

PW 22:
**Cardiomyopathies and
experimental models**

PW22-271	<p>A GENETIC VARIANT OF THE HISTIDINE-RICH CALCIUM BINDING PROTEIN CONFERS SUSCEPTIBILITY TO ARRHYTHMOGENESIS AND SUDDEN CARDIAC DEATH IN DCM PATIENTS</p> <p>ARVANITIS DA¹, SANODOU D¹, KOLOKATHIS F², VAFIADAKI E¹, THEODORAKIS G³, KONTROGIANNI-KONSTANTOPOULOS A⁴, PARASKEVAIDIS IA², ADAMOPOULOS S², DORN II GW⁵, KREMASTINOS DTh², KRANIAS EG⁵</p> <p>(1) Biomedical Research Foundation, Academy of Athens, Athens, GREECE. (2) University of Athens, Medical School, "Attikon" University Hospital, Athens, GREECE. (3) Onassis Cardiac Surgery Center, Athens, GREECE. (4) University of Maryland Baltimore, Baltimore, USA. (5) University of Cincinnati, Cincinnati, USA.</p>
To contact the author:: arvanitd@bioacademy.gr	<p>Abnormal Ca-cycling in the cardiomyocyte is a hallmark of dilated cardiomyopathy (DCM). Furthermore, DCM patients are at substantial risk for sudden cardiac death due to malignant ventricular arrhythmias. The histidine-rich calcium binding protein (HRC) is a low affinity, high capacity, calcium handling protein that has been implicated in the regulation of both sarcoplasmic reticulum calcium release and uptake. To address whether HRC genetic variants may be associated with DCM development and progression, we screened 123 non-ischemic DCM patients and 96 healthy individuals by single strand conformation polymorphism analysis and direct sequencing. Six genetic variants were identified L35L, S43N, S96A, E202_E203insE, D261del and an in frame insertion of 51 amino acid residues at H321. A statistically significant correlation was observed between the S96A polymorphism and the occurrence of life-threatening ventricular arrhythmic events, defined as sustained ventricular tachycardia, ventricular fibrillation or sudden cardiac death, in 28 patients (22.8%). During a follow-up period of 4.02 ± 2.4 years, the risk for ventricular arrhythmias was higher in the S96A homozygous patients. Using multivariate Cox regression analysis, the S96A polymorphism was the only significant arrhythmogenesis predictor in DCM patients, with a hazard risk of 4.1 (95% CI: 2.0 to 8.2; p<0.001). In conclusion, our findings demonstrate that the S96A genetic variant of HRC is associated with life-threatening ventricular arrhythmias in nonischemic DCM and may serve as an independent predictor of susceptibility to arrhythmogenesis in the setting of dilated cardiomyopathy.</p>

PW22-272	<p>EVALUATION OF AN INNOVATIVE THERAPEUTIC APPROACH AND NEW TOOL FOR EARLY DIAGNOSIS OF CARDIOMYOPATHIES. MONGUE-DIN H¹, LIU JM², SALMON A¹, FISZMAN MY¹, WDZIECZAK-BAKALA J², FROMES Y¹ (1) Institut de Myologie, INSERM U582, Paris, FRANCE. (2) ICSN,CNRS, Gif-sur-Yvette, FRANCE.</p>
<p>To contact the author:: h.monguedin@institut-myologie.org.</p>	<p>The chronically failing heart is characterized by alterations in tissue structure, particularly fibrous tissue formation, responsible for the loss of myocardial compliance. Furthermore, rhythm disturbances are commonly observed. The underlying arrhythmogenic mechanisms are multiple, but myocardial fibrosis is frequently observed. Heart rate variability (HRV) reflects the functional status of the autonomic nervous system and its effects on sinus node. Considering autonomic dysfunction playing an important role in the pathogenesis and prognosis of congestive heart failure, HRV analysis may help to identify those who are at risk of cardiac death. Antifibrotic drugs may be considered for the treatment of heart failure.</p> <p>These experiments aimed at studying the diagnostic potential of HRV analysis to detect early modifications in cardiac electrical activity and at investigating the potential benefits of an antifibrotic treatment in CHF147 cardiomyopathic hamsters.</p> <p>HRV analysis was based on quantitative analysis of time series and allowed determining an early dysfunction in cardiac electrical activity in CHF147 cardiomyopathic hamsters. Pharmacological assays, coupled with HRV analysis allowed identifying a high sympathetic activity. Western blot and RT-PCR studies confirmed the neurovegetative imbalance. In a second set of experiment CHF147 hamsters were treated during 42 days with 3 different doses of Ac-SDKP or placebo, using osmotic minipumps. Weight and size of the ventricles were comparable in all groups. Ventricular fibrosis was quantified on Sirius red stained cryostat sections. Whereas fibrotic scar tissue remained comparable, interstitial fibrosis was significantly less pronounced in treated groups. This difference appeared to be more important for the lowest dose of Ac-SDKP. Histological results were confirmed by immunohistochemistry.</p> <p>Thus, HRV analysis appears to be an interesting tool for early diagnosis of cardiomyopathies, detecting decreased heart rate variability and activation of the sympathetic tone. Moreover, an antifibrotic treatment based on low doses of Ac-SDKP might be of therapeutic interest in cardiomyopathies.</p>

PW22-273	<p><u>CARDIAC MYOSIN-BINDING PROTEIN C MODULATES THE TUNING OF THE MOLECULAR MOTOR IN THE HEART</u> LECARPENTIER Y¹, VIGNIER N², OLIVERO P³, GUELICH A⁴, CARRIER L⁵, COIRAUT C⁶ (1) Inserm U689, Cardiovascular Research Center, Paris, FRANCE. (2) Inserm U582, Institut de Myologie, Paris, FRANCE. (3) Inserm U689, Cardiovascular Research Center, Paris, FRANCE. (4) Inserm U689, Cardiovascular Research Center, Paris, FRANCE. (5) Institute of Experimental and Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, GERMANY. (6) Inserm U582, Institut de Myologie, Paris, FRANCE.</p>
To contact the author:: n.vignier@institut-myologie.org.	<p>The precise role of cardiac myosin binding protein C (cMyBP-C) on actomyosin interaction (AMI) remains unknown. We hypothesized that the lack of cMyBP-C impaired cardiac AMI. Experiments were performed on 16 weeks old cMyBP-C^{-/-} (KO) and age-matched wild-type (WT) mice (n=20/group). <i>In vitro</i> mechanical and energetics properties were performed on left ventricular (LV) papillary muscles and Huxley's equations were used to characterize AMI. <i>In vitro</i> motility assays were performed using myosin purified from LV. Myosin-based sliding velocities of actin filaments were analyzed at baseline, after pretreatment of the myosin solution with 10 umol of the catalytic subunit of PKA and/or in the presence of increasing amount of alpha-actinin, an actin-binding protein that acts as an internal load thereby providing an index of relative isometric force. Western-blot analysis was used to quantify cMyBP-C and phosphorylated cMyBP-C. The probability for myosin to be weakly bound to actin was higher in KO than in WT (p<0.05), whereas the number of strongly bound, high-force generated state cross-bridges was lower in KO (p<0.001). The unitary force per AMI was lower in KO than in WT (p<0.01). At baseline, myosin-based velocities of actin were slower in KO than in WT (1.65±0.01 vs. 1.98±0.01 um/s, p<0.01). The minimum amount of α-actinin needed to completely arrest the thin filament motility was significantly higher in WT than in KO (p<0.001). As expected, cMyBP-C was present in WT myosin solution whereas cMyBP-C was not detected in KO. In WT, PKA induced a 1.6-fold increased in cMyBP-C phosphorylation associated with a 53±1% increase in the amount of α-actinin required to arrest thin filament motility (p<0.001). PKA did not modify sliding velocity in WT. In KO, PKA had no effect on myosin sliding. We conclude that cMyBP-C regulates AMI by limiting inefficient cross-bridge formation and by enhancing the power stroke step.</p>

PW22-274	<p><u>ATTEMPTS TO RESCUE A DILATED CARDIOMYOPATHY INDUCED BY CARDIAC-SPECIFIC DISRUPTION OF THE SRF GENE IN THE MOUSE</u> DECAUX JF¹, LAMOTTE L¹, ESCOUBET B², TOUVRON M¹, ROSENTHAL N³, LESSARD J⁴, MERICKSKAY M⁵, LI Z⁵, DAEGELEN D¹, TUIL D¹ (1) INSERM U567, CNRS UMR 8104, Université Paris Descartes, Paris, FRANCE. (2) Faculté de Médecine Xavier-Bichat, Université Paris 7, Paris, FRANCE. (3) Mouse Biology Unit Monterotondo, Rome, ITALY. (4) University of Cincinnati, Cincinnati, USA. (5) CNRS UMR 7079, Université Paris 6, Paris, FRANCE.</p>
To contact the author:: decaux@cochin.inserm.fr.	<p>Serum response factor (SRF) is a transcription factor controlling the expression of many extracellular signal-regulated genes as well as genes encoding sarcomeric contractile proteins. Many SRF target genes are involved in familial hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). By using the tamoxifen inducible Cre/loxP system, we have previously shown that SRF is crucial for adult cardiac function and integrity. Triggering in mice cardiac-restricted knock out of SRF led to impaired left ventricular function with reduced contractility, subsequently progressing to DCM without hypertrophic compensation. Under these conditions, all mutant mice died from heart failure 10 weeks after tamoxifen injections. Functional and structural heart defects were preceded by early alterations in the cardiac gene expression program particularly for cardiac α-actin, IGF-1, muscle creatine kinase and calcium-handling genes.</p> <p>To evaluate the importance of altered IGF-1 and cardiac α-actin genes expression in the progression of DCM and compensate for their loss, we mated our mice model of inducible SRF disruption with transgenic mice overexpressing either IGF-1 or cardiac α-actin specifically in the heart. Cardiac-specific IGF-1 or cardiac α-actin overexpression both attenuates the progression of DCM due to the loss of SRF. Cardiac IGF-1 overexpression improves functional parameters and the survival of the animals until almost 4 months. In the same way, an almost normal cardiac function and a life extension beyond 6 months were associated to cardiac α-actin compensatory expression. Histological and molecular analysis of these new models are in progress.</p>

PW22-275	<p>ISLET-1 CARDIAC PROGENITORS DETECTION IN MOUSE KHATTAR P¹, DECOSTRE V¹, CATELAIN C¹, SCHWARTZ K¹, FISZMAN M¹, BONNE G¹, VILQUIN JT¹ (1) Inserm U582 - Institut de Myologie, UPMC Univ Paris 06, UMR S582, IFR14, Paris, FRANCE.</p>
To contact the author:: jt.vilquin@institut-myologie.org.	<p>Purpose: Islet-1+ cells are cardiogenic and angiogenic progenitors participating to heart formation. The first aim was to locate and quantify these cells in view of potential pre-clinical developments. The second aim was to compare their presence and localisation in normal animals and in a model of dilated cardiomyopathy, the Lmna-H222P mouse carrying a lamin A/C gene mutation.</p> <p>Methods: We set up a time-course study in C57BL/6J, SVJ129, KI-Lmna^{H222P/H222P} mice and wild type littermates. Animals were sacrificed at days 1, 5, 10, 15, 20, 25, then every month (from 3rd to 12th) and Islet-1+ cells detected by immunohistofluorescence on consecutive cryostat sections of whole heart.</p> <p>Results: Islet-1+ cells are gathered in one cluster in C57BL/6J animals. Cells are located in the proximal (basal) portion encompassing 8 to 20% of the organ height in outflow tract, atria, ventricles, septa. Approximately 150 to 300 cells are observed in each heart, from birth to 7 month. In the KI-Lmna mice (C57BL/6J x SVJ129) and wild-type SVJ129, cells are more restricted to atria and septa, and persist beyond 10 months. Importantly, a second cluster located in a more proximal (basal) position contains numerous cells (800-1800) of smaller size, and is no longer observed after the age of 4 months. The cells in this second cluster express sarcomeric actin and are intricated within the resident cardiac tissue. No significant differences are observed between the KI-Lmna^{H222P/H222P} mice and their wild-type littermates. Ki67 expression is not detected, suggesting that islet1+ cells are not proliferating.</p> <p>Conclusions: The presence and localisation of Islet-1+ cells in the heart of young adult mice is linked to the genetic background. However, their low amounts and specific localisation make Islet-1+ cells unlikely to soon become practical tools in a therapeutic perspective.</p> <p>Supported by grants from Leducq Foundation (CaPTAA network) and AFM.</p>

PW22-276	<p>MICRORNA SIGNATURE IN CARDIAC COMMITTED HUMAN EMBRYONIC STEM CELLS NURY D¹, BARBRY P², PUCÉAT M³ (1) INSERM UMR861, evry, FRANCE. (2) IPMC CNRS, nice, FRANCE. (3) INSERM UMR861, evry, FRANCE.</p>
<p>To contact the author:: dnury@istem.genethon.fr.</p>	<p>Genetic cardiomyopathies are predominant among rare diseases. They often originate from mutations in early cardiac transcription factors. Human Embryonic stem (HES) cells represent a key developmental model which recapitulate early cardiogenesis. MicroRNAs are emerging as key regulators of transcriptional pathways, acting as inhibitors of translation initiation. Lineage specification of Human Embryonic stem cells is a tightly regulated process in which miRNAs are likely to play a key role. Using a Chip technology, we compared the expression profile of miRNAs in two distinct HES cell lines (HUES-1 and HUES24). HES cells were then committed toward a cardiac lineage using 10 ng/ml BMP2 for 48hrs. Comparison of HES cells and cardiac specified cells revealed a differential expression of 110 miRNAs (45 were up-regulated and 55 downregulated following BMP2 treatment). Chip data were validated by stem-loop real time PCR. The pattern of miRNA expression was then correlated with the one of mesodermal gene expression induced by the morphogen in both cell lines. Profile of miRNAs under basal or BMP2 conditions will help to comprehend early cardiogenesis under normal or pathological (i.e genetic disease) conditions. This work is supported by the French National Research Agency (grant Blanc06-28138290).</p>

PW22-277	<p><u>CORONARY ARTERY PATTERNING IN TBX1 NULL EMBRYOS.</u> THÉVENIAU-RUISSY M¹, DANDONNEAU M², MIQUEROL L³, KELLY R⁴ (1) Developmental Biology Institute, Marseille, FRANCE. (2) Developmental Biology Institute, Marseille, FRANCE. (3) Developmental Biology Institute, Marseille, FRANCE. (4) Developmental Biology Institute, Marseille, FRANCE.</p>
To contact the author:: thevenia@ibdml.univ-mrs.fr.	<p><i>TBX1</i>, encoding a T-box containing transcription factor, is the major candidate gene for del22q11.2 or DiGeorge syndrome, characterized by craniofacial and cardiovascular defects including tetralogy of Fallot and common arterial trunk.</p> <p>Mice lacking <i>Tbx1</i> have severe defects in the development of pharyngeal derivatives including cardiac progenitor cells of the second heart field contributing to the arterial pole of the heart. The outflow tract of mutant embryos is short and narrow and fails to septate resulting in a common arterial trunk. A series of crosses using transgene markers of the second heart field and coronary artery endothelial cells reveal that <i>Tbx1</i> mutant hearts form in the absence or severe reduction of a specific subpopulation of progenitor cells normally giving rise to subpulmonary myocardium. The <i>Tbx1</i> mutant ventricular outlet thus has an aorta-like morphology with three valve leaflets. This defect is associated with anomalous coronary artery patterning. Both right and left coronary ostia form predominantly at the right/ventral sinus in mutant hearts; as a result, proximal coronary arteries course across the normally coronary free ventral region of the heart. We have identified <i>Semaphorin3c</i> as a <i>Tbx1</i>-dependent gene expressed in subpulmonary myocardium.</p> <p>Our results provide new insights into the association between conotruncal defects and coronary artery anomalies and implicate second heart field derived cells in coronary artery patterning.</p>

PW22-278	<p>GLYCOSAMINOGLYCANS IN THE EXTRACELLULAR MATRIX REMODELLING DURING AGEING AND CARDIAC DISORDER</p> <p>HUYNH M¹, GARCIA-FILIBE S¹, MORIN C¹, BARBIER-CHASSEFIÈRE V¹, BESSE S¹, NARASSIMPRAKASH H¹, JENISKENS G², MARTELLY I¹, PAPY-GARCIA D¹</p> <p>(1) Laboratory CRRET, CNRS UMR-7149, Université Paris 12 / Paris-Est, Créteil, FRANCE. (2) Department of Biochemistry, University Medical Centre, NCMLS, Nijmegen, THE NETHERLANDS.</p>
To contact the author:: papy@univ-paris12.fr.	<p>Despite the increasing evidences demonstrating the diverse roles of glycosaminoglycans (GAGs) in many fundamental biological processes, most studies directed to understand the extracellular matrix implication in cardiac dysfunction focuses mainly in protein components. Age-associated changes in heart structure and function represent the major risk factor in heart failure syndromes. This may be associated to changes in the myocardium intrinsic cardioprotective response to harm. In this study, we were interested to know how the physiological ageing process influences the content and composition of sulfated GAGs in myocardium and how GAG changes can influence important protein functions associated to the response to myocardial ischemia. Sulfated GAGs were isolated from heart left ventricle of healthy rats at different ages. GAGs were quantified according to <i>Barbosa et al 2003</i>. The isolated GAGs were subjected to heparin binding competition assays with some heparin binding growth factors as FGF-2, HARP and VEGF. Extracted GAGs were also tested in a functional assay based on their capacity to potentiate the mitogenic activity of FGF-2. We found that the amount of total sulfated GAG increased gradually with ageing. This increase concerns both, heparan sulfate and chondroitin sulfate. Contrariouly, we observed a clear decrease in these myocardial GAGs abilities to bind to FGF-2, but not to HARP, in aged animals. This low capacity of GAGs extracted from aged hearts to bind FGF-2 was confirmed in a FGF-2 mitogenic activity assay. Immunohistological studies of GAGs on aged and young myocardium are in course. In conclusion, we demonstrated for the first time that the quantity and the quality of the sulfated GAGs in myocardium change during ageing, and that these modifications can differently affect the biological activity of locally expressed growth factors. This might stand for important physiological implications. <i>Acknowledgments: This work was kindly financed by the AFM.</i></p>

PW22-279	<p><u>SIGNIFICANT IMPAIRMENTS IN ION TRANSPORT AND CARDIAC CYTOARCHITECTURE IN THE “HUMANIZED” PHOSPHOLAMBAN MOUSE MODEL</u></p> <p>ARVANITIS DA¹, DONG M², ZHAO W², PAPALOUKA V¹, KRANIAS EG², WANG HS², SANOUDOU D¹</p> <p>(1) Biomedical Research Foundation of the Academy of Athens, Athens, GREECE. (2) University of Cincinnati, Cincinnati, USA.</p>
To contact the author:: dsanoudou@bioacademy.gr.	<p>Phospholamban (PLN), the reversible inhibitor of SERCA2, is a key regulator of calcium homeostasis and cardiac function, and it has been directly implicated in the development of dilated cardiomyopathy. Its amino acid sequence is highly conserved across species except for humans where Asn is replaced by Lys at amino acid position 27. To evaluate the significance of this single nucleotide difference we induced cardiac-specific insertion of the human-PLN in the null background. The “humanized” PLN expressing transgenic (TG) mouse hearts presented increased inhibition of SERCA2, abnormal calcium handling, fibrosis, and hypertrophy. Using microarrays, we identified the global molecular pathways implicated in these processes, including ion transport, muscle contraction, cell cycle and proteolysis. The observed changes in key sodium, potassium and calcium plasma membrane pumps were confirmed at the protein level and suggested an ongoing electrical remodeling process with direct implications in cardiac function. In support of this findings, <i>ex vivo</i> Langedorff perfusion of intact hearts further revealed decreased rates of contraction and relaxation in TGs. Furthermore, patch clamp analysis of isolated cardiac myocytes unveiled significant alterations of their electrophysiological properties. Specifically, the cardiac myocyte action potential duration was significantly prolonged, the transient outward current (I_{to}) was decreased and the sodium/calcium exchanger activity was increased in TG compared to wild-type mice. In conclusion, “human-PLN” directly affects calcium cycling and contractility, which in turn triggers electrical remodeling through differential expression of key ion channels.</p>

