Atrial fibrillation: from Mechanisms to Population Science

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# Atrial Fibrillation: From Mechanisms to Population Science

**Madrid, November 3-4, 2017**

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Atrial fibrillation: from Mechanisms to Population Science

Madrid, November 3-4, 2017
PROGRAMME

Friday, November 3, 2017

08:00-08:30  Registration
08:30  Welcome
08:30-09:00  Opening talk: Borja Ibáñez. CNIC Clinical Research Director

Session I - AF in populations, genomics and epigenetics

Discussion leaders: Guadalupe Sabio, Miguel Manzanares

09:00-09:30  Emelia Benjamin. Boston University School of Medicine, USA
“Can we prevent atrial fibrillation?”
09:30-10:00  Patrick Ellinor. Harvard University, Boston, USA
“Emerging directions in the genetics of atrial fibrillation”

10:00-11:00  Coffee break & poster session

11:00-11:30  Vincent Christoffels. University of Amsterdam, Holland
“Transcriptional control of cardiac electrical patterning”

Selected short talks
11:30-11:45  Rosa Doñate. INMG, University of Lyon, France
“Atrial structural remodeling gene variants in patients with atrial fibrillation”
11:45-12:00  Raquel Rouco. Center for Arrhythmia Research, USA, and CNIC, Spain
“Genomic expression during atrial fibrillation progression in a sheep model of persistent AF”

12:00-13:30  Poster session & Lunch

Session II - Pathogenesis of AF

Discussion leaders: Juan Bernal, Juan Tamargo

13:30-14:00  Ulrich Schotten. University of Maastricht, Holland
“The multifactorial pathogenesis of atrial remodelling and atrial fibrillation”
14:00-14:30  Barbara Casadei. Oxford University, UK
“Atrial fibrillation and cardiomyopathy: the chicken or the egg?”
Programme

Session III - Altered metabolism and atrial adiposis

Discussion leaders: Julian Pérez-Villacastín, Javier Saiz

15:00-15:30 | Sander Verheule. University of Maastricht, Holland
“Metabolic impact of AF”

15:30-16:00 | Stephane Hatem. Sorbonne Université, Paris, France
“Cardiac adipose tissue and atrial fibrillation”

Selected short talks

16:00-16:15 | Alexey Kulikov.
Cardiology Research And Production Complex, Moscow, Russia
“Electrophysiologic study of atrioventricular conduction and electrophysiologic parameters of atria in patients with long-standing persistent atrial fibrillation undergoing surgical correction of mitral valve pathology combined with ‘maze IIIB’ procedure”

16:15-16:30 | Miguel Rodrigo.
Universitat Politecnica de Valencia, Spain. Stanford Hospital and Clinics, USA
“Driver location by body surface and endocardial basket mapping of human atrial fibrillation”

16:30-17:30 | Coffee break & poster session

Session IV - Genes, ion channels and gene therapy in AF

Discussion leaders: Felipe Atienza, David Filgueiras

17:30-18:00 | Eva Delpón. Complutense University, Madrid, Spain
“Novel ion channel modifying genes in atrial fibrillation”
PROGRAMME

18:00-18:30  Dobrīmir Dobrev. University of Duisburg-Essen, Germany
“Molecular basis and role of dysregulated Ca2+-dependent K+ channels in AF patients”

18:30-19:00  Rishi Arora. Northwestern University, Chicago, USA
“Novel gene therapy approaches to target electrical and structural remodeling in atrial fibrillation”

Saturday, November 4, 2017

Session V - The rotor substrate in silico, ex vivo and in humans

Discussion leaders: Francisco García-Cosío, David Calvo

08:30-09:00  Natalia Trayanova. Johns Hopkins University, Baltimore, USA
“AF rotors in the fibrotic substrate”

09:00-09:30  Omer Berenfeld. University of Michigan, USA
“Spatio-temporal AF excitation patterns in the frequency and phase domains”

09:30-10:00  Sanjiv Narayan. Stanford University, USA
“Rotational drivers in clinical atrial fibrillation”

10:00-10:30  Stefan Luther. Max Planck Institute, Göttingen, Germany
“Electromechanical vortex filaments during cardiac fibrillation”

10:30-11:00  Coffee break

Session VI - The real world in today’s AF therapy

Discussion leaders: Jesús Almendral, José Luis Merino

11:00-11:30  John Camm. St George’s University of London, UK
“The classification of atrial fibrillation: research, guidelines, clinical practice and back again”

11:30-12:00  Meleze Hocini. University of Bordeaux, France
“AF ablation guided by noninvasive mapping: from stepwise to rotor ablation”

12:00-12:30  Karl Heinz Kuck. University of Hamburg, Germany
“What have we learned of ablation procedures for atrial fibrillation?”

12:30-13:00  Round up, prizes and farewell
BACKGROUND: Atrial fibrillation (AF) is a common arrhythmia with a well-recognized inherited component. Until now, AF genetic studies mainly focused on the genes involved in electrical remodeling, rather than left atrial muscle remodeling.

OBJECTIVE: To identify rare variants involved in atrial myopathy using mutational screening.

METHODS: A high-throughput next-generation sequencing (NGS) workflow was developed based on a custom AmpliSeq™ panel of 55 genes potentially involved in atrial myopathy. This workflow was applied to a cohort of 94 patients with AF, 66 with atrial dilatation and 28 without. Patients with variants in the selected genes underwent further screening for pathogenic mutations in prevalent arrhythmia-causing genes. Bioinformatic analyses used a pipeline based on NextGENe® software and in silico tools for variant interpretation.

RESULTS: Our AmpliSeq™ custom-made panel efficiently explored 96.58% of the targeted sequences. Based on in silico analysis, 11 potentially pathogenic missense variants were identified that were not previously associated with AF. These variants were located in genes involved in atrial tissue structural remodeling. Three patients were also carriers of potential variants in prevalent arrhythmia-causing genes, usually associated with AF. Most of the variants were found in patients with atrial dilatation (n=9, 82%).

CONCLUSIONS: This NGS approach was a sensitive and specific method that identified 11 potentially pathogenic variants, which are likely to play roles in the predisposition to left atrial myopathy. Functional studies are needed to confirm their pathogenicity.
Atrial Fibrillation (AF) affects over 33 million people globally and is one of the major causes of embolic stroke. While AF usually starts with paroxysmal episodes, in a significant number of patients it evolves to persistent and permanent forms, which likely reflects progressive electrical and structural remodeling in both atria. However, the underlying molecular mechanisms are poorly understood. We have used a well characterized sheep model of persistent AF to conduct a deep transcriptome analysis of the gene expression profile of AF progression in three different sheep groups: sham (control operated animals, n = 3), transition (seven days in self-sustained AF, n = 3), and chronic (one year on longstanding persistent AF, n = 3). For each group, we obtained tissues from the posterior left atrial wall (PLA), the left atrial appendage (LAA) and the right atrial appendage (RAA). In an additional group of 3 sheep, we obtained isolated cardiomyocytes from the LAA and RAA to investigate gene expression changes during AF progression in this cell population. Of 12058 genes expressed in the atrial tissues, 1047 were differentially regulated; similarly of 1266 genes expressed in the cardiomyocytes, 1200 were differentially regulated. Principal component analysis demonstrated clear clustering of genes by tissue group (i.e., sham, transition or chronic), by atrial origin (i.e., left versus right) and by tendency (i.e., sham to transition; transition to chronic), which enabled us to detected changes in related biological processes, especially in pure cardiomyocyte populations. However, differential gene expression changes occurred faster in tissues and cardiomyocytes from the left than the right atrium although the overall changes were similar in tissues and cardiomyocytes from both atria. The largest gene expression changes occurred in both atria during the transition and were typically maintained between transition and chronic stages. Altogether during AF progression transcriptomic analysis demonstrated differential expression changes in genes representing such pathways as ion channel signaling, cell adhesion, extracellular matrix organization, angiogenesis, cell contraction, heart development, and chromatin remodeling. The results improve our understanding of the changes in gene expression associated with atrial remodeling during AF progression. However, more in depth data analysis and additional research will be needed to establish an accurate picture of the molecular mechanisms underlying AF perpetuation.
In the last few years, transcription factors, their targets and transcriptional modifiers have been shown to play important roles in the pathophysiology of atrial fibrillation (AF), the most common cardiac arrhythmia, indicating that dysregulated gene expression is an essential underlying mechanism. However, the underlying gene regulatory networks associated with AF are complex and still not fully understood. In clinical practice AF often coexists with sinus node dysfunction. Based on previous analysis, we have established a functional link between the expression of the homeodomain transcription factor SHOX2, the development of the sinoatrial node (SAN) and arrhythmogenic phenotypes including AF. The homozygous loss of Shox2 in the mouse is embryonically lethal due to cardiovascular defects and severe bradycardia. Shox2-deficiency in zebrafish embryos leads to substantial impairment in pacemaker function with severe bradycardia and irregular heartbeat. For a detailed analysis of Shox2-dependent developmental mechanisms in SAN cells, we established a refined murine embryonic stem cell (ESC)-based cardiac differentiation model. Shox2+/+ and Shox2-/- mouse ESCs lines were generated from Shox2-deficient embryos and differentiated into cardiomyocytes of the SAN subtype. Comparative expression analysis of Shox2+/+ vs. Shox2-/- ESC-derived SAN-like cells identified novel Shox2-dependent genes during pacemaker differentiation including Nppb (Bnp), a well-studied marker in cardiovascular disease. Nppb turned out to be significantly upregulated in Shox2-/- SAN-like cells and in right atrial tissue of Shox2-KO embryos. In turn, elevated BNP levels and higher NPPB mRNA expression have been detected in subjects with AF.

Recently, we linked for the first time heterozygous mutations in the SHOX2 gene to patients suffering from early-onset atrial fibrillation. We identified and validated two novel missense mutations (c.242G>A, c.849C>A) and a significantly associated regulatory variant in the 3’UTR (c.*28T>C) of this gene. These findings contribute to a better classification of AF, since a specific patient cohort with early-onset AF and prolonged PR interval could be determined by genetic predisposition involving the SHOX2 gene. Further, three likely pathogenic (probably damaging) mutations in the SHOX2 gene were identified in an independent cohort of individuals with AF and in a cohort of individuals suffering from sinus node dysfunction.

Developing a mechanistic classification of AF based on genetic susceptibility is essential to improve personalized prevention and management of AF. Thus, the study of the role of SHOX2 and SHOX2-dependent gene regulatory networks in atrial fibrillation will be of utmost importance. To translate the results obtained from the Shox2 mouse ESCs
to humans, we generated a novel cardiac differentiation model utilizing human induced
pluripotent stem cells (iPSCs) reprogrammed from AF patients carrying SHOX2 mutations.
Having established a correlation between SHOX2 and AF, the patient-derived iPSCs will
now give us the unique opportunity to investigate SHOX2-dependent regulatory networks
in a human disease-model. Gaining more insights into the molecular basis of AF will allow
the development of more effective, personalized diagnostic and therapeutic approaches.
SHORT TALK

HYPERTHYROIDISM, BUT NOT HYPERTENSION, IMPAIRS PITX2 EXPRESSION LEADING TO WNT-MICRORNA-ION CHANNEL REMODELING

Estefanía Lozano-Velasco¹; Rosemary Wangensteen²; Andrés Quesada²; Carlos García-Padilla¹;
Julia A. Osorio¹; María Dolores Ruiz-Torres¹; Amelia Aranega¹; Diego Franco¹

¹ Cardiac and Skeletal Muscle Development Group, Department of Experimental Biology,
University of Jaen, Spain
² Department of Health Sciences, University of Jaen, Spain

PITX2 is a homeobox transcription factor involved in embryonic left/right signaling and more recently has been associated to cardiac arrhythmias. Genome wide association studies have pinpointed PITX2 as a major player underlying atrial fibrillation (AF). We have previously described that PITX2 expression is impaired in AF patients. Furthermore, distinct studies demonstrate that Pitx2 insufficiency leads to complex gene regulatory network remodeling, i.e. Wnt>microRNAs, leading to ion channel impairment and thus to arrhythmogenic events in mice. Whereas large body of evidences has been provided in recent years on PITX2 downstream signaling pathways, scarce information is available on upstream pathways influencing PITX2 in the context of AF.

Multiple risk factors are associated to the onset of AF, such as e.g. hypertension (HTN), hyperthyroidism (HTD) and redox homeostasis impairment. In this study we have analyzed whether HTN, HTD and/or redox homeostasis impact on PITX2 and its downstream signaling pathways. Using rat models for spontaneous HTN (SHR) and experimentally-induced HTD we have observed that both cardiovascular risk factors lead to severe Pitx2 downregulation. Interesting HTD, but not SHR, leads to up-regulation of Wnt signaling as well as deregulation of multiple microRNAs and ion channels as previously described in Pitx2 insufficiency models. In addition, redox signaling is impaired in HTD but not SHR, in line with similar findings in atrial-specific Pitx2 deficient mice. In vitro cell culture analyses using gain- and loss-of-function strategies demonstrate that Pitx2, Zfhx3 and Wnt signaling influence redox homeostasis in cardiomyocytes. Thus, redox homeostasis seems to play a pivotal role in this setting, providing a regulatory feedback loop. Overall these data demonstrate that HTD, but not HTN, can impair Pitx2>>Wnt pathway providing thus a molecular link to AF.
**Research objective:** Electrophysiological study of atrial myocardium parameters and characteristics of atrioventricular conduction in attempt to reveal the potential factors contributing to postoperative atrial fibrillation (AF) recurrence in patients with long standing persistent form of AF undergoing Maze III procedure combined with mitral valve operation.

**Methods:** In the study were included 100 adult patients (48 men) with persistent and longstanding persistent forms of AF and valvular pathologies. Average age of patients was 59 years. Average AF duration was 4 years.

Antiarrhythmic therapy in the conditions of cardiological hospitals was tried to all patients, however it was inefficient. Attempts of restoration of sinus rhythm by means of the electric cardioversion were made in 15% of patients, however it was not possible to control sinus rhythm for a long time.

All patients had mitral valve pathology. Also in 80% of patients insufficiency of the tricuspid valve was revealed.

Functional class of heart failure on NYHA 2,7±0,75. The size of the left atrium 5,1±1,5 cm, average left ventricular ejection fraction 61±8,6%.

To all patients the electric cardioversion by a standard technique was made. After successful restoration of a sinus rhythm, a cardiac electrophysiology study (EP) was executed. Then, on the first or second day after EP, correction of valve pathologies combined with “Maze IIIB” procedure was carried out.

**Results:** In our research the study of refractory periods of various atrial areas showed that ERP of AV node is minimal, and the longest duration of ERP was found in HRA. Thus, in patients with long standing persistent form of AF atrial myocardium is heterogeneous on ERP duration.

During the programmed atrial stimulation with single extra stimuli at 26% of patients lengthening of intraatrial conduction time after A2 in a plateau zone was noted. This phenomenon apparently also reflects heterogeneity of electrophysiological characteristics of atrial myocardium.
In 17% of patients atrial vulnerability was revealed. All of them were from the group of 26% patients which had lengthening of the intraatrial conduction time after A2 in a plateau zone, and the start of repeated atrial answers of atrial vulnerability coincided with this lengthening of intraatrial conduction. The zone of atrial vulnerability always adjoined ERP, which in patients with atrial vulnerability was much higher, than at other patients.

The assessment of AV node function showed, that before “Maze IIIB” procedure combined with valve disease correction, all patients had normal AV node function. After the surgery in one patient third-degree atrioventricular block was revealed.

Conclusions:

1. The long existence of mitral valve disease and long standing persistent form of AF lead to anatomic and electrophysiological atrial remodeling that manifests itself as increase of left atrium volume, lengthening of intraatrial conduction time and heterogeneity of the atrial myocardium refractory periods.

2. EP allows to estimate functions of atrioventricular conduction system (AV node and His-Purkinje system) and electrophysiological parameters of atria: to reveal intraatrial conduction disturbance, dispersion of refractory periods and a zone of atrial vulnerability, that can serve as a predictor of AF recurrence.
Background: Isolation of atrial fibrillation (AF) drivers has been proven as an effective therapy for restoration of sinus rhythm in patients refractory to antiarrhythmic medication. These AF drivers can be identified by different mapping techniques involving intracardiac basket catheters or non-invasive recordings (electrocardiographic imaging - ECGI). This study evaluates the correspondence between the driver location estimation from endocardial versus non-invasive recordings for two analysis techniques: Dominant Frequency (DF) and reentrant activity identification.

Methods: Intracardiac electrograms of 15 AF patients (66±12 years, 26% men, 47% persistent AF) were recorded with one 64-pole basket catheter in each atrium simultaneously to 57-lead body surface recordings. Atrial and torso anatomy were reconstructed by using segmented magnetic resonance images. We used activation+phase mapping algorithm (FIRM) to detect endocardial rotational sources (focal sources excluded) and endocardial Dominant Frequency (DF) by spectral analysis to identify fastest activated regions. The ECGI signals were reconstructed by using zero-order Tikhonov regularization and non-invasive identification of atrial sources was carried out by estimation of the highest DF sites.

Results: Endocardial DF analysis in 4.5±5.8 four-second segment/patient showed local maximum DF of 5.1±1.1Hz that stepped down to 3.7±0.4Hz in remaining atrial sites (p<0.01). Stable rotational activity in AF was seen endocardially in 9/15 patients by FIRM. On 3D electroanatomic maps, the site of maximum DF overlay the site of AF rotational activity in 70% cases, although the highest DF regions (<0.5 Hz from the fastest) were considerably wider (35±41% of the analyzed atrium) than the corresponding FIRM sources (16±3%, p<0.01). Epicardial DF analysis obtained by ECGI revealed a similar maximum DF than catheter measurements (5.5±1.8Hz, p=NS versus endocardial). Sites of maximal epicardial DF in AF overlay (a) sites of maximal endocardial DF (Chi-square 4.3, P=0.04) and (b) rotational AF pivots (Chi-square 6.7, P=0.01).

Conclusions: AF rotational sources can be detected endocardially and epicardially, with at least moderate spatial concordance. In many cases, rotors were detected within the
fastest activated atrial regions, suggesting the hierarchical pattern between the reentrant source and the rest of the fibrillatory conduction. This is the first study to systematically compare endocardial and epicardial AF human maps in vivo that suggests a hitherto unreported synchronization of epicardium/endocardium. Further epicardial/endocardial mapping studies are needed to define AF mechanisms and improve AF ablation outcomes.
POSTER 1. MICROSCOPIC OPTICAL MAPPING OF ANATOMICAL AND FUNCTIONAL REENTRIES IN HUMAN CARDIAC CELL CULTURES

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Introduction: Limited knowledge of the mechanisms of perpetuation of fibrillation is hampering the development of effective anti-arrhythmic treatments. The goal of the present study is to present a novel technology to map with high resolution (500x500um) the center of fibrillation drivers in order to characterize the mechanisms of reentry.

Methods: Cell cultures of human cardiomyocytes differentiated from pluripotent stem cells were analyzed with a novel microscopic optical mapping system. Simultaneous voltage and calcium imaging was developed by recording emission light of di-4-ANBDQPO and Fura 2-(AM). During fibrillation the dominant driver was identified (i.e. anatomical vs. functional reentry) and characterized in terms of its dominant frequency (DF). The pharmacological response to verapamil administration of each type of reentry was analyzed.

Results: In all analyzed cell cultures, a reentry was identified as the mechanism of maintenance of the arrhythmia. Microscopic analysis of the reentries allowed their classification into (1) micro-anatomical (46%, N=12) or functional reentries (54%, N=14). Isochronal maps of a representative example of each group are shown in the figure. Anatomical reentries presented lower DFs than functional reentries (i.e. 1.08±0.19 vs. 2.96±0.24Hz, p<0.01). Interestingly, the administration of verapamil produced opposite effects in each group: whereas DF increased in 15±3.4% for anatomical reentries, it decreased in 11.9±6.8% for functional rotors (p<0.01).

Conclusions: Microscopic optical mapping of reentries allows the identification of perpetuation mechanisms which has been demonstrated to be linked with different pharmacological response.
Cardiovascular diseases are the leading cause of death worldwide and congenital heart defects are present in 1% of live births. Understanding heart development regulation is key to determine the onset of those diseases.

Meis1 and Meis2 are transcription factors (TFs) expressed during embryonic development with a very similar expression pattern suggesting redundant functions. Single Meis1 or Meis2 null mice die around ED15.5 due to haematopoietic failure and present ventricular septal defects (VSD) and persistent truncus arteriosus, respectively. Moreover, Meis1 function affects postnatal cardiomyocyte (CMs) proliferation and cardiac regeneration ability. In addition, various GWAS studies have identified variants in the Meis1 locus associated with PR interval alterations in human electrocardiogram.

Here we show that Meis TFs are expressed in differentiated CMs during mid and late cardiac development and we have observed that this expression is sustained in adulthood. Constitutive deletion of Meis TFs in differentiated CMs using a-MHC-Cre causes perinatal death. Atrial malformations, VSD and ventricular apex dysmorphology were found in double knockout (dKOs) embryos. Echocardiographic (Echo) analysis showed significant increase of left ventricular diameter and volume in dKOs embryos by ED16.5 but cardiac function was not yet affected. We are currently performing Echo at ED18.5 to determine the cause of death and studying whether there are changes in CMs proliferation, size and density. We plan to examine by RNA-seq and ChIP-seq the molecular mechanisms that are involved in this phenotype.
Myocarditis is the more frequent manifestation of myocardial infarction with no obstructive coronary arteries (MINOCA), a puzzling clinical entity that occurs in about 10% of all patients with acute myocardial infarction (AMI) criteria. After coronary angiography performed during AMI admission, approximately one third of MINOCA patients had signs of acute myocarditis. Using a transcriptome based approach in myosin heavy chain specific Th17 from experimental autoimmune myocarditis (EAM) induced mice, we identified mmu-miR-721 strongly upregulated in EAM-induced Th17 cells (Th17EAM). Mmu-miR-721 was also found in the serum of EAM mice 21 days after immunization but not in the serum of mice after ligation of the left anterior descending artery (LAD), highlighting this miRNA as a suitable marker between ischemic and non-ischemic pathology. Here we identified the novel hsa-miRNA-Chr8:96a human miR-721 homolog found in the plasma and selectively in extracellular vesicles secreted by Th17EAM cells from patients with myocarditis, as an efficient clinical screening method to distinguish between AMI and acute myocarditis patients.
POSTER 4. ATRIAL FIBRILLATION IS ASSOCIATED WITH HYPERMETHYLATION IN HUMAN LEFT ATRIUM, AND TREATMENT WITH DECITABINE REDUCES ATRIAL TACHYARRHYTHMIAS IN SPONTANEOUSLY HYPERTENSIVE RATS

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ABSTRACT

Atrial fibrillation (AF) is the most common cardiac arrhythmia. As the molecular mechanisms underlying the pathology are largely unknown, this cardiac arrhythmia remains difficult to treat. To identify specific molecular actors involved in AF, we have performed a transcriptomic analysis on left atrium (LA) from patients with valvular heart disease with or without AF. We showed that 1,627 genes had altered basal expression level in LA tissue of AF patients compared with the control group. The significantly enriched gene ontology biological process ‘anatomical structure morphogenesis’ contained the highest number of genes in line with changes in structure that occur when the human heart remodells following AF development (i.e. LA dilatation and interstitial fibrosis). We then focused the study on Pitx2 (paired-like homeodomain 2), being the most altered transcription factor in LA from AF patients and from which compelling evidence have indicated that its reduced expression can be considered as a marker for the disease. In addition, its expression was inversely correlated with LA size. We demonstrated that AF is associated with Pitx2 promoter hypermethylation both in humans and arrhythmic ageing spontaneously hypertensive rats (SHRs). Chronic administration of a DNA methylation inhibitor (i.e. 5-Aza-2’-deoxycytidine) improved ECG arrhythmic profiles and superoxide dismutase activities and reduced fibrosis in the left ventricle of SHRs. Taken together, these data support the notion that AF is associated with epigenetic changes in LA and provide a proof-of-concept that hypomethylating agents have to be considered in the treatment of atrial arrhythmias.
Atrial fibrillation (AF) is the most common arrhythmia. In spite of this, the treatment of AF remains deficient, because of the incomplete knowledge of the complex pathophysiology of this disease. Often, this arrhythmia has been linked to a dysregulation in the dynamics of intracellular calcium. In cardiac cells, calcium is mostly stored inside a network called sarcoplasmic reticulum (SR). Calcium ions can leave the SR through specific channels (ryanodine receptors or RyRs). Clusters of RyRs (CaRU) are placed at the membrane of the SR. Around 50-70 RyRs form one cluster and there are roughly 20000 clusters inside the cell, placed at a distance of \( \sim 1\mu m \). RyRs are sensitive to the calcium concentration, so that, a small spontaneous release of calcium (because of a random RyR aperture), defined as a spark, can turn on to more apertures of neighboring RyRs (in a process called calcium-induced calcium release), leading to a cascade effect that causes an intracellular wave. Exactly how many RyRs participate in a \( \text{Ca}^{2+} \) spark is still debated. However, the number of RyR per cluster and the size and distribution of the clusters has been shown to be different in wild type and cells from samples presenting AF, that also present a higher spark frequency and probability to develop macrosparks.

With this in mind, we have developed a model for the dynamics of intracellular calcium in auricular myocytes that takes into account the detailed intracellular ultrastructure, incorporating data from the size and geometry in the distribution of clusters of RyRs. We consider both calcium concentrations in the cytosol and the SR, using a bidomain model of subcellular calcium, in which the complex sarcoplasmic reticulum (SR) structure is considered through effective diffusion resulting from homogeneization. We have used this model to study the effect of heterogeneities in RyR distribution on the appearance of calcium sparks and waves.

The results of the model reproduce the normal cytoplasmic calcium handling, including the basal level and calcium peak. Monitorization of diastolic levels of SR calcium and fractional release of calcium validate the model. The model also reproduces the typical spark activity in a post-rest potential situation. Finally, we find that an increase in the size of the clusters of RyR, as observed in patients suffering of atrial fibrillation (AF), increases both the spark frequency and occurrence of macrosparks.

In conclusion, we have developed a model of intracellular calcium at the submicron scale that can be used to study the pro-arrhythmic effects of geometrical and functional changes of the cell microstructure.
Atrial fibrillation (AF) is one of the most prevalent cardiac diseases. Although it may have diverse causes, genetic screening has shown that a percentage of patients suffering of AF presents a genetic variant related to dysregulation of small conductance Ca2+ activated potassium (SK) channels. SK channels are potassium channels gated by changes in intracellular calcium. The functional role of these channels in cardiac electrophysiology is still under intense debate. While they do not seem to play an important role in healthy hearts – their associated current, IKCa, is smaller than other potassium currents -, there is increasing evidence that they may become relevant under pathological conditions. In fact, both pro- and anti-arrhythmic effects have been assigned to these channels, depending on the clinical situation. In this work, we have incorporated the current through SK channels, IKCa, into an electrophysiological ionic model of human atria myocyte. This allows us to evaluate changes in the action potential under different parameters affecting the kinetics of these channels. We observe a large dependence of the IKCa with the conductance and gate dynamics of the channel. SK channels are sensitive to changes in intracellular calcium dynamics avoiding or decreasing the pro-arrhythmic effect that events as spontaneous calcium release could produce.
POSTER 7. A DLG1 POLYMORPHISM SHORTENS THE ACTION POTENTIAL DURATION AND THE QT INTERVAL

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Introduction: SAP97 is an scaffolding protein encoded by the DLG1 gene that interacts with several cardiac ion channels including those underlying the fast Na (I_{Na}), the inward rectifier (I_{K1}) and the transient outward (I_{to}) currents, respectively. By next generation sequencing we identified a common [5.3% in the European (non Finnish) population] DLG1 polymorphism (rs34492126) in a man and two sisters diagnosed with Brugada Syndrome, two siblings with familial atrial fibrillation, a man with idiopathic ventricular fibrillation and another with early repolarization syndrome.

Purpose: This work aimed to determine the electrophysiological consequences of the SAP97 p.P888L polymorphism and whether they can contribute to the phenotype of the patients.

Methods: Native (WT) and p.P888L SAP97 tagged with ds-red were cotransfected or not together with the cDNA encoding the alpha and beta subunits underlying human I_{Na}, I_{CaL}, I_{to}, and I_{K1} currents, respectively, in Chinese hamster ovary (CHO) cells. Two cardiac–specific transgenic-like mouse models on the basis of adeno-associated virus gene transfer were created expressing WT and p.P888L SAP97, respectively. Currents and action potentials (APs) were recorded using patch-clamp.

Results: Co-expression of WT SAP97 significantly increased the I_{K1}, I_{Na}, and I_{to} in CHO cells (by 181%, 44%, and 77% respectively, n≥20, P<0.05). These results were confirmed in ventricular myocytes from SAP97 overexpressing mice. Conversely, overexpression of WT SAP97 halved the I_{CaL} densities recorded in both CHO cells and mouse ventricular myocytes. The effects produced by p.P888L SAP97 over the I_{Na} and the I_{CaL} were undistinguishable from those produced by the WT form, results that were confirmed in p.P888L cardiomyocytes. Conversely, in both CHO cells and mouse myocytes, overexpression of p.P888L SAP97 markedly reduced the I_{K1}, i.e., the opposite effect to that produced by SAP97 WT. Regarding the I_{to}, p.P888L also increased the I_{to} peak density, but, more importantly, it doubled the time constant of current inactivation. The slowing of the inactivation process increased the I_{to} charge density (133%) in both CHO cells and mouse myocytes. As a consequence, the AP duration (APD) measured at 20% and 50% of repolarization of the APs recorded in p.P888L SAP97 myocytes was significantly shortened. Electrocardiographic recordings in transgenic-like mice demonstrated that p.P888L overexpression shortened the QT interval compared with WT SAP97 overexpressing mice.

Conclusions: The SAP97 p.P888L polymorphism shortens the QT interval and the APD as a consequence of a marked increase of the Ito charge. Therefore, this polymorphism could exacerbate the phenotypic manifestations in patients affected by arrhythmogenic syndromes characterized by the repolarization acceleration.
POSTER 8. A MUTATION IN THE GENE ENCODING THE TBX5 TRANSCRIPTION FACTOR IS ASSOCIATED WITH THE BRUGADA SYNDROME

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Introduction: Loss-of-function mutations in SCN5A, the gene encoding cardiac Nav1.5 channels, are associated with primary arrhythmogenic syndromes such as the Brugada syndrome. Strikingly, many patients with Brugada Syndrome do not carry SCN5A mutations, pointing to the implication of mutations in other genes affecting expression and/or function of Nav1.5 channels. The transcription factor Tbx5, encoded by the TBX5 gene, plays a key role in cardiac development. Moreover, it has been described that it drives SCN5A expression in the adult mouse heart. In a proband diagnosed with Brugada syndrome, in whom screening for mutations in all described Brugada Syndrome genes was negative, next generation sequencing identified a missense mutation in TBX5 encoding for p.F206L Tbx5. This variation was confirmed by Sanger, predicted as pathogenic and was not previously annotated.

Purpose: We aimed to study the effects of p.F206L Tbx5 on the cardiac sodium current (I_{Na}) to unravel whether it can be associated to Brugada syndrome.

Methods: Human native (WT) and mutated Tbx5 tagged with GFP were transfected in HL-1 cells or included in lentiviral particles for infecting human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Peak and late I_{Na} (I_{Na,L}) were recorded using the whole-cell patch-clamp at room temperature. Luciferase reporter assays were conducted to determine the effects of this mutation on Nav1.5 channel promoter activity.

Results: Transfection of HL-1 cells with WT Tbx5 significantly increased the peak I_{Na} density (from -37.5±5.1 to -62.6±8.2 pA/pF; n≥6, P<0.05), whereas it did not modify the kinetics or voltage-dependence of activation and inactivation of the I_{Na}. Conversely, p.F206L Tbx5 strongly reduced the peak I_{Na} density (-6.7±0.2 pA/pF; n=6; P<0.01) compared to cells transfected or not with Tbx5 WT. However, p.F206L Tbx5 did not modify time- and voltage-dependent properties of the current. Neither WT nor p.F206L Tbx5, modified the I_{Na,L} density (-1.9±0.7 pA/pF at -20 mV; P>0.05). The effects produced by Tbx5 either WT or mutated on HL-1 cells were completely reproduced in hiPSC-CM. Indeed, in hiPSC-CM, WT Tbx5 increased (-27.6±1.9 pA/pF; n=7), while p.F206L Tbx5 decreased (-9.5±1.9 pA/pF) the peak I_{Na} compared to non-infected cells (-19.4±2.8 pA/pF; n=10; P<0.05), leaving the time- and voltage-dependent properties of the current unaffected. Luciferase reporter assays demonstrated that WT Tbx5 doubled the activity of the human SCN5A minimal promoter, whereas p.F206L completely suppressed Tbx5 pro-transcriptional activity over SCN5A.

Conclusions: The p.F206L mutation disables the remarkable Tbx5 pro-transcriptional activity over human SCN5A. Therefore, loss-of-function TBX5 mutations could be associated with the Brugada syndrome.
Atrial fibrillation, the most common arrhythmia seen by the clinician, is a highly morbid condition and the leading cause of hospitalizations for all arrhythmias. Some patients suffer relatively short (<7 days) self-terminating episodes (i.e., paroxysmal) of AF indefinitely, but a large proportion progress to long-lasting forms of AF. When AF lasts continuously for more than 7 days it is considered persistent AF. Sustained AF leads to electrical remodeling and fibrosis of the atria but the mechanism(s) remain poorly understood.

Remodeling can be due to aging, inflammation, underlying cardiac conditions, or AF itself. Current knowledge about the molecular basis of AFs mainly limited to particular genetic variants identified by genome-wide association studies and known mechanisms affecting myocardial structure, electrophysiology or signaling pathways. However, there is a clear demand for more inclusive and large-scale approaches to understand the molecular mechanisms responsible for the disease, as well as its progression and perpetuation.

Here we sought to characterize the dynamic changes of the transcriptome and proteome in an induced model of atrial fibrillation in a sheep model of persistent AF. We collected samples at clinically relevant time points during AF progression, from anatomically distinct regions of both atria, together with cardiomyocytes isolated from these tissues at similar time points. To integrate such multiple layers of information, we explored in detail and compared the data sets, applied different clustering methods, analyzed their concordance and divergence among time points of disease progression and tissues, and between the transcriptional and proteomic level. Moreover, taking advantage of data from isolated cardiomyocytes, we were able to decode signatures coming from other cell types whose roles could reveal new insights on AF-induced remodeling. Altogether, our results are a valuable resource for the study of the molecular dynamics underlying the progression from paroxysmal to persistent atrial fibrillation.
POSTER 10. THE HIGH SENSITIVITY OF PHASE ANALYSIS MIGHT GENERATE FALSE ROTORS

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Background: Atrial fibrillation (AF) is suggested to be driven often by rotors which have become the target of ablation to terminate the arrhythmia. Basket catheters are increasingly being used to localize those rotors. Our objective is to analyze their accuracy of localizing rotors by using phase analysis based on recorded electrograms, through computer simulations.

Methods: We simulated and analyzed rotor detection in a 3D realistic atrial model and an intracardiac basket-type mapping catheter (8x8 electrodes) positioned inside the right atrium. Rotors were detected by localizing phase singularity points on phase maps based on the Hilbert transform of the electrograms.

Results: For a single rotor simulated in the right atrium (Figure A), a basket-type catheter and phase maps (Figure D) detected the rotor with 90% accuracy. Additionally, the basket detected false rotors (IMPS) mainly where the electrodes were separated from the atrial wall. For those false rotors, at least 2 of the 4 electrodes surrounding the phase singularity point tracking the trajectory where at a distance greater than 0.5 cm. False rotors were a consequence of the high sensitivity of the phase analysis, which enhances the contribution of very low amplitude electrograms which may arise also from far field sources (Figure B-C), when the electrodes were either in contact or separated from the atrial wall and the atrial activation was complex.

Conclusions: On the one hand, accuracy of rotor detection by basket catheters and phase analysis increases when the electrodes are in full-contact with, or set at small distances (< 0.5 cm) from the atrial wall. On the other hand, the phase maps of the electrograms might generate false rotors due to the high sensitivity of phase analysis to low electrograms amplitudes which could arise from either local or far sources.
Figure legend. A) A transmembrane voltage (Vm) snapshot of simulated atrial electrical activity. B) Snapshot of electrograms (EGM) voltage calculated on the endocardial surface and color-coded on a magnified scale. C) Traces of the electrograms in points 1 and 2 in B. D) Phase maps on the endocardial surface (left) and basket phase maps (right). LA: left atrium; RA: right atrium; SVC: superior vena cava; IVC: inferior vena cava; RPV: right pulmonary veins; CT rotor: rotor along the crista terminalis; RWE: rotor wave extension; IMPS: imaginary phase singularity; IM-RWE: imaginary rotor wave extension.
**POSTER 11.** EXERCISE TRIGGERS ARHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVC) PHENOTYPE AND GENETIC CHANGES IN MICE HEARTS EXPRESSING A DISEASE-CAUSING MUTATED VERSION OF HUMAN PLAKOPHILIN-2 (PKP2)

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**Abstract:**

Exercise is generally considered a health practice in the general population. However, it is also associated with arrhythmic risk in carriers of arrhythogenic right ventricular cardiomyopathy (ARVC)-associated desmosomal mutations. Interestingly, mutations in desmosomal gene plakophilin-2 (PKP2) remarkably increase the probability of developing ARVC following extreme exercise. This study evaluated that structural and functional cardiac remodelling, following endurance exercise training, is associated with genomic and proteomic changes. We developed a new model of cardiac tissue-specific transgenic–like mice based on adeno-associated (AAV) gene transfer to test the potential of a combination of a human PKP2 mutation (R735X) and endurance training to trigger an ARVC-like phenotype. Here we applied RNA sequencing (RNA-seq) and proteomics to identify genes and proteins signatures for ARVC from the right ventricles of four groups including controls of sedentary and exercise mice expressing wild type PKP2 and R735X mutant. The results from the above-mentioned experiments identified genes and proteins related to mitochondria and cytoskeleton suggesting that both are important for ARVC development following extreme exercise.
POSTER 12. INHIBITION OF PDE3 BUT NOT PDE4 PHOSPHODIESTERASES STIMULATE RYANODINE RECEPTOR PHOSPHORYLATION AT SER2808

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Background: In healthy cardiomyocytes, contraction is activated by calcium induced calcium release from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR2). This occurs when opening of the L-type calcium channel triggers opening of the RyR2. However, under pathological conditions the RyR2 can also open spontaneously, giving rise to calcium waves and spontaneous electrical activity. This phenomenon has been associated with excessive cyclic adenosine monophosphate (cAMP)-dependent phosphorylation of the RyR2. Therefore, the degradation of cAMP by phosphodiesterases (PDE) is expected to play a key role in the regulation of cytosolic cAMP levels and hence RyR2 phosphorylation and activity. However, little is known about the effects of PDEs on the distribution and phosphorylation state of individual RyR2 clusters.

Purpose: The aim of this study was to investigate how inhibition of the phosphodiesterases PDE3 and PDE4, which play a key role in the regulation of cAMP levels in the human heart, affects the distribution of RyR2 clusters and their phosphorylation at ser2808 in human atrial myocytes.

Methods: Human atrial myocytes were isolated from patients undergoing cardiac surgery. The selective PDE3 inhibitor cilostamide (1 µM) was used to assess the role of this PDE and PDE4 was inhibited with RO 20-1724 (10 µM). After incubation with the PDE inhibitor for 10 mins, myocytes were fixed and labelled with mouse anti-RyR2 (C3-33; NR07, Calbiochem) to detect total RyR2 and rabbit anti-phospho-ser2808 (Ser-2808; A010-30, Badrilla) to detect ser2808 phosphorylated RyR2s. A novel approach was used to assess phosphorylation of individual RyR2 clusters at ser2808 based on the ser2808/total RyRs fluorescence intensity ratio. Images were obtained with a 63x objective and resonance-scanning confocal microscopy (Leica SP5 AOBS). RyR2 clusters were detected using a custom-made algorithm.

Results: In control conditions, there was a spatial gradient in the ser2808/total RyR2 (C3-33) fluorescence ratio, with a significantly higher ratio for RyR2 clusters located at the sarcolemma (0.49±0.05) than at the cell center (0.31±0.03, p<0.001). Upon inhibition of PDE3, the fluorescence ratio at the cell center increased from 0.31±0.03 (n=9) in control to 0.73±0.06 with 1 µM cilostamide (n=9, p<0.001). By contrast, 10 µM RO 20-1724 had no significant effect on the ser2808/C3-33 ratio (0.32±0.05, n=10) when compared to control conditions (0.31±0.03, n=10). Neither PDE3 nor PDE4 inhibition had any effect on the spatial gradient of ser2808-phosphorylated RyR2s in human atrial myocytes.

Conclusions: Inhibition of PDE3 but not PDE4 increases RyR2 phosphorylation at ser2808, suggesting that pharmacological control of PDE3 activity may help regulating spontaneous calcium release caused by excessive RyR2 phosphorylation at ser2808.
**POSTER 13. INHIBITION OF THE PHOSPHODIESTERASE PDE3 INDUCE ALTERATIONS IN THE FREQUENCY AND PROPERTIES OF CALCIUM SPARKS THAT FAVOUR THE INDUCTION OF ARRHYTHMOGENIC CALCIUM WAVES AND TRANSIENTS**

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**Background:** Under pathological conditions, spontaneous calcium release from the sarcoplasmic reticulum (SR) can trigger spontaneous electrical activity, and this phenomenon has been associated with excessive cyclic adenosine monophosphate (cAMP)-dependent phosphorylation of the cardiac ryanodine receptor (RyR2). Therefore, phosphodiesterases (PDE) that degrade cAMP are expected to play a key role in the regulation spontaneous calcium release.

**Purpose:** The aim of this study was to investigate how inhibition of the phosphodiesterase PDE3, which plays a key role in the human heart, affects the properties and frequency of local non-propagating spontaneous calcium release events (calcium sparks).

**Methods:** Human atrial myocytes were isolated from 13 patients without a history of atrial fibrillation. The selective inhibitor cilostamide (1 µM) was used to assess the role of PDE3 in the regulation of calcium spark properties and frequency. Calcium sparks were recorded in cal-520 loaded myocytes with a 63x objective and resonance-scanning confocal microscopy (Leica SP5 AOBS). Sparks were detected and characterized using a custom-made algorithm.

**Results:** In control conditions, the densities of calcium sparks (1.48±0.53 sparks/min/1000µm²) and spark sites (2.00±0.68/1000µm²) were modest and calcium waves were not observed. Inhibition of PDE3 with 1 µM cilostamide, strongly increased the density of calcium sparks (to 22.2±5.4 sparks/min/1000µm², p=0.002) and spark sites (to 13.6±4.6 sites/1000µm², p<0.001) suggesting that a larger number of calcium spark sites reaches the threshold for spontaneous calcium release when the PDE3 activity is reduced. The spark density per site was also increased (from 0.43±0.13 to 1.99±0.25 sparks/site, p<0.001) indicating that the refractory period for the spark sites was reduced. The amplitude of the calcium sparks (F/F₀) was not modified by cilostamide (1.78±0.04 vs 1.79±0.07 in control), but there were significant increases in spark width (2.70±0.12 to 3.02±0.2 µm, p<0.05) and decay (36.1±3.9 to 43.6±10.9ms, p=0.02) after exposure to cilostamide. In accordance with the notion that the increase in the spark dimensions and density of spark sites would favor their fusion into larger calcium waves, we observed the appearance of calcium waves and spontaneous calcium transients in 9/13 patients when exposed to cilostamide (1.37±0.51 events/min, p=0.02).

**Conclusions:** Inhibition of the PDE3 activity increases calcium the calcium spark width, duration and density, leading to the induction of spontaneous calcium waves and transients. This suggests that pathological conditions associated with diminished PDE3 activity such as atrial fibrillation may contribute to perpetuate the arrhythmia by favoring arrhythmogenic calcium release.
POSTER 14. MITRAL VALVE DISEASE ALTERS CALCIUM HOMEOSTASIS IN HUMAN ATRIAL MYOCYTES

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Background: Mitral valve disease is a common cardiovascular alteration that, if left untreated, increases cardiovascular morbidity and mortality. The disease is also a known risk factor for atrial fibrillation (AF), an arrhythmia that has previously been associated with alterations in the intracellular calcium homeostasis. However, little is known about the effects of mitral valve disease on the intracellular calcium homeostasis in human atrial myocytes. We therefore tested whether human atrial myocytes from patients undergoing mitral valve replacement (MVR) display alterations in the L-type calcium current ($I_{Ca}$) density or in the frequency of transient inward currents ($I_{TI}$).

Methods: The $I_{Ca}$ density and the $I_{TI}$ frequency were measured in human right atrial myocytes from 388 patients with the perforated patch technique. 82 of the 308 patients underwent MVR while the other 306 patients had surgery for other cardiovascular diseases (no MVR). Mitral valve insufficiency was classified on a scale of 0-4. Results were analyzed with a linear regression model taking into account confounding effects of atrial rhythm, age, cardiovascular risk factors, concurrent cardiovascular disease and pharmacological treatments. For subsequent analysis, patients were divided into three groups according to the atrial rhythm: 1: no AF; 2: paroxysmal AF; and 3: permanent AF.

Results: Overall, 36.6% of all patients underwent MVR. However, only 10.7% of the patients without AF had MVR, while 38% of those with paroxysmal AF and 54% of those with permanent AF had MVR. Linear regression analysis of all patients revealed that MVR had no independent effect on the $I_{Ca}$ density (-1.97±0.26 vs. -2.09±0.23 pA/pF, p=0.47). This was also true when patients were divided into three groups according to the atrial rhythm. By contrast, MVR independently increased the incidence of $I_{TI}$ from 1.50±0.41 to 2.04±0.45 events/min (p<0.05). Moreover, when dividing patients into three groups according to the atrial rhythm, the $I_{TI}$ frequency was elevated in all patients undergoing MVR independently of the atrial rhythm, reaching 2.04±0.52, 2.04±0.67 and 2.22±0.54 events/min in patients without AF, with paroxysmal AF and with permanent AF respectively. By contrast in patients without MVR, there was a progressive increase in the $I_{TI}$ frequency from 1.02±0.44 events/min in patients with no AF (p=0.01 vs. no AF and MVR) to 1.74±0.54 in patients with paroxysmal AF and 2.16±0.50 events/min in patients with permanent AF and no MVR. Analysis of the effect of mitral valve insufficiency in patients without AF showed no independent effect of the degree of mitral valve insufficiency on the $I_{Ca}$ density or the $I_{TI}$ frequency.

Conclusion: Mitral valve disease is associated with a significantly higher incidence of potentially arrhythmogenic $I_{TI}$ currents in human atrial myocytes. This effect is most pronounced in patients without AF and could facilitate induction of AF in this group, contributing to the higher incidence of AF observed in patients undergoing MVR.
POSTER 15. AUTOMATIC DETECTION OF RYR2 CLUSTERS AND THEIR PHOSPHORYLATION STATE IN ISOLATED CARDIOMYOCYTES

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Background: Cardiac arrhythmias have been associated with anomalous calcium handling by the cardiac ryanodine receptor (RyR2). In particular, a higher incidence of spontaneous calcium release events has been associated to excessive RyR2 phosphorylation. While phosphorylation can be determined with western blotting, this technique cannot provide the spatial distribution or state of individual RyR2 clusters.

Purpose: To develop an image-processing tool that allows automatic detection of individual RyR2 clusters and quantification of their phosphorylation state in order to be able to map the distribution and phosphorylation state of the clusters in isolated cardiomyocytes.

Methods: We used isolated ventricle myocytes from genetically modified mice expressing RyR2s labelled genetically with green fluorescent protein (GFP), which allows identifying all the RyR2 clusters in a myocyte. The myocytes were labelled (in red) using primary antibodies detecting all RyR2s (rabbit anti-RyR2, ARR-002, Alamone) or RyR2s phosphorylated at ser2808 (rabbit anti-ser2808; A010-30, Badrilla). Images of the RyR2 clusters were collected with a Leica SP5 confocal microscope. We first convolve the images with a Gaussian template to enhance the fluorescence peaks of the clusters and remove noise. The location of each cluster is then determined by detecting all regional maxima in the image. The detection was further improved by filtering each cluster based on size and intensity. The intensity of each RyR2 cluster was defined as the mean value of a 0.33 by 0.33 µm region of interest around the RyR2. The ratio of the fluorescence emission of the red-labelled RyR2 antibody and the GFP-tagged RyR2 was determined for each GFP-tagged cluster. Finally, algorithm was designed to allow division of the myocyte into concentric layers in order to detect spatial gradients in the RyR2 distribution and or phosphorylation state.

Results: Labeling with anti-RyR2 (in red) revealed that the immunofluorescent labeling was homogeneous and efficient. With a strong correlation between the red fluorescence emission and the GFP fluorescence emission (1.13, r^2=0.70, n=4135, p<0.01). Moreover, detection of immunofluorescently labelled RyR2 clusters was highly efficient with an average of 210±18 clusters/cell compared to a reference value of 223±19 GFP-tagged clusters/cell. To validate the use of the fluorescent intensity ratio as a measure of the RyR2 phosphorylation state, RyR2 phosphorylation was induced by exposing myocytes to the beta-adrenergic agonist fenoterol (FENO, 3μM) for 5 min. Comparison of the anti-ser2808/GFP fluorescence ratio before and after exposure to FENO revealed that the average ratio was doubled from 0.63±0.09 to 1.41±0.08 (p=0.01). Moreover, division of
myocytes into concentric layers revealed that the density of RyR2 clusters was higher near the sarcolemma than in the cell center (0.78±0.05 vs. 0.48±0.02 clusters/μm², p<0.01) while there were no gradients in the RyR2 phosphorylation at ser2808.

**Conclusion:** We have developed an image-processing tool that allows automatic detection of individual RyR2 clusters, which will help identifying pathological changes in the distribution and phosphorylation state of the RyRs and linking them mechanistically to myocardial dysfunction and arrhythmia.
Atrial fibrillation (AF) is the most common arrhythmia, with more than 33 million patients in the whole world. However, the efficacy of antiarrhythmic drugs is limited at the time of terminating AF and maintaining sinus rhythm in different patients. The understanding of the mechanisms responsible of AF maintenance is essential to achieve a personalized treatment for each patient increasing thus, the efficacy. In this work, in-silico simulations representing inter-subject variability are used to find the ionic profile characteristics that explain different responses to antiarrhythmic drug treatments.

Specifically, the aim of this work is to investigate the factors promoting variability in the response of AF dynamics to sodium (INa) and calcium (ICaL) current block.

A population of 173 electrophysiological atrial tissue models capturing variability in experimental measurements from 149 AF patients was used to perform reentry simulations in three scenarios (1) basal conditions, (2) after a 50% decrease in the INa conductance and (3) after a 50% decrease in the ICaL conductance. The relation between the electrophysiological properties and AF termination efficacy of the antiarrhythmic therapies was evaluated.

During basal conditions 126 of the 173 (72.8%) models sustained stable reentries. Partial block of sodium terminated AF by collision between rotors in 64 fibrillation models (50.8%) whereas partial calcium block produced the reentrant termination in 38 (30.2%). With both treatments, termination was associated with an increase in meandering and collision between rotors.

The effectiveness of sodium and calcium block was exclusive in 52 AF models: 39 models (60.9%) which terminated by sodium block did not terminate by calcium block, whereas 13 of the AFs which terminated by calcium block did not terminate with sodium block (34.2%). Effectiveness of sodium or calcium block was dependent on the ionic characteristics of each model. The same reason explained the juxtaposed effects in reentry dominant frequency produced by Calcium block.

As a conclusion, simulations based on a population of models predict that a significant number of AF patients may require completely different pharmacological strategies depending on their specific electrophysiological properties.
Cardiac inflammation and fibrosis are central to cardiovascular diseases including atrial fibrillation (AF). Inflammation is a major indicator of AF in clinical studies but its role in the progression from paroxysmal to persistent AF remains unexplored. Intracardiac levels of the pro-fibrotic protein galectin-3 are greater in patients with persistent than paroxysmal AF and independently predict atrial tachyarrhythmia recurrences after a single ablation procedure. In sheep with tachypacing induced AF, galectin-3 inhibition mitigates structural and electrical remodeling and significantly reduces AF burden. We tested the hypothesis that galectin-3 mediated inflammation early after the onset of atrial tachypacing underlies the structural and electrical remodeling responsible for the progression to persistent AF.

**Methods and results:** Self-sustained AF was induced by atrial tachypacing. Five sheep were euthanized 11.5±2.3 days after AF became self-sustained (transition AF); seven sheep were euthanized after 341.3±16.7 days of self-sustained AF (persistent AF). Six sham-operated animals were in sinus rhythm for 1 year. Expression of Toll-like receptors 4 (TLR4), one of the key receptors for the innate immunity response, was significantly upregulated by 58% in the left atrial appendage (LAA) of transition sheep, and was maintained in persistent AF sheep. TLR2 expression was also significantly increased by 34% and 55% in LAA of transition and persistent AF animals, respectively, compared to sham operated animals. These changes were accompanied by increases in the downstream factor MyD88 of 138.16±13.05% in transition and 141.42±18 in persistent AF sheep, as well as 160.28±16% increase in NFκB phosphorylation in persistent AF sheep. The results suggested that inflammation occurs early in the progression of AF and is sustained thereafter. Importantly, the expression of TLR4 protein was 57% lower in sheep receiving upstream therapy with the galectin-3 inhibitor GMCT-01 (12 mg/kg i.v. twice/week) than animals receiving saline twice per week. TLR2 expression was reduced by only 8%.

**Conclusion:** Galectin-3, a pro-fibrotic protein that is released by fibroblasts during sustained AF activates TLR4 and MyD88 in the myocardium to induce inflammation. We postulate that during sustained AF the resulting inflammatory cascade associated with increased galectin-3 contributes to electrical remodeling, fibrosis and AF perpetuation by modulating the functional expression of genes involved in ion channel and extracellular matrix remodeling.

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POSTER 18. DESIGN OF A METHODOLOGY FOR THE DEVELOPMENT OF PERSONALIZED ATRIAL MODELS

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The development of personalized 3D cardiac models for each patient requires to introduce anatomical, histological, functional and electrophysiological information. However, the acquisition and inclusion of this information in an appropriate and realistic manner requires the implementation of a methodology defining, ordering and adapting the steps needed for the development of personalized atrial models.

To perform the study, computed axial tomography (CT) and delayed-enhancement magnetic resonance imaging (DE-MRI) images were used, belonging to two patients from Hospital Universitario y Politécnico de La Fe. The proposed work consists in the design of a methodology for the development of specific 3D atria models for each patient, so that these models can be used in simulations of cardiac electrophysiology. In addition, we will also model the specific fibrosis regions present in the two patients will be included to each model to obtain more realistic simulations. To do so, the anatomical images provided by computerized axial tomography will be used first to generate the meshes of the surface models (Figure A). CT images were obtained using iodine as a contrast agent, 330 slices and 0.8 mm of slice thickness. These meshes will be divided into the different anatomical zones of the atria, and each one will then be assigned different electrophysiological properties. Subsequently, information related to fibrotic regions (indicated with arrows in the figures) was provided by DE-MRI images using a 3D viability sequence acquisition, which allows to segment such regions and finally include them into the meshes (Figure B). A 3 Tesla scanner was used for the acquisition and was performed 10 minutes after intravenous administration of 0.2 mmol/kg gadobenate of dimeglumine.

From these surface meshes divided into regions and with the built-in fibrosis zone, a hexahedral mesh of finite volume elements will be generated (Figure C). This resulted in a volumetric mesh formed by 2360736 nodes and 1887131 elements, and another mesh formed by 2914102 nodes and 2330322 elements. The orientation of cardiac muscle cells (fibers) will then be defined based on previous histological studies, and will be added to the volumetric model in order to obtain realistic propagation patterns and activation sequence results. Finally, a series of electrophysiological simulations were performed under different conditions for each of the generated models (Figure D). One set of simulations were carried out under control conditions (sinus rhythm) while another correspond to a situation of chronic atrial fibrillation.
POSTER 19. SUBSTRATE CHARACTERIZATION OF ROTATIONAL ACTIVITY SITES IN PERSISTENT ATRIAL FIBRILLATION PATIENTS

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Background: The underlying mechanisms initiating and sustaining atrial fibrillation (AF) are still under debate, and an optimal treatment for AF is not been well established. Spatiotemporal stable sources (rotors) have been proposed as maintenance mechanism of AF. The use of high density electroanatomical mapping with microelectrodes (HDEMM) and a novel rotational activity detection system we can detect rotors and characterize the tissue where rotors are located.

Objective: To characterize the tissue voltage in electro-anatomical maps where rotational activity is detected using HDEMM.

Methods: We included 28 consecutive persistent AF patients referred for first ablation. A novel rotational activity detection system was employed to process the signals in real-time and automatically assessed the presence of rotors. The acquisition and the HDEMM was performed using the PentaRay catheter and Carto3 (Biosense Webster), and maps were obtained in AF. The catheter was required to remain stable and fully spread during the acquisition of the signals. Recordings were acquired for at least 10 seconds per site and the catheter’s position was annotated in the 3D map.

The system detected rotational activity, generating a video for each site containing rotational atrial wavefronts using isochronal maps. Two annotators labeled the detected rotational activity attaining incomplete, complete or multiple or no gyres. Rotational activity was defined for complete or multiple gyres. Electroanatomical maps were exported to measure the bipolar voltages.

Results: We evaluated 603 registers (mean 21 sites/patient). We rejected 214 due to inadequate deployment or noisy signals. Rotational activity was found in 243 sites (the remaining 146 sites exhibited no rotations).

For each rotor site we propose two concentric circle measurements, one of radius 5mm (ϕ=10mm) to analyze the tissue where the rotor spins/anchors and other of radius 15mm (ϕ=30mm) including the catheter acquisition range. Mean bipolar voltages for all labels were 0.65 mV (inner) and 0.61 mV (outer).
Conclusions: The analysis shows evidence of voltage values related to rotational activity beyond bipolar voltage range 0.1-0.5 mV, classically considered for scar definitions. Functional assessment may add incremental value to invasive treatment of AF.

Figure 1. Bipolar voltage distribution of complete and multiple gyre sites for the inner and outer measurement circles.

Table 1. Complete and multiple gyre voltage statistics for the inner ($\phi=10$mm) and outer ($\phi=30$mm) circles.
The mechanisms that contribute to the initiation and maintenance of atrial fibrillation are still unclear. Under chronic atrial fibrillation (cAF), cardiac tissue experiences electrophysiological and structural remodeling. Fibrosis in the atrial tissue has an important impact on myocyte action potential (AP) and its propagation. Recent studies indicate an existing coupling between myocytes and fibroblasts. In the present simulation study, the effects of fibroblasts coupling to atrial myocytes are analyzed under normal sinus rhythm (nSR) and under cAF conditions for four different anatomical region in the atria: pulmonary vein (PV), left atria (LA), left atria appendage (LAA) and the right atria (RA).

Human atrial myocyte and atrial fibroblast electrophysiology were simulated using mathematical models proposed by Koivumaki et al. Atria AP for each anatomical regions we used the modifications proposed by Ferrer et al. Cellular simulations were run using two different resting membrane potentials for the fibroblasts (RMPf) and three fibroblast coupled to a single myocyte for each region. Additionally, AP propagation along a 1D fiber with diffuse fibrosis randomly distributed will be simulated to observe the effects on conduction velocity (CV).

Results show different myocyte's AP duration (APD) for each region in nSR or cAF: RA 233.2 ms, 168.4 ms; LA 213.6 ms, 141.5 ms; PV 241.2 ms, 122.3 ms; and LAA 193.5 ms, 136.2 ms respectively (control). When myocyte was coupled with fibroblasts under nSR and RMPf of -30mV, APD is increased from their control value for RA, LA and LAA (137%, 130%, 135% respectively), but for PV was unmodified. Additionally, when RMPf was -45 mV, APD for PV is reduced to 55% of its control value while APDs in the other regions were less affected. Due to cAF electrical remodeling and fibroblasts coupling, myocyte APD with RMPf of -30 mV RA, and LA APDs were decreased (88%, 97% respectively), while in PV and LAA were slightly increased (103%, 101% respectively). When RMPf was -45 mV, APD in RA was more decreased than in the other three regions.

In 1D simulations, CV in nSR and cAF were RA 70 cm/s, 67 cm/s; LA 82 cm/s, 79 cm/s; PV 120 cm/s, 115 cm/s; and LAA 55 cm/s, 52 cm/s respectively (control). With two different RMPf (-30mV, -45mV) and 10% of fibrosis, CV reduces between 80%-84% for RA, 84%-78% for LA and 75%-70% for LAA in nSR. For both RMPf in PV, CV was 85%. Under cAF and both RMPf, CV was also decreased for RA 60%-48%, for LA 80%-81%, for LAA 71%-77% respectively. However, PV reduction was the same percentage (80%) for both RMPf. When density increases to 20% reduction was 67%-53% for RA, 69%-73% for LA, 61%-66% for PV and 61%-63% for LAA in nSR, and 55%-16% for RA, 60%-64% for LA, 68%-71% for PV and 61%-35% for LAA in cAF.

In conclusion, fibroblasts and their RMP significantly modify myocyte AP and its propagation along cardiac tissue. Under cAF conditions, atrial myocytes seems to be less sensitive to fibroblast coupling and their RMPf variation depending on the region.
POSTER 21. SITES WHERE PERSISTENT ATRIAL FIBRILLATION IS TERMINATED BY LOCALIZED ABLATION HAVE REPETITIVE ACTIVATION PATTERNS ON ISOCHRONAL MAPPING

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Background: The mechanisms by which persistent AF terminates via localized ablation are not well understood. To address the hypothesis that sites where localized ablation terminates persistent AF have characteristics identifiable with activation mapping during AF, we systematically examined activation patterns acquired only in cases of unequivocal termination by ablation.

Methods: We recruited 57 patients with persistent AF undergoing ablation, in whom localized ablation terminated AF to sinus rhythm or organized tachycardia. For each site, we performed an offline analysis of unprocessed unipolar electrograms collected during AF from multipolar basket catheters using the maximum –dV/dt assignment to construct isochronal activation maps for multiple cycles. Additional computational modeling and phase analysis were used to study mechanisms of map variability.

Results: At all sites of AF termination (figure Ai, Bi, Ci), localized repetitive activation patterns were observed (figure ii a-c). Partial rotational circuits were observed in in 26/57 (46%) cases (figure row A), focal patterns in 19/57 (33%) (figure row B), and complete rotational activity in 12/57 (21%) cases (figure row C). In computer simulations, incomplete segments of partial rotations coincided with areas of slow conduction characterized by complex, multicomponent electrograms, and variations in assigning activation times at such sites substantially altered mapped mechanisms.

Conclusions: Local activation mapping at sites of termination of persistent AF showed repetitive patterns of rotational or focal activity. In computer simulations, complete
rotational activation sequence was observed but was sensitive to assignment of activation timing particularly in segments of slow conduction. The observed phenomena of repetitive localized activation and the mechanism by which local ablation terminates putative AF drivers require further investigation.
POSTER 22. HCN4 AND GATA5 PREVIOUSLY UNDESCRIBED VARIANTS IN LARGE KINDRED WITH FAMILIAL ATRIAL FIBRILLATION

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BACKGROUND

Genetic background can be difficult to relate to accepted mechanisms of atrial fibrillation (AF). Mutation of genes encoding ionic channels can result in early onset, familial AF while other variants can be associated to AF risk without clear definition of mechanisms. Mutations in the HCN4 and GATA5 genes encoding the hyperpolarization-activated cyclic nucleotide-gated channel 4 (hc4) and the GATA5 transcription factor, respectively belong to the second category.

OBJECTIVE

Genetic and clinical study of two generations (G) of a large kindred with somewhat high incidence of AF.

First G (G-I) made of 15 siblings, 4 with clinical AF. Second generation (G-II) made of 43 subjects.

METHODS

Long-term ECG monitoring: Full-time, continuous monitoring of 1 ECG lead (average 12 days / 282 ±55 h / 22 ±1.5 h per day) was performed in subjects without clinical AF supported by a new type of textile electrodes mounted on a wearable band, with good tolerance. Full visual review of continuous recordings, supported by analysis software, was done by 2 cardiologists, blinded to genetic testing. Doubtful findings were resolved by wider consensus.

Genetic study: The 4 affected siblings were genotyped by next generation sequencing by means of a Haloplex Custom panel including coding regions and untranslated (UTR) boundaries of 82 genes encoding cardiac ion channels, proteins of cardiac channelosomes, and other proteins that modulate ion channel activity. The confirmation of the variants found and the genetic test were done with the Sanger method. The variants found in the 4 index cases were searched in the rest of the family using the Sanger method.
RESULTS

We studied a total of 42 subjects through genetic study of which 40 were studied through prolonged monitoring, 7 of 13 living G-I and 35 of 43 G-II subjects. Fifty percent of subjects were women (33% in G-I, 53% in G-II, P=0.65). Average age 38±14 y/o G-I subjects were older (63±7 y/o vs 32±8, P<0.001), had more hypertension (50% vs 0%, P=0.004), dyslipidemia in (50% vs 24%, P=0.3), smoking habit (50% vs 7.7%, P=1), overweight (80% vs 38%, P=1) and sleep apnea (50% vs 24%, P=0.034). All 4 G-I subjects with clinical AF shared a heterozygous variant (NM_005477.2:c.3488C>A) at the HCN4 gene, leading to substitution of Pro1163 residue, located at the end of the C-terminus of the channel, to His (p.P1163H hcn4) and a heterozygous variant (chr20:61040536 G,A) at an intronic region of the GATA5 gene (NM_080473.4). The HCN4 variant was also indentified by Sanger in 4 G-I subjects and 6 G-II subjects, of whom, 1 had frequent atrial extrasystoles and 2 had frequent atrial tachycardia. No AF clinically or in Holter was detected. However, no significant differences were found regarding the incidence of atrial arrhythmias or left atrial size in the carriers of the variant. Sinus node dysfunction was not evident in any of the subjects clinically or by Holter. The GATA variant was also identified by sanger in 5 other G-I subjects and 13 G-II subjects, of whom 2 had frequent atrial extrasystoles and 3 had frequent atrial tachycardia. The only subject with AF not previously known also carries the variant. Nevertheless no significant differences were found regarding the incidence of atrial arrhythmias or left atrial size between subjects carrying or not the variant.

CONCLUSION

This large family with a new HCN4 and GATA5 variants and a somewhat high clinical incidence of AF shows a complex genotype/phenotype relationship. The incidence of AF was not related to sinus node dysfunction. The relationship with AF and other clinically silent atrial arrhythmias was not clear in this large kindred. Follow-up of relatives without AF should help clarify the issue.
Atrial fibrillation is the most common sustained arrhythmia and it is expected that its prevalence will increase in next years. Although it is not considered a malignant arrhythmia, its appearance involves risks to the patient’s health and leads to recurrent hospital admissions. However, the physiopathology of the disease it is not known in detail and its treatment is still complex. In this scenario, computer modelling has emerged as a powerful tool in cardiac electrophysiology. For the study of cardiac arrhythmias, it is required a highly-detailed model incorporating anatomical and electrical heterogeneity and an accurate description of fibres orientation. Thus, the objective of this work was to develop a “true” 3D model of the human atria with a realistic description of the wall thickness (from 0.5 to 7 mm), changes in fibres orientation trough the atrial wall and electrophysiological heterogeneity. Two algorithms were implemented with MATLAB for the definition of the wall thickness and the fibres orientation. The model was meshed with hexahedral elements with spatial resolution of 300 µm. The resulting atrial mesh consisted of 1,945,101 elements and 2,174,034 nodes. The Courtemanche model was used to solve the electrical activity and tissue propagation was described by the monodomain formalism. To reproduce the heterogeneity in the atrial properties, nine cellular models and ten atrial tissues were defined. The electrical remodelling was also included through the variation of the maximum conductances of Ito, IcαL, Ik1, Ikur y Iks. The new model was validated by comparing the propagation sequence in sinus rhythm with respect to the experimental local activation times. Then, the electrical remodelling effect was analysed, showing a 56% reduction in the APD90. This shortening in the action potential duration makes the atrial tissue more vulnerable for atrial fibrillation. We compared the fibrillatory activity of the new model with two models less detailed anatomically but with the same electrophysiological properties. The three models reproduce a recurrent fibrillatory patron with the stabilization of a rotor. However, in each model the rotor appears in different areas. It demonstrates that anatomical properties affect the electrical behaviour of the simulation. Hence, in modelling it is very important to get as close as possible to the real system to obtain results that match experimental observation. In conclusion, the obtained results demonstrate that the new model is a useful tool for propagation studies in both physiological and pathological conditions.
Figure. (a) Atrial wall thickness. (b) Local activation times (LATs) on the anterior view of the atrium. (c) Activation during atrial fibrillation. The rotor is located on the superior left pulmonary vein.