IK1, IKr and ICa,L showed high correlations between Cm and current amplitudes or current integrals. For INCX, INa,late and IKs there were low-to-moderate correlations between Cm and these current parameters.

Dividing the original current parameters with Cm either “normalized” the originally non-normal current amplitude or integral distributions or reduced the effect size of non-normality. Furthermore, dividing with Cm showed a tendency to reduce coefficient of variance, reaching statistical significance in some cases.

For most of the cardiac ion currents we found good correlations between ion current amplitudes or integrals and Cm. Limited correlations are likely consequences of spatial inhomogeneity of ion current density and/or non-ideal experimental conditions. This must be considered when interpreting ion current measurements in cardiac cells.
Effect of ageing on heart rate variability in the small primate Microcebus murinus: an emerging model of senescence

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Background/Introduction: Elderly people suffer from maximal heart rate (HR) decrease, which limits aerobic capacity and self-sufficiency. Moreover, reduction of the physiological HR variability (HRV) has been reported in aged humans, horses and mice, with uncertain health consequences. The causes of such HR alterations, which could degenerate into life-threatening arrhythmias, are still unclear, hampering the development of treatments to improve HR in the elderly.

Aim: To clarify why HR and HRV decrease with ageing, we used the small mouse lemur (ML) Microcebus murinus model. This primate shares genetic origins with humans and, unlike rodents, spontaneously develops age-related neurodegeneration that could also affect the autonomic nervous system.

Methods: HR and HRV result from the intrinsic cardiac activity, generated by the sinoatrial node (SAN), and from its autonomic modulation. To investigate HR alterations, we used MLs because they provide an age-related neurodegenerative environment more similar to humans. Using surface electrocardiograms, we recorded ML HR at rest or under mild stress (induced by handling the animals).

Results: We observed a lower HR in aged versus young MLs only under stress (470±10 vs. 493±12 n=7, n=6). We also found reduced HRV in aged versus young animals at rest, whereas stress reduced HRV at equivalent low levels in both groups. This was true for the long-term HRV indices related to the sympathetic modulation. Conversely, short-term HRV, related to parasympathetic modulation, was similar in young and aged MLs at rest, and decreased similarly under stress. According to in vivo results, optical mapping of pacemaker activity in SANs isolated from aged MLs (n=14) showed impaired response to the β-adrenergic agonist isoprenaline and a normal cholinergic response to acetylcholine.

Conclusions: Our preliminary results suggest that β-adrenergic impairment, which may be related to decreased sympathetic sensitivity of the SAN, could explain the maximal HR decrease in aged MLs.
Ablation of axons leading to the AV node and to the sinus node for cardioablation in patients with vagal bradyarrhythmias.

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Functional bradyarrhythmias are caused by parasympathetic hyperactivity in the absence of an organic lesion of the cardiac conduction system. This leads to various types of blocks (atrioventricular block, sinus arrest) and the development of symptoms such as dizziness, severe weakness and syncope. At the moment, ablation of nerve ganglia is practiced in the world to suppress vagal hyperreactivity and serves as an alternative to pacemaker implantation. We have studied a simplified technique of cardioneuroablation in this group of patients and present you the results.

**Purpose:** to evaluate the effectiveness of a simplified technique of cardioneuroablation in patients with vagal bradyarrhythmias.

**Methods:** we performed cardioneuroablation in 15 patients with symptomatic vasovagal bradyarrhythmias. Among them, 6 women, 8 men. Mean age 32±6.3 years. On the daily ECG monitor, were recorded sinus node arrests and atrioventricular blockades of various degrees. All patients underwent ablation according to the following scheme: 1. Stimulation of the carotid sinus (frequency 3000 pulses per minute, amplitude 15 volts, duration 5 ms). 2. Patients with atrioventricular block underwent linear ablation from the oval window to the coronary sinus, with sick sinus node - ablation in the area where the superior vena cava flows into the right atrium along the posterior and septal wall. 3. Re-stimulation of the carotid sinus to confirm the effectiveness.

**Results:** at follow-up for an average of 6.2±1.3 months, patients did not report symptoms. During the control daily ECG monitoring, no blockade was recorded, except for one patient who had 2 episodes of 2nd degree atrioventricular block, type 1, at night.

**Conclusion:** Cardioneuroablation is currently an alternative to pacemaker implantation in certain categories of patients. A simplified version of cardioneuroablation has demonstrated its effectiveness, which may lead to a greater distribution and use of this ablation technique, leveling the risks associated with excessive damage to the ganglia or the risks associated with pacemaker implantation.
Role of protein kinase A and calcium/calmodulin-dependent protein kinase II in beta-adrenergic regulation of potassium channels in canine ventricular cardiomyocytes

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Acute β-adrenergic receptor (β-AR) stimulation robustly regulates cardiac potassium currents to shorten the ventricular action potential (AP). Besides the protein kinase A pathway, downstream effects are also mediated by calcium/calmodulin-dependent protein kinase II (CaMKII). Our aim was to investigate the involvement of CaMKII in mediating β-AR activation on the most important potassium currents flowing during canine ventricular AP.

Experiments were carried out on isolated cardiomyocytes originating from canine left ventricles. Rapid (IKr) and slow delayed rectifier potassium current (IKs), transient outward potassium current (Ito1) and inward rectifier potassium current (IK1) were measured under action potential voltage clamp conditions. Data were collected in six study groups: [1] Control (CTRL), [2] β-AR stimulation with 10 nM isoproterenol (ISO), [3] CaMKII inhibition (1 µM KN-93), [4] PKA inhibition (1 µM H-89), [5] KN-93+ISO, [6] H-89+ISO.

Neither Ito1 nor IKr differed significantly between the groups studied. IKs was prominently larger in ISO than under CTRL or KN-93 or H-89 conditions having an about 6-fold larger current amplitude and carrying about 8 times as much total charge. In KN-93+ISO and in H-89+ISO, IKs showed an about 2.5-3 times smaller amplitude and carried roughly half as much total charge compared to the ISO group.

IK1 amplitude did not differ between the studied groups, whereas the carried charge was about 25 % larger in ISO compared to CTRL, and about 15 % larger compared to KN-93+ISO. Under β-AR stimulation, IK1 started to activate earlier during the AP plateau. IK1 density at 0 mV was about 2 times greater in ISO compared to CTRL; and about 90 % larger in H-89+ISO compared to H-89.

CaMKII activation plays an important role in beta-adrenergic stimulation of potassium currents. Beta-adrenergic enhancement of IKs is partly, whereas enhancement of IK1 is mainly mediated by CaMKII activation.
Intravenous nicotine infusion potentiates atrial refractoriness shortening during obstructive respiratory events in pigs.

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Background: Cigarette smoking is associated with obstructive sleep apnoea (OSA). Both OSA and nicotine consumption as part of cigarette smoking impairs cardiac electrophysiology. Whether nicotine is an additive arrhythmogenic risk-factor for atrial fibrillation (AF) in OSA is unknown.

Methods: In seven spontaneously-breathing pigs (sedation; 4%-alpha-chloralose and 5µg/kg/hour fentanyl), obstructive respiratory events were simulated by intermittent negative upper-airway pressure (INAP) applied via a pressure device connected to the intubation tube. INAP was applied for 75-seconds with a 10-minute resting period in-between. Atrial effective refractory period (AERP) and AF-inducibility were measured following an S1S2-protocol before (Pre-INAP), during (INAP) and after INAP (Post-INAP). Atrial electrophysiological responses to INAP were assessed as means of two INAP-applications; during vehicle and in the presence of three intravenous nicotine (15µg/kg) boli (N1; N2; N3; continuous nicotine (10µg/kg) infusion between each boli). Additionally, heart rate (HR), sinus node recovery time (SNRT) and atrioventricular ERP (AVERP) were measured after completing each respective bolus.

Results: Nicotine infusion dose-dependently increased HR (Vehicle: 82.5bpm; N1: 85.6bpm; N2: 93.1bpm; N3: 97.6bpm (p=0.035)) and reduced both SNRT (Vehicle: 1460ms; N1: 1305ms; N2: 1268ms; N3: 1111ms) and AVERP (Vehicle: 236ms; N1: 215ms; N2: 195ms; N3: 181ms). When subjected to INAP, AERP shortened predominantly during Post-INAP (Vehicle: Pre-INAP 128ms vs INAP 113ms (p=0.26); - vs Post-INAP 94ms (p=0.056)). In the presence of IV-nicotine infusion, AERP shortened more pronouncedly already during INAP (Pre-INAP vs INAP; N1: 127ms vs 99ms (p=0.003); N2: 120ms vs 102ms (p=0.051); N3: 120ms vs 95ms (p=0.02)), whereas Post-INAP shortening remained. Early INAP-induced AERP-shortening was associated with increased AF-inducibility (Vehicle: 43% (p=0.23); N1: 57% (p=0.047); N2: 64% (p=0.018); N3: 43% (p=0.23)).

Conclusion: IV-infusion of nicotine revealed a dose-dependent increase in HR, and reduction of SNRT and AVERP. However, AERP remained stable before each INAP. INAP unmasked increased AF-susceptibility demonstrated by potentiated AERP-shortening and increased AF-inducibility.
Idiopathic ventricular fibrillation as an inherited channelopathy?

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Background: Inherited arrhythmias are often associated with variants in genes encoding cardiac ionic channels. Similar genetic variants can be also detected in some patients suffering from “true” idiopathic ventricular fibrillation (VF).

Aim: This is a pilot study to reveal proarrhythmic potential of selected genetic variants associated with “true” idiopathic VF in our patients. Two probands are going to be investigated, the first one carrying the Y4734C-RYR2 variant, the second one a combination of two KCNH2 variants, S1021Qfs*98 and A228V.

Methods: Patient-specific cardiomyocytes have been prepared from a sample of peripheral blood of Y4734C-RYR2 proband and investigation of the functional defect has been started (whole-cell patch clamp, microelectrode array). The functional analysis of KCNH2 variants expressed in Chinese hamster ovary cells is being prepared, control KCNH2 data are being collected (whole-cell patch clamp).

Results: The first experimental data showed a tendency of the patient-specific Y4734C-RYR2 cardiomyocytes to irregular electric activity at specific conditions (e.g., increased temperature, decreased extracellular K+, beta-stimulation). The ongoing analysis comparing properties of the patient-specific cardiomyocytes to control differentiated cardiomyocytes (independent control and “healthy” relative) should elucidate the origin of proarrhythmic activity in the proband. The control KCNH2 data are in agreement with the data published so far.

Conclusions: It is surprising to observe a physiological ECG both at rest and during exercise in patients with rare variants in cardiac channel genes, even in a proband with two rare KCNH2 variants and repeated episodes of VF (appearing during alarm ringing). Detailed functional analysis is needed to reveal a possible relationship between the identified genotype and phenotype. It should reveal if the “true” idiopathic VF can be an inherited channelopathy at least in some of our patients. Identification of provoking circumstances that can result in unmasking of the phenotype would be very useful from the clinical point of view.

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Electrophysiological Effects of the Transient Receptor Potential Melastatin 4 Channel Inhibitor (4-Chloro-2-(2-chlorophenoxy)acetamido) Benzoic Acid (CBA) in Canine Left Ventricular Cardiomyocytes

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Introduction: The electrophysiological role of calcium activated transient receptor potential melastatin 4 (TRPM4) ion channels in ventricular myocytes is not clarified yet. CBA (4-chloro-2-(2-chlorophenoxy)acetamido benzoic acid) has been chosen as a possible selective inhibitor to study TRPM4 function.

Aim: Study the expression of TRPM4 in canine heart and to examine the selectivity and the effect of CBA in ventricular cardiomyocytes.

Methods: Ionic currents were recorded with conventional or action potential (AP) voltage-clamp technique in whole-cell configuration at 37 °C in enzymatically isolated canine left ventricular cardiomyocytes. 10 mM BAPTA was used to avoid the activation of TRPM4. AP was recorded with conventional sharp microelectrodes and 10 µM CBA was used. Expression of TRPM4 protein was studied by Western blot.

Results: TRPM4 was expressed in the wall of all chambers of the canine heart as well as in isolated left ventricular cells. AP duration measured at 90% of repolarization and its short-term variability were reduced by CBA. AP amplitude was increased and the maximal rates of phase 0 and 1 were reduced by CBA. In AP clamp measurements, CBA-sensitive current contained a short, early outward and mainly a long, inward current. Transient outward potassium current (Ito) and late sodium current (INa,L) but not L-type calcium current was reduced in the presence of CBA. These effects of CBA were mostly reversible upon washout.

Summary: CBA induced reduction of Phase-1 slope and the slight increase of AP amplitude can be due to the inhibition of Ito. The AP shortening can be explained by the inhibition of inward currents seen in AP-clamp recordings during the plateau phase, which was identified as INa,L. CBA seems to be not entirely selective for TRPM4 channels, so it can only be used with caution to test the role of TRPM4 channels in cardiac electrophysiology of native ventricular cells.
Orange flavonoid Hesperetin prolonged action potential duration and inhibits the slow delayed rectifier potassium current (IKs) in dog and rabbit cardiac ventricular muscle preparations and isolated myocytes

Background:
Hesperetin is the main flavonoid in oranges. Flavonoids are known to reduce cardiovascular mortality, however, their effects on cardiac electrophysiology may have both antiarrhythmic and proarrhythmic consequences as they can attenuate the repolarization reserve.

Aim:
The present work aimed to study the additive inhibitory effect of Hesperetin on the repolarization of the action potential duration (APD) in dog and rabbit ventricular preparations with normal and attenuated repolarization reserve. Hesperetin effect on transmembrane slow delayed rectifier K+ current (IKs) was also investigated.

Method:
Action potentials were recorded in dog and rabbit right ventricular preparations using conventional microelectrode techniques. The repolarization reserve was attenuated using the IKr blocker Dofetilide and the late Na+ channel activator Veratrine. Transmembrane IKs was measured using the whole-cell configuration of the patch-clamp technique at 37°C.

Results and discussion:
Hesperetin 10 µM alone has no notable effect on APD, however, applying 10µM hesperetin after attenuating the repolarization reserve with Dofetilide 100nM and Veratrine 50 µg caused significant prolongation of the steady APD (from 466±18 ms to 512±23 ms (n=12)). In agreement with APD data, a moderate but statistically significant effect of 10 and 30 µM hesperetin was observed in the magnitude of transmembrane IKs.

Conclusion:
Hesperetin alone has no or negligible effect on action potential duration, therefore the risk of arrhythmia is low for healthy people. However; if the repolarization reserve has been attenuated due to some pathological conditions such as heart failure or some variable abnormalities such as adverse effects, genetic mutations, a high amount of orange juice consumption might lead to ventricular arrhythmia due to the inhibition of IKs and prolongation in the action potential duration.
ABT-333 (dasabuvir) increases the duration of canine left ventricular cardiomyocyte action potential

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Introduction: ABT-333 is an antiviral agent used against hepatitis C which has a methanesulfonamide group. The arrhythmia inducing effect of ABT-333 has been previously reported. In clinical practice, ABT-333 produced this property when its plasma concentration was increased due to inhibition of its degrading enzyme.

Aims: Our goal was to investigate the acute effects of ABT-333 on the cells enzymatically isolated from canine heart left ventricles, which is electrophysiologically a good model of the human heart.

Methods: Action potentials were recorded using a sharp microelectrode technique at 37 °C. In our experiments, ABT-333 was first applied at a concentration of 1 μM for 15 min, followed by a 20 min washout. Later we used increasing concentrations (1, 3, 10 and 30 μM, 5-5 min) in a cumulative manner.

Results: 1 μM ABT-333 reversibly increased the length of the AP with approximately 8%. When used in increasing concentrations, the drug also increased the action potential duration in a dose-dependent and reversible manner. The elongation was 7, 21, 37, and 50%, respectively. In addition, early afterdepolarizations occurred in some cells in the presence of higher ABT-333 concentrations (10 and 30 μM). ABT-333 reduced the maximal rate of the early repolarization phase of the action potential as well, but this effect was only partially reversible. At higher concentrations (10 and 30 μM), the value of the membrane potential measured at 20% of the action potential duration (plato20) was reversibly increased by 5 and 8 mV.

Summary: In light of our results, it is likely that the effect of ABT-333 on action potential is achieved primarily through the inhibition of potassium currents, mainly Ik. Slowing down early repolarization and increasing plateau20 is likely due to the inhibition of transient outward potassium current.
Molecular, electrophysiological and mechanical characterization of human induced pluripotent stem cell-derived atrial and ventricular cardiomyocytes

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BACKGROUND: Atrial and ventricular cardiomyocytes (aCM, vCM) exhibit distinct functional profiles. Generation of homogeneous populations of aCM differentiated from hiPSC is crucial to understand better atrial-specific disease mechanisms and develop chamber-specific drugs. This study aims to provide new insights into the development of human atrial lineages from hiPSC that enable generation of enriched, functional cardiomyocyte populations.

MATERIAL AND METHODS: hiPSC differentiation into aCM and vCM was initiated at the same passaging step. Differentiation into aCM was induced by adding 1uM retinoic acid from day 3 to day 8. On day 30, the two CM populations were compared using qPCR, immunostaining, and flow cytometry. Electrophysiological properties were assessed using patch clamp technique at room temperature, while active mechanical properties were analysed by nanoindentation at 37°C.

RESULTS: aCM at day 30 presented higher expression of atrial biomarkers such as CACN1D, KCNA5, KCNJ3, and NR2F2 and lower expression of ventricular genes GJA1, MYL2, IRX4, and HEY2 compared to the vCM. The two chamber selective markers MLC2v and COUP-TFII were further investigated. Flow cytometry analysis confirmed that 64.23% (±10.35) of aCM were COUP-TFII+, versus 34.83% (±08.22) of the vCM. In addition, vCM were 92.1% (±09.31) MLC2V+ versus 38.42% (±11.26) of the aCM. This was confirmed by confocal microscopy. Electrophysiological characterisation showed that aCM had shorter action potential duration compared to vCM, 77.3±17.4 ms and 264.0±53.4 ms, respectively. Contractions were also significantly shorter for aCM.

CONCLUSION: Retinoic acid treatment allows differentiation of hiPSC into atrial cardiomyocytes characterized by fast action potential and cell contraction. We envision that the ability to differentiate aCM provides a unique opportunity to study atrial physiology and pharmacological responses in a human-relevant in-vitro model.

FUNDING: This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No.860974.
Introduction: It is widely known that regular exercise helps people to live a healthy life. However, based on accumulating evidence, long-term heavy training beyond an optimal dose may also have cardiac electrophysiological adverse effects under certain circumstances.

Aims: To develop animal models with significant translational value for human athlete’s heart and to investigate potential cellular electrophysiological causes of cardiac arrhythmias because of long-term heavy endurance training in in vitro studies.

Methods: Twenty-four dogs and twenty-six guinea pigs were randomized into sedentary (‘Sed’) and exercised (‘Ex’) groups (n=12-12; n=13-13). The latter groups underwent an intensive several weeks-long training program. Characteristics of athlete’s heart were validated in in vivo studies. Degree of interstitial fibrosis was quantified in histopathological study. Left ventricular myocytes were enzymatically isolated, and ionic currents and action potential duration (APD) were recorded using patch-clamp technique. Density of transmembrane ionic channel subunits was determined in immunocytochemistry study.

Results: The 90 percent of APD was significantly lengthened in left ventricular myocytes of ‘Ex’ dogs (472.8±29.6 ms; n=29 vs. 369.3±31.4 ms; n=24 p=0.023), however, there was no difference between the groups in case of guinea pigs. The amplitude of the transient outward potassium current (Ito), which is not expressed in the guinea pig heart, was decreased in the ‘Ex’ dogs (‘Ex’ vs. ‘Sed’ 7.6±0.6 pA/pF, n=54 vs. 10.2±1.0 pA/pF, n=42, p<0.05). The HCN4 protein density was increased in ventricular myocytes obtained from ‘Ex’ dogs. Mild ventricular fibrosis was observed in ‘Ex’ animals from both species.

Conclusion: The in vivo results correspond to the human endurance-trained athlete’s heart features. The increased repolarization dispersion, the changed HCN4 protein expression profile, and the enhanced level of fibrosis may contribute to life-threatening arrhythmias in a vulnerable period. Further studies are warranted to clarify this hypothesis in more detail.

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On the Role of Immune Cells in Persistent Atrial Fibrillation Maintenance

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Background: Atrial Fibrillation (AF) shows a steady increase in prevalence worldwide, yet the cellular and molecular mechanisms underlying its long-term maintenance have not been fully addressed.

Aim: To understand the role of inflammatory cells and their interactions with other non-myocytes that may explain regional differences in AF dynamics.

Methods: We used a translational pig model of Persistent AF (PersAF) with underlying infarct-related atrial substrate to simulate a highly prevalent clinical scenario. The myocardial infarction was performed using ischemia-reperfusion in the proximal left circumflex artery, including the left atrial branch. Two months after the infarction, pigs underwent high-rate atrial pacing to develop PersAF. After an average of 8.72±2.86 months of atrial pacing and a minimum of 6 months in self-sustained PersAF, four pigs underwent in-vivo high-density electroanatomical mapping. Atrial regions with higher than surrounding average instantaneous frequency modulation were considered leading-drivers, associated with AF maintenance. Then, single-cell RNA transcriptomics were performed in driver and remote regions (n=2 pigs).

Results: Twenty distinct cell populations were identified in driver regions and remote samples based on differential expression of established lineage markers. Among the cell types that were identified (fibroblasts, smooth muscle cells, endothelial cells, macrophages, and other immune cell populations as granulocytes, B and T cells, and natural killer cells), T cells were the most predominant population in non-driver regions, encompassing 21.6% of all non-myocytes. In driver regions, macrophages were the most abundant population (11.3%). Within the macrophage cell population, we defined 6 subsets. Macrophages expressing a protective and anti-inflammatory tissue resident signature were more predominant in driver vs. non-driver regions. Transmission electron microscopy analysis further indicated that resident macrophages may support mitochondrial homeostasis in driver regions.

Conclusions: These results provide new cellular and transcriptomic data associated with regional differences in non-myocyte populations during AF maintenance in a clinically-relevant pig model of PersAF.
PO 070

Role of RyR2-S2030 phosphorylation on Calcium handling in the cardiac pacemaker

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PO 071

Pro-resolving mediators prevent Ca2+ mishandling and cardiac dysfunction induced by experimental myocarditis

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PO 072

Ca2+ handling in induced-pluripotent cardiomyocytes from a CPVT family harbouring the RyR2 R420Q mutation

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PO 073

Investigation of electrocardiographic repolarization parameters in patients with polycystic ovary syndrome

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PO 074

Electrophysiological effects of IQM-266 on cardiac Itof. Pharmacological implications on Kv4.3 channelosome

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PO 075

Electrophysiological study of a new MOG1 gain-of-function variant, L18F, found in a patient with LQT episodes

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PO 076

An in-silico analysis of the dynamic regulation of cardiac electrophysiology by Kv11.1 ion channel trafficking

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PO 077

The equine TASK-1 channel ortholog: functional expression and pharmacological characterization.

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PO 078

A dynamic clamping approach using in silico IK1 current for discrimination of chamber-specific hiPSC-derived cardiomyocytes

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PO 079

Human and Large Mammal Myocardial Slices model Plateform for Atrio-Ventricular Proarrhythmic Mechanistic Exploration

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PO 080

Formation of peripheral and dyadic junctions during cardiomyocyte maturation evaluated by electrophysiology, immunofluorescence and electron microscopy

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PO 081

Atrial fibrillation in arrhythmogenic right ventricular cardiomyopathy (ARVC) patients

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PO 082

Three-dimensional modelling of mutant Kir2.1 channel – PIP2 interactions help stratify arrhythmia severity in Andersen Tawil syndrome type 1

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PO 083

The consequences of the DPP6p.R274H variant on the Purkinje and ventricular action potential: an in-silico study

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PO 084

Optogenetic dissection of self-generating T-tubular action potential

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PO 085

Extracellular cysteine disulfide bond break disrupts PiP2-dependent Kir2.1 channel function in Andersen-Tawil Syndrome

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PO 086

The reverse mode of Na\(^{+}\)/Ca\(^{2+}\) exchanger contributes to the ‘coupled clock’ mechanism of sinoatrial pacemaking

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PO 087

SNTA1 restores the NaV1.5-Kir2.1 channelosome and improves electrical function in iPSC-CMs from DMD patients with life-threatening arrhythmias

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PO 088

Human induced pluripotent stem cell derived cardiac myocytes, a tool in molecular forensics to study sudden arrhythmic death

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PO 089

NOVEL ACTIVE FIXATION LEAD GUIDED BY ELECTRICAL DELAY CAN IMPROVE RESPONSE TO CARDIAC RESYNCHRONIZATION THERAPY IN HEART FAILURE.

DR. PAOLO VINCIGUERRA\textsuperscript{1}, MATTEO CASALE\textsuperscript{2}, MAURIZIO MEZZETTI\textsuperscript{3}, PAOLO BUSACCA\textsuperscript{2}, FRANCESCA PARISI\textsuperscript{1}, LORENZO PISTELLI\textsuperscript{1}, ROSALBA DE SARRO\textsuperscript{1}, PASQUALE CREA\textsuperscript{1}, GIUSEPPE DATTILO\textsuperscript{1}

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PO 095

Disparity between calcium transient duration and action potential duration as the basis for ectopic beats and non-sustained ventricular tachycardia

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PO 096

Angiotensin-converting enzyme inhibition in heart failure induced by volume overload in rats

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PO 097

Modeling and Simulation of the Effects of nNOS Inhibition on the Right and Left Atrium: Implications for Atrial Fibrillation from Single-Cell to Body-Surface Level

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PO 098

Decreasing microtubule detyrosination by parthenolide restores sodium current in mdx cardiomyocytes

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PO 099

Human myofibroblasts alter the function of human induced pluripotent stem cell-derived cardiomyocytes through distinct paracrine and contact mechanisms

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PO 100

Assessment of cardiac toxicity of manganese chloride for cardiovascular magnetic resonance

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PO 101

SK channels characterization in the human ventricle: differences in expression levels and electrophysiological role in non-diseased and heart failure ventricles

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