Resumen Proyecto

La fibrilación auricular (FA) es la arritmia cardíaca más común que afecta a 38 millones de personas en todo el mundo. Duplica la mortalidad, aumenta el riesgo de accidente cerebrovascular cinco veces y disminuye la calidad de vida. Además, se desarrolla progresivamente con la edad y se vuelve cada vez más difícil de tratar con el tiempo. Por lo tanto, hay una creciente necesidad de un tratamiento personalizado más eficiente para los pacientes con FA, con el fin de reducir la inmensa carga socioeconómica de esta enfermedad. En este contexto, se han asociado más de 150 polimorfismos de un solo nucleótido (SNP) con un mayor riesgo de FA en los últimos 15 años.

Esto ofrece nuevas oportunidades para mejorar la prevención, la estratificación del riesgo y el tratamiento personalizado de la FA, pero la alta frecuencia y el impacto modesto de estas variantes complican su utilidad clínica. Para resolver este problema, uno de los principales desafíos radica en la identificación de variantes que aumenten sinérgicamente el riesgo de FA. Debido a la ubicación intronica o intergénica de la mayoría de estas variantes, los mecanismos que vinculan un SNP con el riesgo de FA siguen siendo virtualmente desconocidos, dificultando la identificación de SNPs que afectan a una vía de señalización común y que aumente el riesgo, obstaculizando así su uso en la medicina de precisión.

Por lo tanto, para avanzar en el estado actual de este campo de investigación y facilitar un uso eficiente de los SNP asociados con el riesgo de FA en la práctica clínica y la medicina de precisión, nuestro objetivo es probar la hipótesis de trabajo que establece que *la identificación de polimorfismos de un solo nucleótido (SNP) vinculados a la señalización defectuosa de AMP cíclico y la homeostasis del calcio; y organizarlos en grupos con efectos sinérgicos, aditivos o antagonistas proporcionará nuevos criterios para mejorar la estratificación del riesgo y personalizar la prevención y el tratamiento de la FA*.

Para lograr este objetivo, nuestro proyecto se divide en dos subproyectos que utilizan análisis genómicos y proteómicos de muestras auriculares humanas, combinados con técnicas micoscopía confocal en tiempo real y patch-clamp en miocitos auriculares humanos nativos y derivados de células madre, así como modelización matemática. Esto nos permitirá determinar cómo la interacción entre múltiples SNP afecta la señalización local subcelular de AMP cíclico y calcio, identificar las combinaciones con efectos sinérgicos que causan disfunción electrofisiológica celular, y validar los mecanismos moleculares subyacentes como objetivos terapéuticos específicos para las respectivas combinaciones de SNP.

Posteriormente, para validar los hallazgos a nivel molecular y celular en entornos clínicamente relevantes, demostraremos que los SNPs que inducen alteraciones funcionales en miocitos también inducen alteraciones correspondientes en la función auricular, que pueden detectarse con herramientas de diagnóstico actuales como ECG, resonancia magnética cardíaca, mapeo electro-anatómico o registros Holter. Finalmente, para evaluar la utilidad clínica de estos hallazgos, determinaremos el impacto de las combinaciones de SNP de alto riesgo en la función auricular mediante análisis bioestadísticos de grandes conjuntos de datos y el análisis de pacientes con FA referidos para la terapia de ablación.

Project Summary

Atrial fibrillation (AF) is the most common cardiac arrhythmia affecting 38 million people worldwide. It doubles mortality, increases the risk of stroke 5-fold and decreases life-quality. Moreover, it develops progressively with age and becomes increasingly difficult to treat with time; and there is therefore a growing need for better more efficient personalized treatment of patients with AF to reduce the immense socio-economic burden of this disease. In this context, more than 150 Single Nucleotide Polymorphisms (SNPs) have been associated with increased risk of AF over the past 15 years.

This offers new opportunities for improving prevention, risk stratification and personalized treatment of AF, but the high frequency and modest impact of these variants complicates their clinical utility. To resolve this issue, one of the main challenges lies in the identification of variants that synergistically increase the risk of AF. Due to the intronic or intergenic location of most SNPs, the mechanisms that link a SNP to risk of AF remain virtually unknown, hampering the identification of SNPs affecting a common signaling pathway expected to increase risk and hence impeding their use in precision medicine.

Therefore, to advance the current state-of-the-art and facilitate an efficient use of SNPs associated with risk of AF in clinical practice and precision medicine, we here aim to test the working hypothesis stating that *Identification of single nucleotide polymorphisms (SNPs) linked to defective cyclic AMP signaling and calcium homeostasis; and organizing them into groups with synergic, additive or antagonistic effects will afford novel criteria for improving risk stratification and personalizing the prevention and treatment of AF.*

To achieve this, our project is divided into two subprojects using genomic and proteomic analyses of human atrial samples, combined with imaging and electrophysiological analyses in native and stem cell-derived human atrial myocytes and mathematical modeling. This will allow us to determine how cross-talk between multiple SNPs affect local subcellular cyclic AMP and calcium signaling and identify combinations that cause synergic derangements of cellular electrophysiological function and validate the underlying molecular mechanisms as therapeutic targets specific to the respective SNP combinations.

Subsequently, to validate findings at the molecular and cellular levels in clinically relevant settings we shall show that the SNP-associated derangements in myocyte function coincides with corresponding alterations in atrial function, which can be detected with current diagnostic tools such as ECG, cardiac MRI, electro-anatomical mapping or Holter recordings. Finally, to assess the clinical utility of these findings, we shall determine the impact of high-risk SNP combinations on atrial function using bio-statistical analyses of big data sets and analyses of patients with AF referred for ablation therapy.

Research consortium

This proposal is the fruit of multiple on-going and established research collaborations between the laboratories of the project leader and scientists participating in the two subprojects. The working hypothesis and design of the proposed project are supported by publications as well as preliminary data from ongoing collaborations between members of the research teams, currently funded by grants from the Spanish Government to the two PIs and grants to participating international collaborators. In these collaborations, we have combined electrophysiological and live-cell imaging techniques with the development and implementation of detailed mathematical myocyte models in order to predict how the properties of one specific or several Ca^{2+} regulatory mechanisms affect Ca^{2+} dynamics in resting and stimulated cardiomyocytes. Thus, the PIs of this proposal have already benefited mutually from the multidisciplinary research collaborations, as reflected in their publication record. Moreover, the know-how emerging from our collaboration over the past five years has allowed us to participate in a growing number of international research collaboration with international collaborators or authors from both subprojects, underscoring the benefits of a coordinated multidisciplinary project.

In the present proposal we intend to strengthen further the benefits of working in a multidisciplinary research team, including international scientists with whom we are already collaborating. This is shown in figure 1, displaying the highly complementary skill sets of the two research teams and outlining how the subprojects will interact and complement each other in the current proposal. This will not only benefit each of the subprojects, but strongly increase the quality of the experimental data and facilitate visualization and translation of the results.

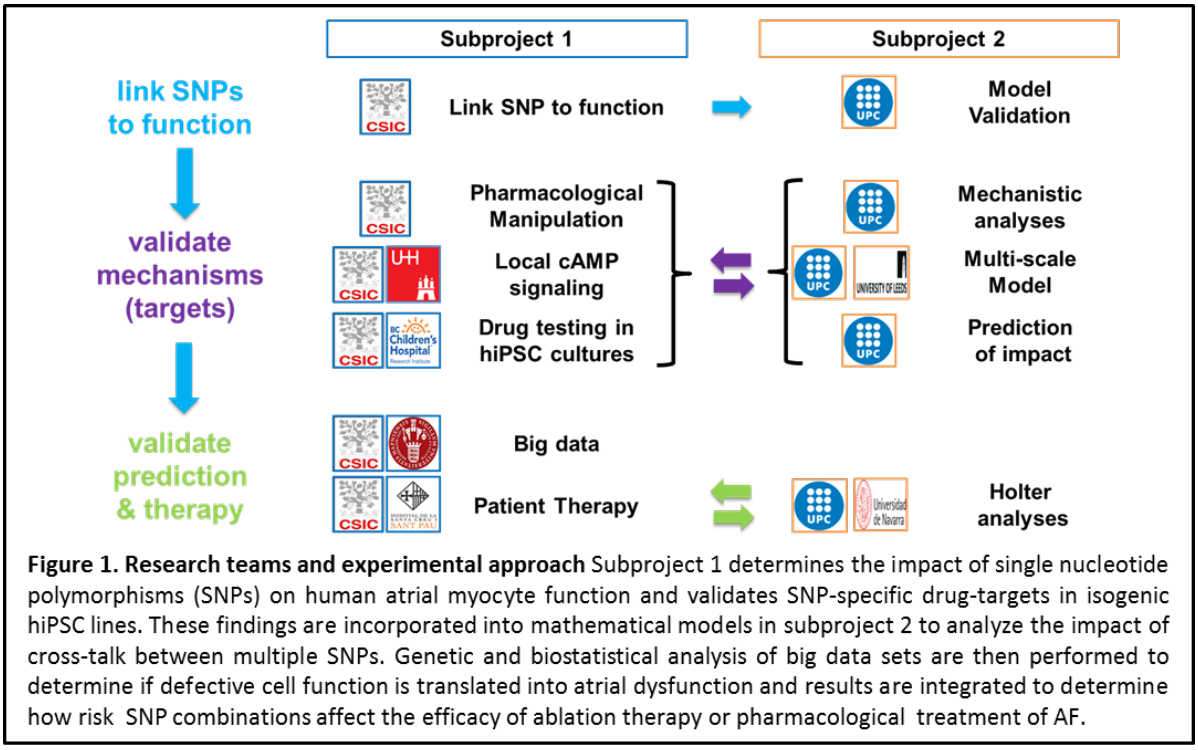

Figure 1. Research teams and experimental approach Subproject 1 determines the impact of single nucleotide polymorphisms (SNPs) on human atrial myocyte function and validates SNP-specific drug-targets in isogenic hiPSC lines. These findings are incorporated into mathematical models in subproject 2 to analyze the impact of cross-talk between multiple SNPs. Genetic and biostatistical analysis of big data sets are then performed to determine if defective cell function is translated into atrial dysfunction and results are integrated to determine how risk SNP combinations affect the efficacy of ablation therapy or pharmacological treatment of AF.

Organization

To achieve the goals of this proposal, it is structured in two multidisciplinary subprojects, using complementary state-of-the-art techniques and experimental protocols designed to determine how genetic modulation of cyclic AMP (cAMP) and calcium homeostasis contribute to the development of atrial fibrillation (AF).

The object of our research is atrial fibrillation (AF), the most common cardiac arrhythmia affecting 38 million people worldwide. Its prevalence increases with age, affecting 5% of the population older than 65 years and reaching 20% in octogenarians. The incidence of cerebrovascular accidents is 5-fold higher in patients with AF, and the prognosis for cardio-embolic stroke is worse than for other types of stroke. Thus, AF not only increases mortality (2-fold) but is also a key contributor to decreasing the life-quality of more than 120.000 people suffering a stroke in Spain yearly (https://ictusfederacion.es/infoictus/codigo-ictus/). In addition, AF is a self-perpetuating disease with a continuous need for better treatments to reduce its immense socio-economic burden.
In this context, the exponential increase in single nucleotide polymorphisms (SNPs) associated with increased risk of AF (from 2 in 2007 to more than 150 in 2023) and the molecular mechanisms contributing to the development of atrial electrophysiological dysfunction and AF, calls for precision medicine with personalized therapy based on the underlying risk factors and molecular mechanisms. As outlined in figure 2, **reduced L-type calcium current** (I_{Ca}) and **spontaneous calcium release from the sarcoplasmic reticulum** (SR) through the ryanodine receptor (RyR2) **are two hallmarks of AF** contributing to the development of atrial electrical reentry and ectopic activity respectively.

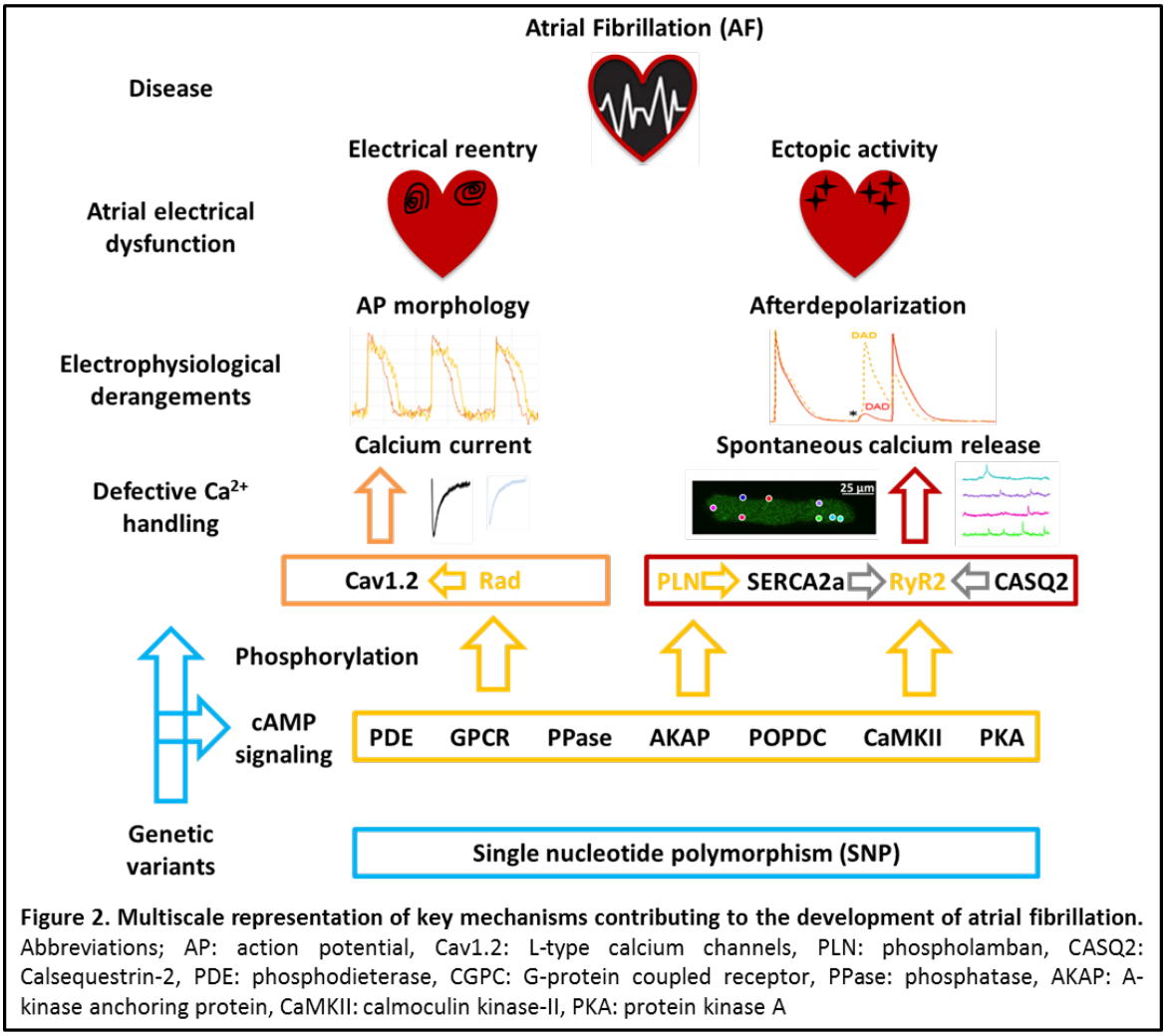

Figure 2. Multiscale representation of key mechanisms contributing to the development of atrial fibrillation. Abbreviations; AP: action potential, Cav1.2: L-type calcium channels, PLN: phospholamban, CASQ2: Calsequestrin-2, PDE: phosphodieterase, CGPC: G-protein coupled receptor, PPase: phosphatase, AKAP: A-kinase anchoring protein, CaMKII: calmoculin kinase-II, PKA: protein kinase A

Because cyclic AMP (cAMP)-signaling plays a key role in the regulation of both I_{Ca} and spontaneous calcium release, this proposal will focus on genetic modulation of cAMP and calcium homeostasis. Specifically, to advance the state-of-the-art within this field, our proposal aims to test the hypothesis that the identification and organization of risk SNPs into groups with synergic, additive or antagonistic effects on cAMP and calcium homeostasis, will afford novel criteria for improving risk stratification and personalizing the prevention and treatment of AF (figure 3).

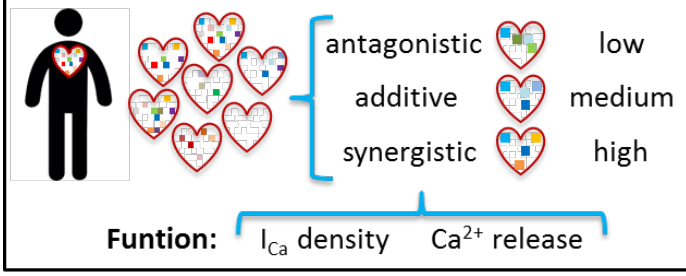

Figure 3. Identifying synergies among risk SNPs affords new therapeutic opportunities. The novelty of this proposal lies in identifying synergies among risk SNPs. Thus, SNPs with an antagonistic, additive or synergistic impact on cell function are expected to cause a small, moderate or strong increase in the risk of AF. Moreover, the underlying mechanisms represent therapeutic targets specific to the corresponding SNP-combination.

Description of the methodology.

HUMAN ATRIAL MYOCTE STUDIES

Perforated patch-clamp techniques. The candidate will learn these techniques in order to determine whether SNPs associated with risk of AF modify the I_{Ca} density or the incidence of spontaneous arrhythmogenic calcium release events. Experiments will be performed using the voltage-clamp configuration to quantify the impact of the SNPs on I_{Ca} density and properties and the incidence of spontaneous calcium release-induced transient inward currents.

L-type calcium current (I_{Ca}): Specific protocols shall be used to determine the I-V relationship, the voltage-dependent inactivation and the recovery of I_{Ca} from inactivation. The impact of the stimulation frequency on the beat-to-beat response (the I_{Ca} amplitude and its stability on a beat-to-beat basis) will also be determined using a stepwise or gradual increase in the stimulation frequency from 0.5 to 3 Hz.

Transient inward current (I_{TI}) amplitude and frequency will be measured at -80 mV for 8 x 30s and the caffeine-releasable SR calcium load will be determined at rest (-80 mV). Reloading of the SR will be determined with a second caffeine pulse applied after 3, 10 and 30 stimulation pulses at 1 Hz.

To determine the impact of the SNPs on action potential morphology and the incidence of afterdepolarizations myocytes will be subjected to current-clamp, using holding currents that clamp the membrane potential at -80 and -60 mV. Action potentials will be measured at different pacing rates, using stepwise increases in the stimulation frequency from 0.5 to 3 Hz.

To assess modulation of ion channel activity by calcium or cAMP signaling, patch-clamp techniques will be combined with intracellular perfusion and calcium imaging techniques. Protocols similar to those employed previously used to address the impact of adenosine signaling on calcium homeostasis and ion currents will be used.

Live cell calcium imaging. To address the impact of SNPs on spontaneous calcium release events and their spatial distribution, the candidate will learn and use validated standard techniques developed and used routinely in our laboratory. Briefly, myocytes will be loaded with calcium sensitive fluorescent dyes such as Cal-520 and confocal calcium images (512x140 pixels) recorded at 90 Hz. To minimize cytotoxic effects and minimize bleaching of fluorescent dyes, image stacks of 10s will

be acquired once every minute for 6 minutes. Myocytes will be transiently exposed to 10 mM caffeine for 10s and the amplitude of the transient will be used as a measure of SR calcium load.
Our patented custom-made wavelet-based detection algorithms (running in MatLab) will be used to detect calcium sparks. Sparks that coincide spatially (within a radius of 1.5 µm) are pooled into a common spark site. Each spark is characterized by its amplitude, full duration at half maximum, decay time-constant and its full width at half maximum. The spark density is calculated as the number of sparks/cell/s or normalized to the cell area within the confocal plane. Additionally, the spark sites are normalized to the cell area and given as sites/mm^2. The average spark frequency per site is given as sparks/site/s. Propagating calcium release calcium waves and transients will also be analyzed with custom-made algorithms to determine their incidence and properties.
Immunofluorescent labeling. The candidate will learn and apply this technique in order to determine define the impact of SNPs on the distribution of ion channels and proteins regulating cAMP and intracellular calcium levels. Validated techniques, currently used in our laboratory shall be used in double immuno-labeling experiments, focusing on the localization of the L-type calcium channel or the RyR2 (total and phosphorylated at Ser-2808 or Ser-2814) with respect to proteins encoded by *CASQ2, POPDC, AKAP6, PDE4* or *CAMK2D*. The spatial distribution of proteins and/or their phosphorylation will be determined using custom-made algorithms developed and validated in our currently funded project as shown in the figure below.

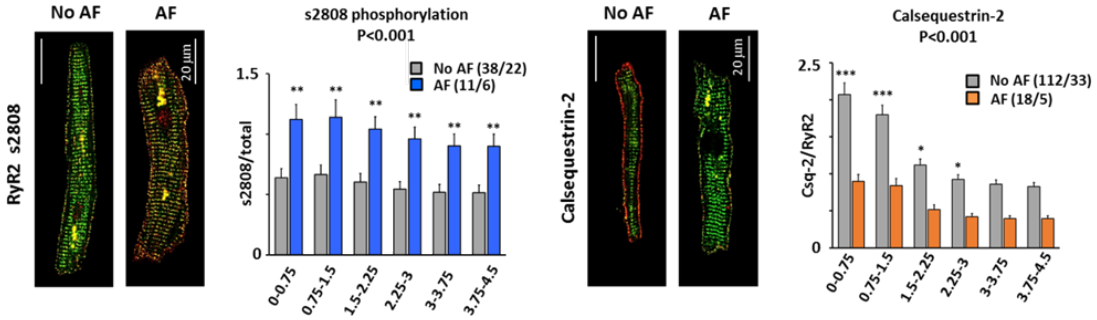

Figure 6. Immunofluorescent analyses of protein phosphorylation and distribution. Left: distribution of Ser-2808 relative to total RyR2. Right: Distribution of calsequestrin-2 relative to total RyR2 (ref. 16, section 4.5).

Activity of PDEs, protein kinases and phosphatases. The activity of these proteins will be determined using commercial kits comparing activities before and after selective inhibition of the target protein.
Pharmacological testing in myocytes. To identify and validate molecular mechanisms linked to SNPs as specific molecular targets, we shall determine the impact of pharmacological manipulation on ionic currents or calcium homoeostasis. Standard protocols for patch-clamp recordings and calcium imaging, described above, will be carried out for each myocyte in control conditions. Subsequently, the protocol will be repeated in the same myocyte after it is exposed to compounds targeting the molecular mechanism. To avoid run-down of currents, cytotoxicity or bleaching of fluorescent dyes, dose-response curves will employ shorter protocols focusing on specific features such as membrane potential and spontaneous activity at rest, SR calcium load or the response (current amplitude, action potential morphology, etc.) with steady-state stimulation, pertinent to the compound to be tested.

Previous results of the team in the theme of the proposal.

Team members have extensive experience within the field of this proposal. As illustrated by selected publications from the team members over the past five years, they have all contributed to advancing the state-of-the-art within the field of the proposal. Moreover, their skill-sets are highly

complementary, addressing all conceptual and methodological aspects of this proposal, affording guarantees of a successful execution of the work plan and its specific tasks. Publications have been grouped according to the different topics, aims and tasks of the proposal.

Genetic variants, Pitx2 and Bioinformatics. Over the past five years we published the first study on the impact of a 4q25 risk SNP on calcium homeostasis and electrical activity in human atrial myocytes, showing that it closely mimics alterations observed in myocytes from patients with atrial fibrillation. We have also shown in a transgenic mouse model that heterozygous atrial specific deletion of Pitx2c, thought to mediate the effects of 4q25 risk SNPs, induce alterations in calcium homeostasis and electrical activity similar to those of the 4q25 risk SNP. Finally, our collaborator Prof. Morten S Olesen has used bioinformatics analyses to identify SNPs associated with risk of AF that also affect left atrial volume or function. We believe that combining these techniques is a powerful approach to investigate how SNP-associated alterations in cAMP-signaling or calcium homeostasis in human atrial myocyte models translate into an impact on atrial rhythm or function in the present proposal.

1. Pitx2c deficiency confers cellular electrophysiological hallmarks of atrial fibrillation to isolated atrial myocytes. Tarifa C, Serra SA, Herraiz-Martínez A, Lozano-Velasco E, Benítez R, Aranega A, Franco D, **Hove-Madsen L**. Biomed Pharmacother. 2023 Jun;162:114577. doi: 10.1016/j.biopha.2023.114577
2. Increased Ca^{2+} Transient Underlies RyR2-Related Left Ventricular Noncompaction. Ni M, Li Y, Wei J, Song Z, Wang H, Yao J, Chen YX, Belke D, Estillore JP, Wang R, Vallmitjana A, Benitez R, **Hove-Madsen L**, Feng W, Chen J, Roston TM, Sanatani S, Lehman A, Chen SRW. Circ Res. 2023; 133:177-192. doi: 10.1161/CIRCRESAHA.123.322504.
3. Identification of atrial-enriched lncRNA Walras linked to cardiomyocyte cytoarchitecture and atrial fibrillation. García-Padilla C, Domínguez JN, Lodde V, Munk R, Abdelmohsen K, Gorospe M, **Jiménez-Sábado V**, Ginel A, **Hove-Madsen L**, Aránega AE, Franco D. FASEB J. 2022; 36:e22051. doi: 10.1096/fj.202100844RR
4. Cardiac ryanodine receptor calcium release deficiency syndrome. Sun B, Yao J, Ni M, Wei J, Zhong X, Guo W, Zhang L, Wang R, Belke D, Chen YX, Lieve KVV, Broendberg AK, Roston TM, Blankoff I, Kammeraad JA, von Alvensleben JC, Lazarte J, Vallmitjana A, Bohne LJ, Rose RA, Benitez R, **Hove-Madsen L**, Napolitano C, Hegele RA, Fill M, Sanatani S, Wilde AAM, Roberts JD, Priori SG, Jensen HK, Chen SRW. Sci Transl Med. 2021; 13(579):eaba7287. doi: 10.1126/scitranslmed.aba7287.
5. The 4q25 variant rs13143308T links risk of atrial fibrillation to defective calcium homoeostasis. Herraiz-Martínez A, Llach A, Tarifa C, Gandía J, **Jiménez-Sabado V**, Lozano-Velasco E, Serra SA, Vallmitjana A, Vázquez Ruiz de Castroviejo E, Benítez R, Aranega A, Muñoz-Guijosa C, Franco D, Cinca J, **Hove-Madsen L**. Cardiovasc Res. 2019 Mar 1;115(3):578-589. doi: 10.1093/cvr/cvy215
6. Genome-wide association study identifies 18 novel loci associated with left atrial volume and function. Ahlberg G, Andreasen L, Ghouse J, Bertelsen L, Bundgaard H, Haunsø S, Svendsen JH, **Olesen MS.** Eur Heart J. 2021; 42:4523-4534. doi: 10.1093/eurheartj/ehab466.

Human atrial myocytes, Atrial fibrillation and Ablation therapy. Over the past five years we have published several studies on the impact of mechanisms and clinical factors on calcium homeostasis, myocyte function and ablation therapy that have allowed us to identify potential confounders of the statistical analyses and settled the basis for the experimental design for the proposed tasks.

7. Beta-blocker treatment of patients with atrial fibrillation attenuates spontaneous calcium release-induced electrical activity. **Jiménez-Sábado V,** Casabella-Ramón S, Llach A, Gich I,

Casellas S, Ciruela F, Chen SRW, Guerra JM, **Ginel A,** Benítez R, Cinca J, Tarifa C, **Hove-Madsen L**. Biomed Pharmacother. 2023 Feb;158:114169. doi: 10.1016/j.biopha.2022.114169.

8. Influence of sex on intracellular calcium homoeostasis in patients with atrial fibrillation. Herraiz-Martínez A, Tarifa C, **Jiménez-Sábado V**, Llach A, Godoy-Marín H, Colino-Lage H, Nolla-Colomer C, Casabella-Ramon S, Izquierdo-Castro P, Benítez I, Benítez R, Roselló-Díez E**, Rodríguez-Font** E, Viñolas X, Ciruela F, Cinca J, **Hove-Madsen L**. Cardiovasc Res. 2022 Mar 16;118(4):1033-1045. doi: 10.1093/cvr/cvab127.
9. Abnormal Calcium Handling in Atrial Fibrillation Is Linked to Changes in Cyclic AMP Dependent Signaling. Reinhardt F, Beneke K, Pavlidou NG, Conradi L, Reichenspurner H, **Hove-Madsen L, Molina CE**. Cells. 2021; 10:3042. doi: 10.3390/cells10113042
10. Reassessment of the electrical connection between the pulmonary veins and the left atrium: A study to determine the different contributions of myocardial fibers along the standard ablation circumference. Campos-García B, Alonso-Martín C, Guerra JM, Moreno-Weidmann Z, Méndez-Zurita F, Montiel-Quintero R, Betancur-Gutiérrez A, Viñolas-Prat X, **Rodríguez-Font E**. Front Cardiovasc Med. 2023; 10:1162197. doi: 10.3389/fcvm.2023.1162197.

Cyclic AMP signaling and subcellular compartmentalization. Similarly we, and our collaborator Prof. Cristina E Molina, have published data underscoring the necessity of undertaking the proposed tasks aiming to determine how cAMP regulatory proteins cause compartmentalization of cAMP-signaling; how this translates into local subcellular calcium regulatory domains, and how risk SNPs affect this compartmentalization and the propensity of human atrial myocytes to present arrhythmogenic responses.

11. Expression and Impact of Adenosine A_3 Receptors on Calcium Homeostasis in Human Right Atrium. Tarifa C, **Jiménez-Sábado V**, Franco R, Montiel J, Guerra J, Ciruela F, **Hove-Madsen L**. Int J Mol Sci. 2023 Feb 23;24(5):4404. doi: 10.3390/ijms24054404
12. RyR2 Serine-2030 PKA Site Governs Ca^{2+} Release Termination and Ca^{2+} Alternans. Wei J, Guo W, Wang R, Paul Estillore J, Belke D, Chen YX, Vallmitjana A, Benitez R, **Hove-Madsen L**, Chen SRW. Circ Res. 2023; 132:e59-e77. doi: 10.1161/CIRCRESAHA.122.321177.
13. Impact of R-Carvedilol on β2-Adrenergic Receptor-Mediated Spontaneous Calcium Release in Human Atrial Myocytes. Casabella-Ramón S, **Jiménez-Sábado V**, Tarifa C, Casellas S, Lu TT, Izquierdo-Castro P, Gich I, Jiménez M, **Ginel A**, Guerra JM, Chen SRW, Benítez R, **Hove-Madsen L**. Biomedicines. 2022 Jul 21;10(7):1759. doi: 10.3390/biomedicines10071759
14. β2-adrenergic stimulation potentiates spontaneous calcium release by increasing signal mass and co-activation of ryanodine receptor clusters. Nolla-Colomer C, Casabella-Ramon S, **Jimenez-Sabado V**, Vallmitjana A, Tarifa C, Herraiz-Martínez A, Llach A, Tauron M, Montiel J, Cinca J, Chen SRW, **Benitez R, Hove-Madsen L**. Acta Physiol (Oxf). 2022 Apr;234(4):e13736. doi: 10.1111/apha.13736
15. Adenosine A_{2A} Receptors Are Upregulated in Peripheral Blood Mononuclear Cells from Atrial Fibrillation Patients. Godoy-Marín H, Duroux R, Jacobson KA, Soler C, Colino-Lage H, **Jiménez-Sábado V**, Montiel J, **Hove-Madsen L**, Ciruela F. Int J Mol Sci. 2021 Mar 27;22(7):3467. doi: 10.3390/ijms22073467
16. Spatial distribution of calcium sparks determines their ability to induce afterdepolarizations in human atrial myocytes C Tarifa, A Vallmitjana, **V Jiménez-Sábado,** M Marchena, A Llach, A Herraiz-Martínez, H Godoy-Marín, C Nolla-Colomer, **A Ginel**, X Viñolas, J Montiel, F Ciruela, **B Echebarria**, R Benítez, J Cinca, **L Hove-Madsen**. Basic to Translational Science 8(1), 1-15, 2023
17. Phosphodiesterase 8 governs cAMP/PKA-dependent reduction of L-type calcium current in human atrial fibrillation: a novel arrhythmogenic mechanism. Grammatika Pavlidou N, Dobrev

S, Beneke K, Reinhardt F, Pecha S, Jacquet E, Abu-Taha IH, Schmidt C, Voigt N, Kamler M, Schnabel RB, Baczkó I, Garnier A, Reichenspurner H, Nikolaev VO, Dobrev D, **Molina CE.** Eur Heart J. 2023; 44:2483-2494. doi: 10.1093/eurheartj/ehad086.

18. Live Cell Imaging of Cyclic Nucleotides in Human Cardiomyocytes. Beneke K, **Molina CE.** Methods Mol Biol. 2022;2483:195-204. doi: 10.1007/978-1-0716-2245-2_12

Stem cell derived cardiomyocytes. Our collaborator Prof. Glen Tibbits has over the past five years set up a state-of-the-art facility for research with human stem cell derived cardiomyocytes, and publications in collaboration with him document the utility of this preparation for drug discovery and disease modeling, including research addressing the impact of SNPs in isogenic stem cell lines. We shall take advantage of this methodology to test drugs targeting specific risk-SNP combinations.

19. HiPSC-derived cardiomyocytes as a model to study the role of small-conductance Ca^{2+}-activated K^+ (SK) ion channel variants associated with atrial fibrillation. **Babini H, Jiménez-Sábado V**, Stogova E, Arslanova A, Butt M, Dababneh S, Asghari P, Moore EW, Claydon T, Chiamvimonvat N, **Hove-Madsen L, Tibbits GF**. Frontiers in Cell and Developmental Biology. 2024; 12. doi: 10.3389/fcell.2024.1298007
20. Atrial-specific hiPSC-derived cardiomyocytes in drug discovery and disease modeling. Gharanei M, Shafaattalab S, Sangha S, Gunawan M, Laksman Z, **Hove-Madsen L, Tibbits GF**. Methods. 2022; 203:364-377. doi: 10.1016/j.ymeth.2021.06.009
21. In vitro analyses of suspected arrhythmogenic thin filament variants as a cause of sudden cardiac death in infants. Shafaattalab S, Li AY, Lin E, Stevens CM, Dewar LJ, Lynn FC, Sanatani S, Laksman Z, Morin RD, van Petegem F, **Hove-Madsen L**, Tieleman DP, Davis JP, **Tibbits GF**. Proc Natl Acad Sci U S A. 2019; 116:6969-6974. doi: 10.1073/pnas.1819023116

Training program planned in the context of the requested Project.

The scientific team has been training students and post-doctoral fellows within the field of molecular and cellular electrophysiology, employing the latest technological advances combining electrophysiological and live cell imaging techniques. Our close collaboration with the Cardiology and Cardiac Surgery Departments at Hospital de Sant Pau also allows students to understand the translational aspects of cellular and molecular research. Participation in national research networks and established international European and transatlantic research collaborations has allowed short-term exchange during pre-doctoral training, and longer-term exchange for post-doctoral training. Finally, it has been laboratory practice that pre- and postdoctoral fellows present progress on their projects at the most relevant national and international meetings within cardiovascular research in order to get experience in communicating their data to the relevant scientific communities. The coordinator and PI has been Adjunct Professor at Simon Fraser University Vancouver, Canada since 2004, undertaking research collaborations and contributing to the training of pre- and postdoctoral fellows at this institution as reflected in the publications done in collaboration with Professor Tibbits over the past decade. This collaboration will continue in the present proposal, where the project coordinator is on the supervisory committee of a PhD student in professor Tibbit's team that will contribute to the pharmacological testing in hiPSC-derived stem cells in task 3.3. Similarly, PhD students from the international collaborators' teams at Copenhagen and Hamburg Universities will contribute to tasks 2.1 and 4.1.

Training program for the PhD student:

The PhD student will be trained by group members and learn state-of-the-art techniques by participating in the execution of tasks and work packages of the project. The student will first apply existing experimental protocol supervised by group members, and then learn to edit, design and validate new protocols. To be able to work independently, the student will first learn to isolate human atrial myoctes and estimate sample sizes, followed by the experimental techniques applied in the myocytes. This includes:

- Immuno-fluorescent labeling and confocal microscopy to visualize the distribution and phosphorylation of proteins involved in cAMP signaling, calcium homeostasis and electrical activity.
- Confocal live-cell imaging to visualize local changes in cytosolic calcium or membrane potential.
- Patch-clamp techniques in the voltage- and current-clamp configurations to record whole membrane currents and membrane potentials respectively.
- Combination of patch-clamp and live cell imaging techniques.

The student will also learn to use commercially and custom-made programs for data analysis. For the interpretation of the results, the student will learn statistical analyses and evaluation of the potential impact of clinical confounders. For this, the student will interact with medical doctors involved in the project and collaborators in the other subproject of this coordinated research project. The student will also interact with international collaborators in order to communicate and understand the significance of the results obtained within the context of the coordinated project. This, and the preparation of results for their presentation at national and international meetings or in publications will help the student learn communicating findings in a multi-disciplinary context.

Short CV; Project coordinator and PhD supervisor

PERSONAL INFORMATION

First name	Leif		
Family name	Hove-Madsen		
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CV SUMMARY

General indicators of quality of scientific production:
5 sexenios de investigación; most recent: 2020.
9 PhD thesis directed since 2010
Total citations: 4986; h-index: 44; Publications Q1: 61. (Google Scholar)
Last 5 years (2019-): 1678 citations; h-index: 22; Publications D1: 11; Q1: 23.
Trajectory and lines of research:
International pre-and postdoctoral training. 1989-93. PhD Studies in the laboratories of professors Hans Gesser, Denmark and Donald M. Bers, USA. Each of the four publications in the PhD thesis have been cited more than 100 times. **1993-95**. Postdoc with Prof. Rodolphe Fischmeister, Faculté de Pharmacie, Université de Paris-Sud, learning patch-clamp and its combination with intra- and extracellular perfusion techniques.
First line of own research (1995-2001). Postdoc. with Prof. Lluis Tort, Univ. Autónoma de Barcelona. Calcium homeostasis in cardiomyocytes of vertebrate ectotherms. Studies were the first to use patch-clamp to question the dogma that the sarcoplasmic reticulum is rudimentary in teleosts.

Pioneering studies on defective calcium homeostasis in human atrial myocytes (2001-08). Ramon y Cajal Scholarship followed by the I3 program with Prof. Juan Cinca, Hospital de Sant Pau. In 2002, we founded the Cellular Physiology Laboratory, dedicated to the study of arrhythmogenic mechanisms in human cardiomyocytes. In 2004 we published the article "Atrial fibrillation is associated with increased spontaneous calcium release from the sarcoplasmic reticulum in human atrial myocytes" (Circulation. 2004, 110:1358-63). This was the first publication to associate atrial fibrillation with excessive spontaneous calcium release in human atrial myocytes. It currently accumulates 395 citations. We next directed our studies towards mechanisms underlying the increase in spontaneous calcium release in patients with atrial fibrillation. In 2006 we were first to show that A_{2A} adenosine receptors are expressed in the human atrium and stimulate spontaneous calcium release.

Development of new lines of research on human atrial myocytes (2008-). Científico Titular at CSIC. Head of the Heart Rhythm and Contraction Group, Biomedical Research Institute IIBB-CSIC

Neuro-hormonal modulation of calcium homeostasis (2008-present) We continue to study the modulation of calcium release by membrane receptors and secondary messengers, showing that both adenosine A_{2A} receptors (article #10) and type 4 phosphodiesterase (article #9) can intervene in the dys-regulation of intracellular calcium in atrial fibrillation.

Local modulation of arrhythmogenic calcium release (2003-). Being pioneers in 2D calcium imaging in cardiomyocytes we have, in collaboration with experts at UPC, continues to develop and patent new analytical tools for processing, analysis and event detection in large confocal image stacks (patents # 2 and 3), which has allowed us to address how the spatial distribution of spontaneous calcium release determines their ability to trigger afterdepolarizations (articles #4-5)

Impact of clinical and genetic risk factors on calcium homeostasis and electrical activity (2010-). This line of research has addressed how the impact of the transcription factor Pitx2 and common genetic variants (articles #3 and #7) as well as sex and aging on calcium homeostasis in human atrial myocytes (articles #6 and 8); fields that will help improve the prevention, stratification and personalized treatment of atrial fibrillation.

Research and international collaborations:

We have since 1997 established and maintain international collaborations on development and stem cell-derived cardiomyoctes with Prof. Glen Tibbits (Since 1997, Article #1), on ventricular arrhythmias with Prof. Wayne Chen (since 2011, article #2). Research performed in foreign laboratories include

- 1989-92: Predoctoral studies with Profesor Donald M. Bers, Division of Biomedical sciences, University of California Riverside, USA. (36 months)
- 1992-93: Posdoctoral studies with Profesor Donald M. Bers, Stritch School of Medicine, Loyola University, Maywood, Chicago, USA. (6 months)
- 1993-95: Posdoctoral studies with Profesor Rodolphe Fischmeister, Faculté de Pharmacie, Université de Paris-Sud, Chantenay-Malabry, France. (28 months)
- Visiting Professor (july-agust) since 2004, BC Childrens Hospital & Simon Fraser University, Vancouver, Canada.

Adjunct Professor at Simon Fraser University since 2004

10 Publications relevant to the project

* corresponding author

1. Babini H, Jiménez-Sábado V, Stogova E, .., **Hove-Madsen L**, Tibbits GF*. HiPSC-derived cardiomyocytes as a model to study the role of small-conductance Ca2+-activated K+ (SK) ion

channel variants associated with atrial fibrillation. Frontiers in Cell and Developmental Biology. 2024; 12. doi: 10.3389/fcell.2024.1298007.
2. Wei J, Guo W, Wang R, .., **Hove-Madsen L**, Chen SRW*. RyR2 Serine-2030 PKA Site Governs Ca2+ Release Termination and Ca^{2+} Alternans. Circ Res. 2023; 132:e59-e77.
3. Tarifa C, Serra SA, Herraiz-Martínez A, Lozano-Velasco E, Benítez R, Aranega A, Franco D, **Hove-Madsen L***. Pitx2c deficiency confers cellular electrophysiological hallmarks of atrial fibrillation to isolated atrial myocytes. Biomed Pharmacother. 2023; 162:114577. doi: 10.1016/j.biopha.2023.114577
4. Tarifa C, Vallmitjana A, Jiménez-Sábado V, .., **Hove-Madsen L***. The Spatial Distribution of Calcium Sparks Determines Their Ability to Induce Afterdepolarizations in Human Atrial Myocytes. JACC Basic to Translational Science 8(1), 1-15, 2023.
   Editorial comment: doi: 10.1016/j.jacbts.2022.10.002
5. Nolla-Colomer C, Casabella-Ramon S, Jimenez-Sabado V, .., Benítez R, **Hove-Madsen L***. β2-adrenergic stimulation potentiates spontaneous calcium release by increasing signal mass and co-activation of ryanodine receptor clusters. Acta Physiol (Oxf). 2021 Oct 28:e13736. doi: 10.1111/apha.13736.
   Editorial comment: doi: 10.1111/apha.13798
6. Herraiz-Martínez A, Tarifa C, Jiménez-Sábado V, .., **Hove-Madsen L*.** (2022) Influence of sex on intracellular calcium homeostasis in patients with atrial fibrillation. Cardiovasc Res. 2022;118(4):1033-1045. doi: 10.1093/cvr/cvab127.
7. Herraiz-Martínez A, Llach A, Tarifa C, .., **Hove-Madsen L*.** (2019) The 4q25 variant rs13143308T links risk of atrial fibrillation to defective calcium homeostasis. Cardiovasc Res. 115(3): 578-589 doi: 10.1093/cvr/cvy215.
   Editorial comment: doi:10.1093/cvr/cvy283
8. Herraiz-Martínez A, Álvarez J, Llach A, .., **Hove-Madsen L***. (2015) Ageing is associated with deterioration of calcium homeostais in isolated human right atrial myocytes. Cardiovasc Res;106(1):76-86.
9. Molina CE, Leroy J, Richter W, .., **Hove-Madsen L**, Conti M, Vandecasteele G, Fischmeister R* (2012). Cyclic adenosine monophosphate phosphodiesterase type 4 protects against atrial arrhythmias. J Am Coll Cardiol; 59(24): 2182-90.
10. Llach A, Molina CE, Prat-Vidal C, .., **Hove-Madsen L***. (2011). Abnormal calcium handling in atrial fibrillation is linked to up-regulation of adenosine A2A receptors. Eur Heart J; 32(6): 721-9.

Relevant research projects

1. **Title:** Genetic Modulations of Cyclic AMP signaling and calcium homeostasis as biomarkers for personalized treatment in atrial fibrillation
   Financing entity: National R + D + I Plan. **PID2023-152610OB-C21**.
   Coordinator and PI: Leif Hove-Madsen. From: Dec. 2024 – Dec. 2027
2. **Title:** Impact of Genetic and Clinical Risk on Molecular Signaling and Electrophysiological Dysfunction in Atrial Fibrillation
   Financing entity: National R + D + I Plan. **PID2020-116927RB-C21**.
   Coordinator and PI: Leif Hove-Madsen. From: 01/09/2021 – 31/08/2024
3. **Title:** Using hiPSC-derived atrial tissue to understand better the role of SK ion channel variants in atrial fibrillation
   Financing entity: Canadian Institutes of Health Research **CIHR- 506438** $1,162,800
   IP: Glen F Tibbits; Investigador Colaborador: Leif Hove-Madsen, 2024-2029 (5 years)
4. **Title:** Catecholaminergic polymorphic ventricular tachycardia (CPVT): arrhythmogenic mechanisms and personalized intervention

Financing entity: Canadian Institutes of Health Research **CIHR- 451191** 924,120 $Cnd
IP: Glen F Tibbits; Investigador Colaborador: Leif Hove-Madsen, 2021-2026 (5 years)

5. **Title:** Filling the translational gap: Single nucleotide polymorphisms as biomarkers and targets for personalized treatment of electrophysiological defects in atrial fibrillation
   Financing entity: National R + D + I Plan. **SAF2017-88019_C3-1-R**. 121,000€
   Coordinator and PI: Leif Hove-Madsen. From: 01/01/2018 - 31/08/2021
6. **Titel: CIBER CARDIOVASCULAR, CB16/11/00276**
   Investigador colaborador: Leif Hove-Madsen. Since: 01/01/2017
7. **Title:** Adenosine receptors as new targets for the treatment of atrial fibrillation: Biomarker, Risk-Stratification and Therapy
   Financing entity: Fuandació Marató de TV3. **MARATO2015_20_30** 186.442 €
   Coordinator and PI: Leif Hove-Madsen. From: 03/2016 - 31/12/2019
8. **Title:** Identification of molecular and Cellular Electrophysiological Risk factors That confer patients a high risk for Atrial fibrillatioN
   Financing entity: Plan Nacional I+D+I. **SAF2014-58286-C2-1-R** 108.900 €
   Coordinator and PI: Leif Hove-Madsen. From: 01/01/2015 to: 31/8/2018

Relevant Patents

1. Inventors: R.Franco, F.Ciruela, C.Lluis, C.Muller, J.Cinca, **L.Hove-Madsen**.
   Title: Compuestos para el tratamiento de la fibrilación auricular.
   Patent number (grant date): ES-2273599-B1 (14-3-2008); EP-1949903-B1 (18-4-2012); US-8012973-B2 (6-9-2011); CN-101325956-B (02-29-2012); RU-2445099-C2(20-3-2012)
   Entity holder: Cima S.L. Biomedicine Project
2. Inventors (signature p.o): R.Benítez, E.Álvarez Lacalle, **L.Hove-Madsen.**
   Title: Método de detección de eventos locales de liberación de calcio intracelular.
   Patent number: ES-2345089_B1 Concession Date: 09-20-2011
   Entity holder: Universitat Politècnica de Catalunya
   Inventors (signature p.o.): **L.Hove-Madsen**, Alexander Vallmitjana, R. Benitez.
3. Title: Método implementado por ordenador para caracterización dinámica de células en cultivos celulares y programas informáticos para llevar a cabo el método.
   Patent Number: P201330974 Date of concession: 07-01-2016
   Entity holder: Universitat Politècnica de Catalunya