models. Ultimately these studies may identify new therapeutic targets for CKD patients, to reduce their cardiovascular risk.

216 IDENTIFYING A NOVEL ROLE FOR PMCA1 (ATP2B1) IN HEART RHYTHM INSTABILITY

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10.1136/heartjnl-2017-311726.214

Arrhythmias continue to be a leading cause of death and disability across the world, and genetics are one of the mechanisms that are known to increase susceptibility. By identifying new genetic influences and further understanding the pathways involved in heart rhythm control we can begin to tackle some of the main challenges facing treatment development.

Here we aim to identify a new role for a gene linked to several features of heart failure *Atp2b1* (Plasma membrane calcium ATPase 1, PMCA1). Along with its role in hypertension and other aspects of cardiac physiology, we believe PMCA1 may also influence heart rhythm stability and consequently the development of arrhythmias.

To investigate the role of PMCA1 in cardiac rhythm, cardiomyocyte-specific knockout mice (PMCA1^{CKOÃ,Â}) were generated. *In vivo* electrocardiography showed PMCA1^{CKO} displayed signs of cardiac repolarisation dysfunction related to prolonged QT and JT intervals. Supplementary analysis using Langendorff-perfused hearts revealed PMCA1^{CKO} hearts have prolonged action potential duration compared to controls. Additionally using the methods highlighted above, PMCA1^{CKO} mice were shown to have an increase arrhythmia susceptibility to both *in vivo* and *ex vivo* programmed electrical stimulation. Further echocardiography and histological analysis showed these heart rhythm abnormalities occur in the absence of detectable structural heart disease with PMCA1^{CKO} cardiac structure and function being comparable to controls.

Our findings suggest a novel role for PMCA1 in heart rhythm stability, distinct from other cardiac disease. Furthermore, alterations in expression of *Atp2b1* could influence an individuals susceptibility to developing arrhythmias.

217 GENETIC ABLATION OF MICROTUBULE-ASSOCIATED PROTEIN 1S (MAP1S) PROTECTS THE HEART FROM PATHOLOGICAL HYPERTROPHY VIA REGULATION OF AUTOPHAGY

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10.1136/heartjnl-2017-311726.215

Background Autophagy is a process essential in maintaining cellular homeostasis, by degrading and recycling unwanted materials such as misfolded proteins and dysfunctional organelles. In the heart, autophagy is key in mediating pathological processes such as hypertrophy and remodelling. Autophagy is tightly regulated by a number of proteins and defective autophagy in response to pathological stimuli may lead to the development of adverse remodelling and eventually heart failure. In this study we investigated the role of microtubule-associated protein 1S (MAP1S) in regulating autophagy during a number of cardiac pathological conditions. MAP1S has previously been identified as an interacting partner of the major autophagy regulator LC3; however, its role in the heart is unknown.

Results We used siRNA gene silencing to knockdown MAP1S in neonatal rat cardiomyocytes (NRCM) and detected the autophagic flux using GFP-LC3 expressing adenovirus. Following stimulation with rapamycin (5 uM) and chloroquine (3 uM) for 2 hours, NRCM lacking MAP1S exhibited an increase in autophagy as indicated by a significant elevation in GFP-LC3 puncta formation. To confirm this finding we cultured fibroblasts from MAP1S knock out (MAP1S-/-) mice and induced autophagy using the same stimulus. Consistently, MAP1S-/- fibroblasts also showed increased autophagy after rapamycin/chloroquine treatment. Interestingly, the expression of autophagic modulators LC3II, Beclin and p62 did not differ between cells lacking MAP1S and controls, suggesting that MAP1S might affect autophagosome elongation and not the initiation process. *In vivo*, we confirmed higher autophagy in MAP1S-/- mice following rapamycin/chloroquine intraperitoneal injection as indicated by the number of amphisomes detected by electron microscopy. Next, to test the effects of pathological stimuli we subjected MAP1S-/- mice to transverse aortic constriction (TAC, 2 weeks) or myocardial infarction (MI, 4 weeks). Following TAC, MAP1S-/- mice displayed less hypertrophy as indicated by heart weight/body weight ratio, cardiomyocyte surface area (histology) and the expression of hypertrophic markers. Consistently, after MI MAP1S-/- mice also showed a reduction in hypertrophy.

Conclusions Our findings suggest that genetic inhibition of MAP1S induces autophagy in cardiomyocytes, whereas *in vivo* ablation of this gene in mice reduces cardiac hypertrophy in response to pathological stimuli such as TAC and MI.

218 KNOCKOUT P47PHOX REDUCES ANGIOTENSIN II-INDUCED CARDIAC OXIDATIVE STRESS AND HYPERTROPHY

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10.1136/heartjnl-2017-311726.216

Oxidative stress due to the activation of a Nox2-containing NADPH oxidase is involved in Angiotensin II (AngII)-induced cardiovascular dysfunction. p47^{phox} is a key regulatory subunit of Nox2. However, the role of p47^{phox} in AngII-induced cardiac damage remains unclear. In this study, we used littermates of C57BL/6 wild-type (WT) and p47^{phox} knockout (KO) mice (n=7) at the age of 10~12 months to investigate the effect of p47^{phox} knockout on AngII-induced cardiac oxidative stress and hypertrophy. In WT mice, AngII infusion (1 mg/kg/day for 14 days) increased significantly the systolic blood pressure (SBP) from 127±13 to 172±11 mmHg, and this was accompanied with significant indication of cardiac hypertrophy (heart/body weight ratio increased ~17.9±0.1%) as compared to vehicle infused controls ($p<0.05$). However, in p47^{phox} KO mice, AngII infusion caused a mild increase in SBP (from 119 ±9 to 149±10 mmHg, $p<0.05$) without significant increase in heart/body weight ratio. Cardiac production of reactive oxygen species (ROS) was examined by both lucigenin-chemiluminescence and DHE fluorescence. Compared to vehicle controls,

AngII infusion caused two-fold increase in ROS production of WT hearts ($p<0.05$) (but not $p47^{phox}$ KO mice), which was inhibited significantly by diphenyleneiodonium (DPI, a flavoprotein inhibitor) or superoxide dismutase, significantly but slightly by NG-nitro-l-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor), but not by rotenone (mitochondrial respiratory chain inhibitor) or oxypurinol (xanthine oxidase inhibitor). Increased ROS production in WT AngII-infused hearts was accompanied by significant phosphorylation of ERK1/2. In conclusion, $p47^{phox}$ and $p47^{phox}$ signalling through ERK1/2 play an important role in AngII-induced cardiac hypertrophy.

219 GALANGIN, A DIETARY FLAVONOID REDUCES MITOCHONDRIAL DAMAGE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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10.1136/heartjnl-2017-311726.217

Introduction Hyperglycemia-induced ROS generation within mitochondria plays a major role in the development of diabetic complications. Mitochondria are one of the most important cell organelles in diabetes research because of its crucial role as a regulator of energy balance. The present study was aimed to evaluate the effect galangin, a flavonoid, on oxidative mitochondrial damage in in streptozotocin (STZ)-induced diabetic rats.

Materials and methods Diabetes was induced by intraperitoneal administration of low dose of STZ (40 mg/kg body weight (BW)) into male albino Wistar rats. Galangin (8 mg/kg BW) or glibenclamide (600 µg/kg BW) was given orally daily once for 45 days to normal and STZ-induced diabetic rats.

Results Diabetic rats showed a significant ($p<0.05$) increase in kidney and heart mitochondrial oxidant (Thiobarbituric acid reactive substance) levels and a significant decrease in enzymatic (superoxide dismutase, glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidants levels as compared to control rats. The activities of mitochondrial enzymes such as isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase and mitochondrial respiratory chain enzymes such as NADH dehydrogenase and Cytochrome c-oxidase were decreased significantly ($p<0.05$) in diabetic rats as compared to control rats. Administration of galangin to diabetic rats resulted in the following findings as compared to diabetic control rats: the oxidant levels decreased significantly ($p<0.05$); the enzymatic and non-enzymatic antioxidants levels increased significantly ($p<0.05$); and the function of mitochondrial enzymes and the mitochondrial respiratory chain enzymes increased significantly ($p<0.05$).

Conclusion From the results, we conclude that galangin could maintain kidney and heart mitochondrial function in diabetic rats.

220 DAPHNIA MAGNA AS A MODEL FOR QUANTIFYING CHAOS IN CARDIAC ARRHYTHMIA

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10.1136/heartjnl-2017-311726.218

Introduction *Daphnia magna* are an established model in ecology for the investigation of toxins in freshwater systems, as well as an emerging model in medical science. *Daphnia* have a myogenic heart, exhibiting responses comparable to that of the human heart to a range of established therapeutics, and displaying varying arrhythmias on exposure to pro-arrhythmic agents. Given the multitude of mathematical methods put forward to predict arrhythmia, it is surprising as yet none are in clinical use. This study aims to rectify this issue.

Methods *D. magna* cardiac action was captured on HD film for periods of 24 s (120+ heart contractions) both prior to and following chemical induction of cardiac arrhythmia. A novel semi-automatic process gave heart area values over the full 1440 frames per film. Along with time domain data, this gave parameters for heart rate and cardiac output after parabolic peak interpolation. Data were analysed in linear terms, including ellipse fitting[1] and standard deviation of successive differences;[2] and in non-linear terms including complex correlation,[3] multi-scale ratio analysis,[4] median stepping increment[5] and finite time growth.[6]

Results Results demonstrate that non-linear analysis methods are superior to linear methods in differentiating cardiac arrhythmias from both one another and from normal rhythm. While most published methods do not differentiate arrhythmic heart conditions with significance, finite time growth, by contrast, may offer some headway toward a robust method of quantifying cardiac arrhythmia.

Implications The *Daphnia* filmatographic model presents an opportunity to examine heart action *in vivo*; offering highly accessible means of assessing both current and developing models for the prediction of arrhythmias.

REFERENCES

1. Mohebbi M, Ghassemian H. Prediction of paroxysmal atrial fibrillation based on non-linear analysis and spectrum and bispectrum features of the heart rate variability signal. *Comput Methods Programs Biomed* 2012;105(1):40–9.
2. Galland BC, Taylor BJ, B, *et al*. Heart rate variability and cardiac reflexes in small for gestational age infants. J App Physiology 2006;100(3):933–9.
3. Karmakar CK, Khandoker AH, *et al*. Complex Correlation Measure: a novel descriptor for PoincarÃƒÂ© plot. *BioMed Eng OnLine* 2009;8(17).
4. Huo C, Huang X, *et al*. A multi-scale feedback ratio analysis of heartbeat interval series in healthy vs. cardiac patients. *Med Eng Phys* 2014;36(12):1693–1698.
5. Gong Y, Lu Y, *et al*. Predict Defibrillation Outcome Using Stepping Increment of Poincare Plot for Out-of-Hospital Ventricular Fibrillation Cardiac Arrest. *BioMed Res Int* 2015;(493472):7.
6. Wessel N, Voss A, *et al*. Nonlinear analysis of complex phenomena in cardiological data. Herzschr Elektrophys 2000;11:159–173

221 ALTERED BIOPHYSICAL PROPERTIES OF THE VOLTAGE-GATED SODIUM CHANNELS IN MOUSE ATRIAL AND VENTRICULAR CARDIOMYOCYTES

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10.1136/heartjnl-2017-311726.219

Introduction Several antiarrhythmic drugs target the cardiac sodium current I_{NA}. There is an increasing interest in atrial-