Performance evaluation of each mouse proceeds on a one-way-treadmill equipped with calorimetric system (Phenomaster, TSE, Germany).

The following parameters were measured:
- oxygen consumption/carbon dioxide production (VO2, VCO2), respiratory exchange rate (RER = VCO2/VO2).

We analyzed mice performances with 2 protocols:
- the first protocol consisted in increasing the speed by 1 cm.s-1 every 15s; VO2 peak was determined as the highest value of VO2 reached over 15s. The second protocol was carried out to 75% of their best speed reached during the previous test (Vmax).

Results:
- KO mice had a body weight (g) significantly higher (31±3.3 vs 28.5±3.3; p>0.05). Lean mass (g) was significantly more important both in young (22.3±0.4 vs 18.9±0.8; p>0.001) and in old (27.7±2.6 vs 20.2±0.8; p=0.01) KO mice. Their fat mass was also significantly reduced in both groups. The ejection fraction is significantly lower in KO mice than in WT at any age (in young: 72.3±3.4 vs 81.0±1.4 %, p>0.05; in old: 58.5±4.4 vs 72.1±4.5 %, p>0.01).

Results of the first protocol indicated that VO2 peak relative to lean mass (ml.h-1.g-1) was significantly reduced in KO mice. RER peak was lower in old (0.85±0.04) than in young (0.96±0.06) mice (p>0.001). The results of the second protocol were similar to those of the first one with regards to the RER peak and RER mean. During the second protocol, the time (sec) of race and the run distance (m) observed for WT were significantly higher than those of the KO mice (1634±1108 vs 978±667; p=0.05 and 504±316 vs 306±187; p>0.05 respectively).

Conclusions:
- HFE-/- KO mice presented a decrease in oxygen uptake and a reduced capacity in endurance performance. This loss of performance seemed to be amplified with age. The cardiac function was greatly decreased in aged HFE-/- KO mice. In perspectives, in these mice, we will analyse the effect of endurance training on performance.

Animal models- #3275

P03- 49- FGF/MAPK-dependent spatio-temporal patterning of the ascidian cardio-pharyngeal mesoderm

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FGF signals transduced by the Ras/MAPK pathway are crucial for the development of numerous tissues, from limbs to nervous system and teeth. Similarly, several FGF ligands are necessary for the correct development of the cardio-pharyngeal (CP) mesoderm, from which both the pharyngeal and the cardiac muscles arise. FGF signaling both promotes Tbx1 expression and pharyngeal muscle development and opposes early cardiac differentiation. Nonetheless, how sequential FGF/MAPK signals are integrated into the progressive deployment of the CP gene regulatory network remains elusive in vertebrates.

The tunicate Ciona intestinalis has emerged as a chordate model to study conserved aspects of CP development in a simple context with cellular resolution. Here, we built upon the exquisite knowledge of the cell lineage in Ciona to study the contribution of FGF/MAPK signals in the cell fate decisions of the multipotent CP progenitors called the trunk ventral cells (TVCs).

We show that MAPK signaling is first active in the TVCs and is then restricted to their lateral progeny as it is excluded from the medial heart precursors. Gain and loss-of-function assays show that FGF/MAPK signaling is both necessary and sufficient to promote the pharyngeal muscle identity at the expense of the heart fate. Remarkably, in the heart precursors, inhibition of FGF/MAPK transcriptional inputs occurs at the level of Ras activation and might require the Ets repressor Erf/Etv3, which is normally inhibited by MAPK-mediated phosphorylation. Finally, positive feed-forward circuits involving FGF/MAPK signaling and the transcription factors Hand-related, Tbx1/10 and COE(Collier/Olf/Ebf) drive the progressive pharyngeal muscle specification and its restriction to a subset of the CPM

Altogether, our results describe how the progressive restriction of FGF/MAPK signaling impinges on the regulatory programs underlying heart vs. pharyngeal muscle specification and patterns the CP mesoderm in a simple chordate.

P04- Cardiac stem cells, cardiogenesis and cardiomyopathies- N° 50 to N° 65

Cardiac stem cells and cardiogenesis- #2396

P04- 50- Discovery and cardioprotective effects of the first non-peptide agonists of the G protein-coupled prokineticin receptor-1.

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Prokineticins are angiogenic hormones that activate two G protein-coupled receptors: PKR1 and PKR2. PKR1 has emerged as a critical mediator of cardiovascular homeostasis and cardioprotection. Identification of non-peptide PKR1 agonists that contribute to myocardial repair and collateral vessel growth hold promises for treatment of heart diseases. Through a combination of silico studies, medicinal chemistry, and pharmacological profiling approaches, we designed, synthesized, and characterized the first PKR1 agonists, demonstrating their cardioprotective activity against myocardial infarction (MI) in mice. Based on high throughput docking protocol, 250,000 compounds were computationally screened for putative PKR1 agonistic activity, using a homology model, and 10 virtual hits were pharmacologically evaluated. One hit internalizes PKR1, increases calcium release and activates ERK and Akt kinases. Among the 30 derivatives of the hit compound, the most potent derivative, IS20, was confirmed for its selectivity and specificity through genetic gain- and loss-of-function of PKR1. Importantly, IS20 prevented cardiac lesion formation and improved cardiac function after MI in mice, promoting proliferation of cardiac progenitor cells and neovascularogenesis. The preclinical investigation of the first PKR1 agonists provides a novel approach to promote cardiac neovascularogenesis after MI.

Cardiac stem cells and cardiogenesis- #2548

P04- 51- A novel E3 ubiquitin ligase required for early cardiac morphogenesis

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Ubiquitin-mediated proteolysis comprises the major proteolytic pathway in Eukaryotes and ensures that specific protein functions are turned off at the right time and in the right place through the selective targeting of proteins to proteasome. In this pathway, E3 ubiquitin ligases are key players because they interact with the selected protein and provide specificity to the system. E3 ubiquitin ligases identified as containing a SOCS box domain (Ankyrin repeat-containing protein with a Suppressor of Cytokine Signaling box) were thought to act as substrate-recognition modules of E3 ubiquitin ligase complexes. Despite counting 18 members, the identity of the physiological targets of the ASB proteins remains largely unexplored.

Our group demonstrated that both isoforms encoded by the ASB2 gene, ASB2a and ASB2b, are the specificity subunits of E3 ubiquitin ligase complexes and that ASB2 proteins trigger polyubiquitylation and drive proteasome-mediated degradation of the actin-binding proteins, filamins. ASB2b is mainly expressed in hematopoietic cells and ASB2b in heart and skeletal muscles of human adults. Using several cellular model systems, we demonstrated that ASB2 proteins, through induced proteasomal degradation of filamins, can regulate integrin-dependent functions such as cell spreading, cell adhesion and cell migration. During development, ASB2 gene is expressed first in the heart and later in the myotome both in mouse and chicken. To investigate the in vivo function of ASB2 gene, we analyzed the impact of the total ASB2 knock-out in mice. ASB2+/− offspring were viable, fertile and appeared normal. However, no viable ASB2−/− offspring were obtained in litters from ASB2+/− intercrosses, indicating that the mutation was embryonic lethal. We showed that this lethality results from major cardiovascular defects at 9.5 dpc. Our results indicate that the ASB2 gene is critical for cardiac development and provide the first evidence that the proteasomal degradation of filamin controls key steps of early heart morphogenesis.

ASB2, proteasome, filamin

Cardiac stem cells and cardiogenesis- #2963

P04- 52- Activin A Promotes Cardiomyocyte Differentiation of Human Pluripotent Stem Cells by Enhancing Mesendodermal Lineages

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Patient-specific cardiac tissue is difficult to obtain and manipulate for basic research and clinical applications. Human induced pluripotent stem cells (iPSCs) offer an alternative source as patient-specific cardiovascular cells for cardiac-related disease model systems and large-scale drug screening.

Several protocols have been described to differentiate pluripotent stem cells (PSCs) towards cardiomyocytes (CMs) in two-dimensional (2D) systems. However, these 2D methods show high variability in efficiency both within experimental replicates and among different cell lines. Moreover, the obtained CMs are immature. Here we describe the effect of the growth factor Activin A (ActA) in early human embryonic stem cell (ESC) fate determination, as well as the importance of the presence of HNF4a+ endoderm-like cells and their contribution to the generation of CMs. The cardiac differentiation process is based on the formation of embryoid bodies (EBs) in a serum containing chemically defined media and results in a reliable induction of cMyHC+ CMs. Addition of different concentrations of ActA during early CM differentiation leads to a stronger mesoderm induction. High concentrations of ActA upregulate endoderm-associated genes, including Sox17 and HNF4a. Interestingly, a dose-dependent increase in the co-receptor expression of the TGF-β superfamily Cripto-1 (Tdgf1) is observed in response to ActA.
Together, our results suggest that intercellular interactions occur in the EB clusters between cells derived from the meso- and endodermal developmental lineages that contribute to a better maturation phenotype of differentiating CMs. In conclusion, our study identifies an ActA-induced HNF4a+ cell population that promotes CM differentiation and their functionality. Moreover, we demonstrate an important interplay between ActA and Cripto-1 to promote CM generation from human ESCs.

**Human Pluripotent Stem Cells, Cardiomyocyte Differentiation, Activin A, HNF4a Endoderm-like Cells, Cripto-1**

**Cardiomyopathies- #2391**

**P04- 53- Heart looping and chamber identity in zebrafish requires UNC45b to fold cardiac transcription factors.**

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The sarcomere- basic contractile unit of muscle tissue- is a highly organized structure that relies on several assembly and maintenance proteins for its formation and integrity. The zebrafish model system is advantageous for the study of muscle development because of the availability of molecular techniques and because zebrafish embryos can survive for a short period of time without a functional heart. Furthermore, studying the heart phenotype does not require dissection due to the transparent embryos. unc45b encodes a myosin chaperone, and unc45b mutants have distinct defects in their cardiac and skeletal muscle. UNC45b is required for the formation of the thick filaments within the sarcomere; however, unc45b mutants have a unique heart phenotype similar to that of another chaperone mutant, smyd1b. unc45b hearts do not loop and show a loss of atrial and ventricular identity. Interestingly, in mice, UNC45b has been shown to be responsible for folding GATA 4, one of the key transcription factors in the heart morphogenesis pathway. We have found that UNC45b does not fold Gata4 in the zebrafish model. We are continuing genetic analysis of the unc45b mutant heart defect and the role UNC45b has in zebrafish heart morphogenesis by studying potential transcription factor targets. We are studying the expression levels of these potential targets and partially rescuing the mutant phenotype with mRNA microinjections.

**UNC45b, chaperone, dual function, transcription factor**

**Cardiomyopathies- #2482**

**P04- 54- Early cardiac alteration in golden retriever dogs with muscular dystrophy**

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Cardiomyopathy is a leading cause of mortality in patients with Duchenne muscular dystrophy (DMD). Previous studies have shown that golden retriever muscular dystrophy (GRMD) dogs, a natural model of DMD, also develop cardiomyopathy. However, it remains to be determined whether cardiac alterations develop early at young age. The study objective was to assess cardiac function of GRMD (n=23) and their control littermates (n=7) at 2-month old by echocardiography. Left ventricular (LV) two-dimensional grayscale images of the parasternal short-axis at the level of papillary muscles and apical views were obtained in awake dogs in standing posture for conventional and longitudinal speckle tracking analysis. Tissue Doppler imaging (TDI) images were obtained in the parasternal short-axis view at the level of papillary muscles for calculating radial systolic velocities of LV subendocardium (Endo) and subepicardium (Epi). At 2-month old, GRMD had smaller body weight (3.0±0.2 kg) than control dogs (4.4±0.4 kg, p>0.001) but similar heart rate (165±6 vs 160±5 beats/min, respectively). GRMD dogs had smaller LV end-diastolic diameter (2.03±0.04 vs 2.28±0.13 cm in control dogs, p>0.05) and volume (calculated by biplane method: 6.3±0.4 vs 10.8±0.8 ml in control dogs, p=0.001). GRMD exhibited normal fractional shortening and ejection fraction as compared to control dogs (37.6±1.2% vs 38.5±2.5% and 57.4±1.1% vs 58.8±1.4%, respectively). However, TDI revealed a reduced radial systolic velocity in the subendocardium of GRMD (7.0±0.2 vs 7.9±0.3 cm/s in control dogs p=0.01). Endo-Epi gradient of radial systolic velocity was also decreased in GRMD (2.9±0.1 vs 3.9±0.1 cm/s in control dogs, p>0.001) and this phenomenon (i.e. >mean value-2SD of control dogs) was observed in 52% GRMD. In addition, speckle tracking imaging showed slightly but significantly smaller global longitudinal strains in apical 3-chamber, 4-chamber and 2-chamber views in GRMD dogs (-19.4±0.4%, -19.6±0.5% and -19.5±0.5%, respectively) than control dogs (-22.4±0.9%, -21.5±0.5% and -21.9±0.7%, respectively). The reduced longitudinal strains appeared in 22%, 35% and 50% GRMD in each view, respectively, and 5 GRMD dogs had an abnormal segment kinetic (>10% strain). Thus, cardiac alteration is an early event in GRMD dogs, which can be detected by TDI and speckle tracking echocardiography and is characterized by slightly decreased subendocardial contractile function and longitudinal strain.

**Duchenne muscular dystrophy, golden retriever muscular dystrophy dog, cardiomyopathy, Cardiac function, echocardiography**

**Cardiomyopathies- #2483**

**P04- 55- Alteration of NAD+ metabolism participates in the development of dilated cardiomyopathy caused by mutations in A-type lamins gene**

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Together, our results suggest that intercellular interactions occur in the EB clusters between cells derived from the meso- and endodermal developmental lineages that contribute to a better maturation phenotype of differentiating CMs. In conclusion, our study identifies an ActA-induced HNF4a+ cell population that promotes CM differentiation and their functionality. Moreover, we demonstrate an important interplay between ActA and Cripto-1 to promote CM generation from human ESCs.
Mutations in the lamin A/C gene (LMNA), encoding nuclear envelope proteins, cause dilated cardiomyopathy by mechanisms that remain incompletely understood. LMNA dilated cardiomyopathy is characterized by an increase in both myocardial mass and volume. The ventricular walls become thin and stretched, compromising cardiac contractility and ultimately resulting in poor left ventricular function. Despite current strategies to manage LMNA dilated cardiomyopathy, the disorder remains a common cause of heart failure.

Energy failure is a characteristic feature of the failing heart. We found that nicotinamide adenine dinucleotide (NAD+), a key player in energy metabolism in eukaryotic cells, was significantly decreased (25%) in heart from a mouse model of LMNA dilated cardiomyopathy (LmnaH222P/H222P mice) compared to control mice. NAD+ availability is determined by the relative rates of NAD+ biosynthesis and degradation. In mammals, nicotinamide (NAM) can be a NAD+ precursor through its metabolism into NAM mononucleotide (NMN) by the rate-limiting enzyme nicotinamide phosphoribosyltransferase (Nampt), NMN can be converted into NAD+ through a single additional reaction catalyzed by the nicotinamide mononucleotide adenyllyltransferases (Nmnat) enzyme. Nicotinamide riboside (NR) metabolism constitutes an additional pathway for NAD+ biosynthesis. NR is phosphorylated by the NR kinase 2 (Nrk2) generating NMN. After generation of NMN, Nmnat enzymes can then catalyze the formation of NAD+. We showed that the expression of Nampt was decreased while Nrk2 was increased in heart from LmnaH222P/H222P mice compared to control mice (RNA and protein levels). We next asked whether increasing the NAD+ content in heart could attenuate the disease progression. We modulated NAD+ content (pharmacologically) in LmnaH222P/H222P mice. Treatment was delivered in the food. After 2 months of treatment (initiated at 3 months of age at the incipience of the disease), the LmnaH222P/H222P mice showed a significant increase of their cardiac NAD+ content and exhibit an improvement of both left ventricular dilatation and contractile function (echocardiography).

We showed that increasing NAD+ level in the heart of LmnaH222P/H222P mice significantly delayed disease progression. These results emphasize the role of NAD+ metabolism in the pathogenesis of LMNA dilated cardiomyopathy.

Cardiomyopathie, LMNA, A-type lamins, Nicotinamide adenine dinucleotide

Cardiomyopathies- #2550
P04- 56- Contribution of Multiplex amplification and NGS sequencing of five major genes of Hypertrophic Cardiomyopathy: Spectrum of mutations and CNVs in 1259 patients.
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Hypertrophic Cardiomyopathy (HCM) is an impairment of the heart cell structure with a prevalence estimated to be 0.2%. About 60 % of HCM are familial, with autosomal dominant inheritance and incomplete penetrance. About twenty genes have been implicated but five genes (MYH7, MYBPC3, TNNT2, TNNI3, and MYL2) are responsible of the majority of diagnoses (90%). The objective of this study was to evaluate the diagnostic performance of this approach including the detection of mutations (SNVs, Indel) and copy number variations (CNVs) in 1259 patients.

Analysis of 1259 HCM patients was performed by multiplex amplification of genes MYH7, MYBPC3, TNNT2, TNNI3, and MYL2 (HCM MASTRTM Assay, Multiplicom). The assay is composed by 132 amplicoms (22.7Kb), including 16 control fragments. Patients are sequenced by sets of 96 on MiSeq on a 2x 300 cycles Flow Cell V3.0 (Illumina). The bioinformatic analysis was performed using the software SeqPilot for detecting common SNVs. The CNVs detection is performed by an algorithm comparing the sequenced amplicoms coverage after inter- patient and intra- series normalization (Genodiag, IPEPS- ICM).

The analysis of these five genes has resulted in the identification of pathogenic SNVs in 37.8 % of patients (N = 476). 90% (N=429) of patients carrying a single dominant mutation and 10 % (N = 47) carrying two mutations either heteroallelic in the same gene or in two distinct genes. MYBPC3 is the most frequently involved gene with 57.6 % of mutated patients, then MYH7 (26.2 %), TNNT2 (9.0 %), TNNI3 (5.6 %) and MYL2 (1.6 %). A total of 271 different mutations have been identified: 147 in MYBPC3, 84 in MYH7, 20 in TNNT2, 13 inTNNI3 and 7 in MYL2. Patients with complex genotypes correspond to 10 % of patients including 43% of MYH7 / MYBPC3 digenism carriers.

Although not described, the detection of CNVs confirms that this mechanism may be responsible for HCM and increases the rate of patients diagnosed by the analysis of these five genes. This high throughput multiplex amplification allows making a quick diagnosis in about 40% of the patients by analyzing 96 patients simultaneously. The advantage of this approach is to detect frequent mutations as SNVs, Indel as well as more complex rearrangements involving one or more exons. This addition, it makes possible to broaden the spectrum of the molecular defects responsible for HCM.

Cardiomyopathy, genetics, high throughput sequencing

Cardiomyopathies- #2557
P04- 57- Role of Nicotinamide Riboside Kinase 2 in Dilated Cardiomyopathy and cardiac fibrosis
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Background: Dilated cardiomyopathy (DCM) is a severe heart disease characterized by reduced systolic function and metabolic defects. In a mouse model of DCM, we found an alteration in the nicotinamide adenine dinucleotide (NAD) homeostasis in the heart and a strong induction of the nicotinamide riboside kinase 2 (Nmrk2) implicated in the synthesis of NAD, a major player in energy metabolism in eukaryotic cells, was significantly decreased (25%) in heart from a mouse model of LMNA dilated cardiomyopathy (LmnaH222P/H222P mice) compared to control mice.
coenzyme in energy metabolism and a signaling molecule used by sirtuins. Nmrk2 is also known as the muscle integrin binding protein (MIBP).

Aims: We want to understand the role of Nmrk2 in: i) maintenance of cardiac functions and structure, ii) pathways regulating NAD homeostasis, iii) response to the hypertrophic agonist Angiotensin II; reported to alter cardiac NAD levels.

Methods and results: We generated Nmrk2 KO mice that were viable. Echocardiography at 5, 8, 12 and 24 months revealed a decrease in the ejection fraction (EF) and the development of mild DCM phenotype stabilized after 12 months. Effort tests indicate a strong reduction of endurance. At the histological level, red sirius staining of collagen fibers showed the presence of cardiac fibrosis in the KO. Electron microscopy confirmed the presence of collagen deposits. We also observed an accumulation of lysosomes in Nmrk2 KO cardiomyocytes suggesting an alteration of the autophagy. Western blot analysis of the autophagic markers showed an increased LC3BII/LC3B1 ratio and decrease of p62 expression confirming the stimulation of autophagy in the KO heart.

Echocardiography of control and KO mice treated with Ang II for 15 days, showed a similar increase in the LV mass index. RTQPCR analysis showed an increase in BNP stress marker and decrease in genes regulating NAD homeostasis (Nampt, Sirt1) and integrin signaling (Itga7, Melusin) in both genotypes.

Conclusion: Nmrk2 enzyme is required to preserve cardiac function and structure. Molecular characterization of compounds modulating this pathway could give future therapeutic prospects for DCM.

Cardiomyopathies- #2617

P04- 58- Positive inotropic effect of IL-13 on the perfused rat heart

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IL-13 is a cytokine produced during sepsis, but its pro- or anti-inflammatory effects, especially at the heart level, are still not clear. The aim of this study was to clarify the impact of IL-13 on heart contraction, and on voltage-dependent Na+ channels NaV1.4 and NaV1.5 which are responsible of the membrane excitability essential for excitation/contraction coupling.

For this study, rat hearts were perfused ex vivo in Langendorff columns with IL-13 at 10ng/ml during 30min. Contractile force, heart frequency and coronary flow were recorded. Expression of NaV1.4 and NaV1.5 was analysed by western blot after membrane and cytosol protein extraction from ventricular cells.

IL-13 induced an increase of the contractile force (+28.3%), and in both maximal speeds of contraction (+35.5%) and relaxation (+38.9%). Concerning heart frequency and coronary flow, IL-13 has no significant effect. By using PKA inhibitor we have shown that IL-13 acted by a pathway involving PKA activation. The hearts perfused with IL-13 had more NaV1.4 (+37.4%) and NaV1.5 (+52.2%) at the membrane level. In addition, the ratio of membrane/cytosol proteins were also increased too after IL-13 perfusion for NaV1.4 (+281.4%) and NaV1.5 (+214.4%), when compared to hearts perfused without the cytokine.

Here we demonstrate that IL-13 has a positive inotropic effect on perfused heart. This cytokine can increase NaV1.4 and NaV1.5 membrane targeting, and then increase membrane excitability.

Heart, IL-13, Contractile force, Voltage gated sodium channels, Protein expression

Cardiomyopathies- #2868

P04- 59- High prevalence of arrhythmic and haemodynamic complications in patients with cardiac glycogenosis secondary to PRKAG2 mutations

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Background: The PRKAG2 cardiac syndrome is an autosomal dominant glycogenosis caused by mutations in PRKAG2 gene. Clinical manifestations affect the heart and may associate a ventricular pre-excitation (VPE) with hypertrophic cardiomyopathy (HCM). This, sometime, results in potentially lethal arrhythmic and haemodynamic complications. Several patients were reported with mild myopathic symptoms associated with cardiac manifestations.
Goals: Performing a retrospective time-to-event study of the clinical manifestations associated with PRKAG2 syndrome.

Methods: A cohort of 34 patients from 9 families was recruited between 2001 and 2010 at the laboratory for molecular diagnosis of HCM at ?Hôpitaux Universitaires Pitié-Salpêtrière?.

Results: Overall, 4 families carried the recurrent p.Arg302Gln mutation (NM_016203.3 (PRKAG2):c.905G>A). In this cohort, for individuals aged 40 or older, the risk of VPE was 70% (99%-CI: 50%-87%) and HCM was 61% (99%-CI: 41%-81%). In addition, 53% of the patients presented complications at 50 years old and median age at death was 60. Ablation procedure attempts of VPE were complicated by severe conduction defects in 4/6 patients, requiring cardiac device implantations. A genotype-phenotype correlation study performed between the recurrent mutation (c.905G>A) versus private ones did not demonstrate significant differences regarding the occurrence of VPE, ablation complications or death incidence.

Conclusion: This study is the first timeline description of the natural history of the PRKAG2 syndrome, demonstrating a high risk of HCM, VPE and ablation procedure complications. This study highlights the importance of a close follow-up of mutation carriers and the high risk of iatrogenic complications during VPE ablation.

Cardiomyopathies- PRKAG2 syndrome, PRKAG2, Wolff Parkinson White, Ventricular pre-excitation

P04- 60- Impact of ?-cardiac actin on myocardial integrity and oxidative stress in a dilated cardiomyopathy mouse model

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Background:
Dilated cardiomyopathy (DCM) is the most common of non-ischemic cardiomyopathy, which is characterized by dilation of left and right ventricles, leading to heart failure. We previously developed a DCM mouse model, by cardiac-specific and inducible disruption of the Serum Response Factor gene (SRF HKO). Our findings demonstrated that ?-cardiac actin, main sarcomeric actin isoform, is the first target gene affected by SRF loss before many more. To distinguish the effects linked to ?-cardiac actin independently to SRF on cardiac gene program leading to DCM development, we created and used a new mouse model.

Methods and results:
This transgenic model named SRF HKO/Actc1+, allows SRF loss and cardiac ?-actin compensatory expression simultaneously by Cre/LoxP method. Our major data indicate that the presence of cardiac ?-actin: 1)- improves cardiac functions, delays DCM development and heart failure issue ; 2)- maintains cardiomyocyte cytoarchitecture with regular sarcomere organization and energetic balance are efficient.

The data obtained from the Affymetrix screening showed that more than 50% of genes which are usually affected during DCM, were rescued, in particular those involved in oxidative stress regulation. Then, we demonstrated that Reactive Oxygen Species (ROS) production was substantially reduced when the expression of cardiac ?-actin is maintained in heart of SRF HKO/Actc1+ mice compared with SRF mutant mice. Moreover, in our vitro studies (HEK293 cells) strongly suggest that cardiac ?-actin overexpression was able to buffer hydrogen peroxide-induced ROS production by preserving the mitochondrial activity and also the stoichiometry of redox enzymes.

Conclusions:
All these data point out that cardiac ?-actin plays a crucial role in cardiac function and integrity. In addition, we showed for the first time that cardiac ?-actin is able to decrease the level of oxidative stress in cardiomyocytes. Now the next step will be to understand the mechanisms involved in this process.

P04- 61- Evidence for genetic heterogeneity in left ventricle non compaction by next generation sequencing of 110 genes in 95 unrelated patients.

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Non compaction of the left ventricular cardiomyopathy (NCVG) is characterized by ventricular hypertrabeculation and clinical presentation may vary from asymptomatic patient to heart failure or sudden death. NCVG is considered as frequently of familial/genetic origin and some mutations have been described in various genes. However, the exact spectrum, prevalence of these genes and the impact of screening in clinical practice screening has not been evaluated.

For this purpose, a cohort of 95 index cases (PHRC Ref: 2011-A-00987-34) was sequenced on a large panel of 110 genes (1800 target, 550 KB) after targeted capture (SeqCap EZ, Nimblegen, Roche). This genes were selected because described to be involved in cardiomyopathies and/or rhythm disturbances.
After bioinformatics analysis, only non-synonymous variants in the coding regions or splicing and with frequency >0.1%, were considered potentially pathogenic. For the titin (TTN) gene, only nonsense mutations were considered.

Among 95 index case patients enrolled, 52 (56%) are carriers of mutations in a cardiomyopathy related gene. A unique dominant mutation was found in 33 patients (35%), two potentially pathogenic variants are found in 14 patients (15%) and 5 patients (6%) carry a TTN mutation. Moreover 12/52 patients showed the presence of additional mutations in ?arrhythmia? genes (not related with NCVG until now) or a TTN mutation No definitive pathogenic mutation was identified in 35 patients (37%). The remaining index cases (7%) carry a variant of unknown significance (VUS) or mutations in genes considered until now as related to arrhythmia.

Of the 110 genes tested, at least 35 can account for the phenotype with high variability of frequencies, the most prevalent being the TTN gene (16%) and 6% for MYH7, MYH6 and HCN4 finally come LDB3, MYBP3, RYR2, ACTC1, ACTN2, DSP, FBN1 and FLNC. A total of 79 pathogenic variants have been found in cardiomyopathies related genes including 54 new variants and 9 variants of unknown significance. In the TTN gene, 16 nonsense mutations were identified.

In conclusion, molecular analysis of 110 genes in 95 cases index with NCVG shows a mutation detection rate of 56%. These mutations are, for the vast majority of them, new mutations, their interpretation and causal link with the disease must be confirmed by a close cooperation with the Cardiologists and the possibility of segregation in families. Only this approach will achieve a reliable genetic counseling to the families.

**Cardiomyopathy, genetics, high throughput sequencing, Left ventricle non compaction**

**P04- 62- mTOR inactivation in myocardium of infant mice rapidly leads to dilated cardiomyopathy due to translation defects and p53/JNK-mediated apoptosis**

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Mechanistic target of rapamycin (mTOR) is a central regulator of cell growth, proliferation, survival and metabolism, as part of mTOR Complex 1 (mTORC1) and mTORC2. While partial inhibition of mTORC1 using rapamycin was shown to be cardioprotective, genetic studies in mouse models revealed that mTOR is essential for embryonic heart development and cardiac function in adult. However the physiological role of mTOR during postnatal cardiac maturation is not fully elucidated yet. We have therefore generated a mouse model in which cardiac mTOR was inactivated at an early postnatal stage. Mutant mTORcmKO mice rapidly developed a dilated cardiomyopathy associated with cardiomyocyte growth defect, apoptosis and fibrosis, and died during their third week. Here we show that reduced cardiomyocyte growth results from impaired protein translation efficiency through both 4E-BP1-dependent and -independent mechanisms. In addition, infant mTORcmKO hearts displayed marked increased apoptosis linked to JNK kinase activation and p53 accumulation. mTORcmKO hearts showed a strong downregulation of myoglobin content, thereby suggesting intracellular hypoxia. Nevertheless, they lacked HIF1a-mediated adaptive response, consistently with mTOR being required for hypoxia-induced HIF-1α activation. Altogether, our results demonstrate that mTOR is critically required for cardiomyocyte growth, viability and oxygen supply in early postnatal myocardium, in addition to provide insights into the molecular mechanisms involved in apoptosis of mTOR-depleted cardiomyocytes.

mTOR, heart postnatal development, signal transduction, translation, cardiomyocyte apoptosis, myocardial metabolism.

**P04- 63- Increase in ROS, inhibition of mitochondrionally-driven apoptosis and autophagic fluxes downregulation bridge the gap between tafazzin mutations and cardiomyocyte defects in the Barth syndrome.**

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Mutations in the gene that encodes the monolys-o-transacylase, TAZ, leads to Barth syndrome. Individuals affected by this X-linked multi-system disorder present with cardiomyopathy, skeletal muscle weakness, neutropenia, growth retardation and methylglutaconic aciduria (3-MGA). The previous research on various species models or cells derived from the Barth syndrome have established deep roots laying on top of biochemical characterization of the cellular defects associated to TAZ mutations. Biopsies from the heart, liver and skeletal muscle of patients exhibited malformed mitochondria and dysfunctional mitochondria. Our recent work has focused on basic aspects of the Barth syndrome and also on recent developments into cardiopin and tafazzin research which provide some clues to better understand of the link between mitochondrial dysfunction exhibiting defect in oxidative phosphorylation, respiratory supercomplexes disorganization, lower ATP production, slight increase oxygen radical production and the Barth syndrome pathology. Increase in ROS production linked to tafazzin mutation was previously described in in lymphoblasts1 or fibroblasts2 derived from Barth syndrome patients. The presence of an elevated level of non-mature cardiolipin may explain the down regulation of mitophagy whereas other autophagic processes are not affected. These results together with the previously observed inhibition of mitochondrionally-driven apoptosis3 may certainly highlight multiples cellular disorder leading to skeletal muscle and cardiomyocytes defect (left ventricular non-compaction).
We believe that the link between ROS production and sarcomeres dysorganization could effectively be straightforward and related to both a toxic effect of ROS but also on the dysregulation of apoptosis. However, they are some clues linked with autophagic flux homeostatic destabilization which might be involved in the pathophysiology of the disease. Our results are discussed in light of the the elegant work of Wang's team, that bridge the gap between abnormal ROS production in BTHS derived-iPSCs and the mechanical defect of the heart-on-chip? reconstitution of the sarcomeric structure associated with mechanical defects.


Barth syndrome, cardiomyopathy, mitochondria, bioenergetic, ROS, apoptosis, autophagy

Cardiomyopathies-#3032

P04-64-Cardiac involvement in glycogen storage disease type III

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Background

Glycogen storage disease type III (GSD III) is an autosomal recessive disease, due to deficiency of glycogen debranching enzyme (GDE), a key enzyme involved in glycogen degradation. Clinical presentation includes hepatomegaly, myopathy, hypoglycemia and cardiomyopathy.

Aims

We performed a longitudinal study in order to describe the natural history of heart involvement in patients with GSD III

Methods and materials

We included all consecutive patients with GSD III followed in our center and collected clinical, genetic, electrocardiogram and echocardiography data.

Results

47 patients were included our study (16 male/31 female). Mean age was 25.7 years +15.4. All patients were in sinus rhythm expect one patient (atrial fibrillation). 9 patients/ 47 disclosed abnormal repolarization. Electrical left ventricular hypertrophy was found in 22 patients /47. Symptomatic heart failure was found in 10 patients /47 and 2 patients/47 disclosed left ventricular dysfunction. Hypertrophic cardiomyopathy (HCM) was found in 23 patients /47 and 8.5% of patients disclosed obstructive hypertrophic cardiomyopathy.

Conclusion

Hypertrophic cardiomyopathy is frequent in patients with GSD3 and is associated with cardiac clinical events.

Cardiomyopathies-#3210

P04-65-Axial stretch-dependent cation entry in dystrophic cardiomyopathy: involvement of several TRPs channels

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In Duchenne muscular dystrophy (DMD), deficiency of the cytoskeletal protein dystrophin leads to well-described defects in skeletal muscle but also to dilated cardiomyopathy (DCM). In cardiac cells, the subsarcolemmal localization of dystrophin is thought to protect the membrane from mechanical stress. The dystrophin deficiency leads to membrane instability and a high stress-induced Ca2+ influx due to dysregulation of sarcolemmal channels such as stretch-activated channels (SACs). In this work, divalent cation entry has been explored in isolated ventricular Wild Type (WT) and mdx cardiomyocytes in two different conditions: at rest and during the application of an axial stretch. At rest, our results suggest that activation of TRPV2 channels participates to a constitutive basal cation entry in mdx cardiomyocytes. Using microcarbon fibres technique, an axial stretch was applied to mimic effects of physiological conditions of ventricular filling and study on cation influx by the Mn2+-quenching technique demonstrated a high stretch-dependent cationic influx in dystrophic cells, partially due to SACs. Involvement of TRPs channels in this excessive Ca2+ influx has been investigated using specific modulators and demonstrated both sarcolemmal localization and an abnormal activity of TRPV2 channels.

In conclusion, TRPV2 channels are demonstrated here to play a key role in cation influx and dysregulation in dystrophin deficient cardiomyocytes, enhanced in stretching conditions.
Congenital muscular dystrophies (other than dystroglycanopathies) N° 66 to N° 75

P05- Congenital muscular dystrophies / Dystroglycanopathies- N° 66 to N° 75

**P05-65** Investigation of calcium current properties and leak conductance in mouse muscle fibers overexpressing a type 1 Hypokalemic Periodic Paralysis mutant. L-type calcium channel suggests a role of acidification in attacks of periodic paralysis

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Missense mutations in the gene encoding the alpha1 subunit of the L-type calcium channel CaV1.1 induce type 1 Hypokalemic Periodic Paralysis (HypoPP1). These mutations mainly occur at arginine residues in the fourth transmembrane segment of voltage-sensor domains. Very few studies have investigated the acute effects of these mutations on channel function and muscle membrane electrical properties because of the difficulty to express Cav1.1 in heterologous systems. In the present study we successfully transferred by electroporation the genes encoding the turboGFP-tagged human wildtype (WT) and R1239H HypoPP1 mutant Cav1.1 into hind limb mouse muscles. The expression profile of the two channels showed a regular striated pattern indicative of the localization of the channels in the t-tubule membrane. Measurement of the L-type current using the silicone-clamp technique showed that the maximal conductance and the voltage-dependence of the Cav1.1 channel were significantly reduced and shifted towards negative potentials respectively in fibers expressing R1239H Cav1.1 as compared to fibers expressing WT Cav1.1. Applying voltage ramps from a holding potential of 0, -20, -40 or -60 mV to -120 mV in the presence of an external low-chloride, sodium-free and potassium-free solution revealed a significant higher leak conductance measured between -80 and -120 mV in fibers expressing R1239H Cav1.1. Acidification of the external solution significantly increased the leak inward current, leak conductance and the fluorescence of an internally loaded pH indicator in fibers expressing R1239H. These data suggest that an elevated leak inward current, likely carrying protons, flows at resting membrane potentials in fibers expressing R1239H Cav1.1 and that muscle acidification could contribute to favor the onset of muscle paralysis in HypoPP1.

**calcium channel, periodic paralysis, cell electrophysiology**

Congenital muscular dystrophies (other than dystroglycanopathies) N° 66 to N° 75

**P05-67** YAP mediated mechanosensing defects of LMNA mutant myoblasts also affect cell-cell contacts

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The mechanisms underlying the cellular response to mechanical forces are critical for muscle development and functionality. The LINC (Linker of the Nucleoskeleton and Cytoskeleton) complex, enables transmission of forces between the nucleus and the extracellular matrix via the actin cytoskeleton. Mutations in LINC-complex associated proteins, including lamins cause human muscular dystrophies but the molecular mechanisms still remain to be elucidated. Recently, mechanosensing defects have been reported in patient-derived immortalized myoblasts carrying mutations in A-type Lamins (Bertrand et al., 2014). These mutant myoblasts were shown not to adapt their cytoskeletal organisation to the stiffness of their environment. Also MKL-1 and Yes-Associated Protein (YAP) signaling, two important mechanotransducers, were shown to be misregulated on low stiffness substrates in mutant myoblasts. In this study, we hypothesized that mechanosensing defects in LMNA mutant myoblasts also affect cell-cell contact through deregulation of YAP. Cell-cell contact induced the cytoplasmic relocalization of YAP in WT but not in LMNA myoblasts. Further investigations revealed no defects in the HIPPO pathway signaling upstream of YAP. YAP expression and activity but also the LATS mediated phosphorylation of YAP at serine 127 were increased in LMNA mutant myoblasts. In conclusion, our results indicate that the mechanical regulation of YAP as sensor and mediator of mechanical inputs from cell-cell interaction is impaired in LMNA mutant myoblasts, presumably through a disturbed actin-dependent regulation of YAP.

**mechanotransduction, yap, hippo, LaminA/C**

Congenital muscular dystrophies (other than dystroglycanopathies) N° 66 to N° 75

**P05-68** Inactivation of Myostatin: a potential therapeutic tool against Autosomal Dominant Centronuclear Myopathy.

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Context: The unique mouse model for autosomal dominant centronuclear myopathy (Kl-Dnm2R465W/+), associated to mutations of dynamin 2 gene (Dnm2) reproduce some of the clinical features reported in human, notably muscle atrophy and weakness. Myostatin (Mstn), a member of TGFβ family, is a master negative regulator of skeletal muscle mass. We hypothesized that inactivation of Mstn could limit muscle atrophy and weakness reported in the Kl mouse model. To test this hypothesis, we intercrossed Kl mice with mice inactivated for Mstn (KO-Mstn) to generate a double mutated lineage (KIKO mice).

Results: Animals were followed over a 12 months period. Muscle force (grip strength test) and motricity (rotator test) were significantly reduced in 1-month old Kl mice. A significant loss of muscle mass and volume (microMRI) were observed in Kl from 2 months of age. The analysis of tibialis anterior muscle mass was correlated with the decrease of muscle volume determined by microMRI (r=0.9). From 2 to 12 months, all these parameters remained below control values.

**mechanical atrophy, myostatin, centronuclear myopathy**