

2

RESEARCH AT THE CENTER

The CNIC is organized into two departments, one focused on Basis Research and the other on Clinical Research. Research in these fields is fully interconnected through three multidisciplinary Research Areas.

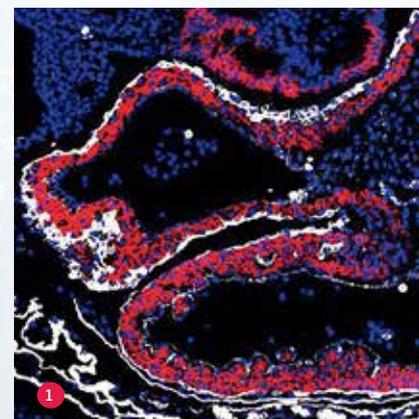
VASCULAR PATHOPHYSIOLOGY

Coordinator: **Francisco Sánchez Madrid**

The focus of the Vascular Pathophysiology Area is the biology of the vascular system in health and disease, investigated through multidisciplinary approaches that include molecular and cellular biology, animal models of disease, and translational and clinical studies. The Area hosts three technical units- Genomics, Proteomics, and Bioinformatics-, which provide state-of-the art technology to CNIC scientists while actively participating in the Area's research projects. Our research takes advantage of high-throughput genomics, proteomics and metabolomics coupled to bioinformatic analysis, as well as state-of-the art imaging technologies, all applied to both animal models and population studies with the aim of opening up new diagnostic and prognostic avenues. We conduct translational research into atherosclerosis, the main underlying cause of heart attack and stroke. We are interested in dissecting the regulatory pathways involved in vessel wall and cardiac remodeling and the contribution of aging to these events, including both physiological and premature aging. We also investigate the role of smooth muscle and endothelial cells and the interplay between the hematopoietic and cardiovascular systems during atherosclerosis development, as well as the signaling pathways that regulate cardiovascular development and disease and age-associated alterations of muscle cells that could account for the decline in tissue regeneration with age. Another important area of interest is the immune and inflammatory component of cardiovascular disease, including the role in atherosclerosis of somatic mutation-driven clonal hematopoiesis and the antibody immune response, the mechanisms of intercellular communication between immune cells, and the role of T cell and immunomodulatory molecules in the development of myocarditis.

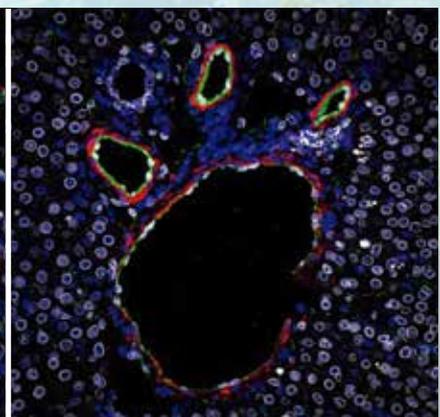
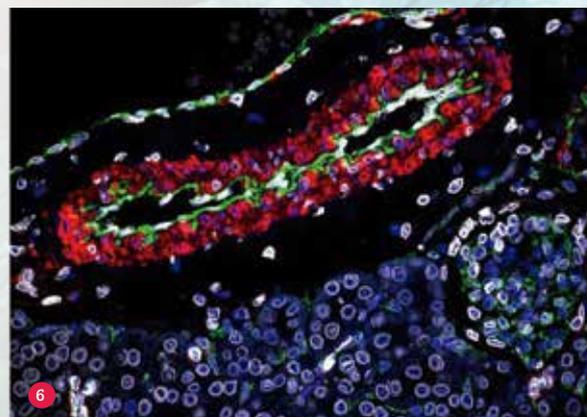
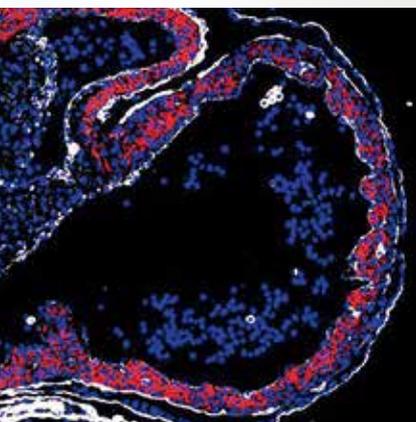
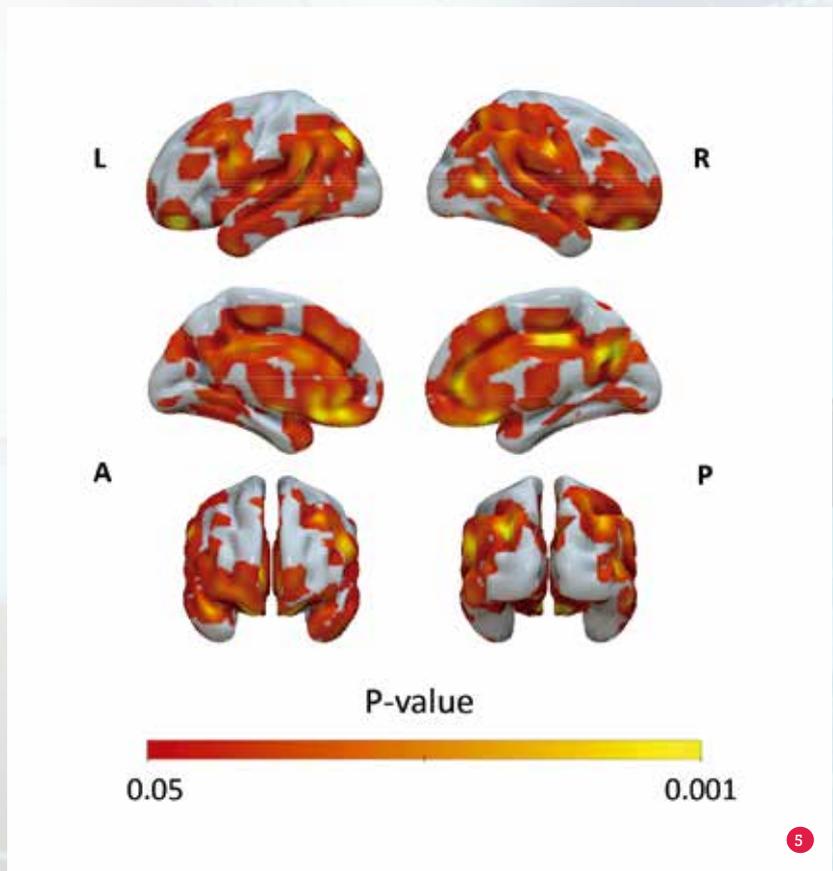
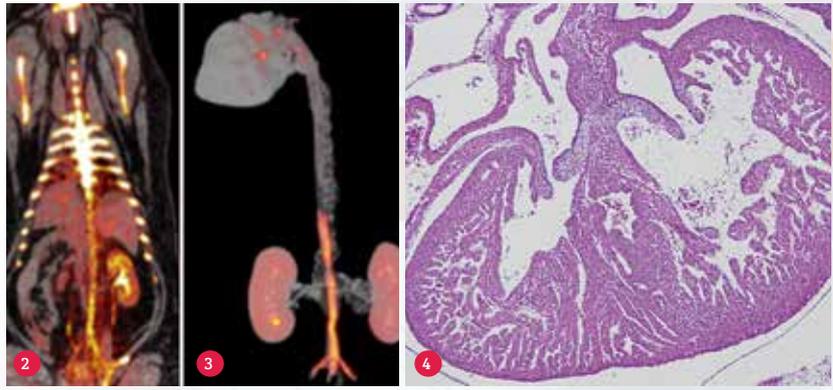
TECHNICAL UNITS

- [Genomics](#)
- [Proteomics](#)
- [Bioinformatics](#)



RESEARCH GROUPS

- [Vicente Andrés](#)
- [Jacob F. Bentzon](#)
- [José Luis de la Pompa](#)
- [José J. Fuster](#)
- [Valentín Fuster](#)
- [Pilar Martín](#)
- [María A. Moro](#)
- [Pura Muñoz](#)
- [Almudena Ramiro](#)
- [Juan Miguel Redondo](#)
- [Francisco Sánchez Madrid](#)
- [Jesús Vázquez](#)



CELL AND DEVELOPMENTAL BIOLOGY

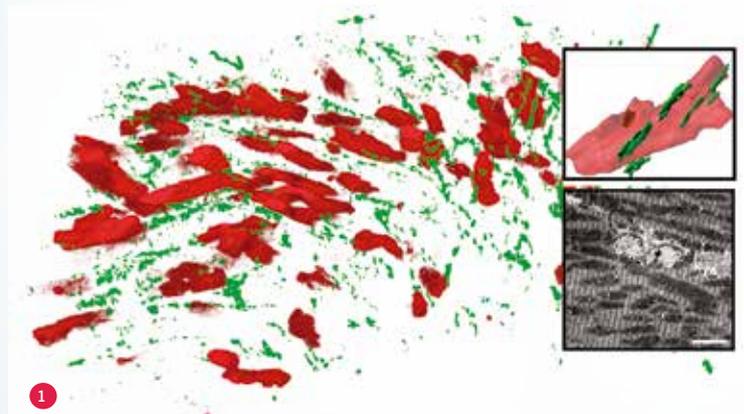
Coordinator: **Miguel Ángel del Pozo**

The Cell and Developmental Biology (CDB) Area comprises eight research groups and three technical units devoted to basic studies and their translational projection in vascular development, homeostasis, and disease. Some groups seek to understand how the spatiotemporal regulation of genome architecture and expression determines cell decisions in the early embryo and heart development, thus contributing to the advancement of cardiovascular (CV) regenerative medicine. Other groups investigate cell and tissue mechanisms that determine CV function, such as angiogenesis, inflammation, and repair, and explore the principles controlling the mechanical function and adaptability of the CV system. This research line deploys multidisciplinary programs integrating cell and systems biology, biophysics, and single-molecule techniques. Efforts are specifically devoted to building bridges between basic research and cardiovascular medicine, with a focus on cardiomyopathies, atherosclerosis, and cerebrovascular disease.

The CDB's three core technical units provide support on state-of-the-art visualization techniques and develop solutions covering different scales and biological processes. The Microscopy Unit offers advanced confocal, multiphoton, and super-resolution imaging technologies, together with approaches for quantitative biology. The Cellomics Unit provides cytometry and separation services (including state-of-the-art spectral cytometry), as well as a high-content functional genomics screening platform. Both units provide support for tailored image analysis and data processing. The Advanced Imaging Unit offers a portfolio of cutting-edge preclinical imaging services for small animals (ultrasound, magnetic resonance, PET/CT, optical imaging, and radiochemistry). The unit provides support to the Center's research groups in the assessment of various animal models and performs its own technical research on advanced molecular imaging techniques. The Advanced Imaging Unit was awarded ISO 9001:2015 quality accreditation in June 2018.

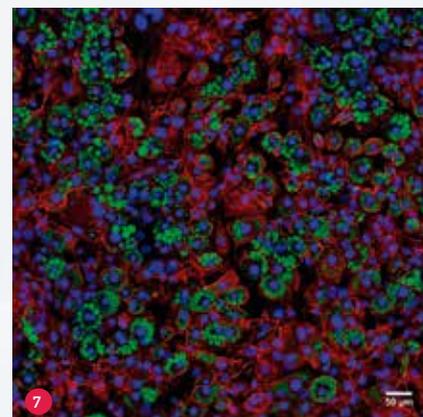
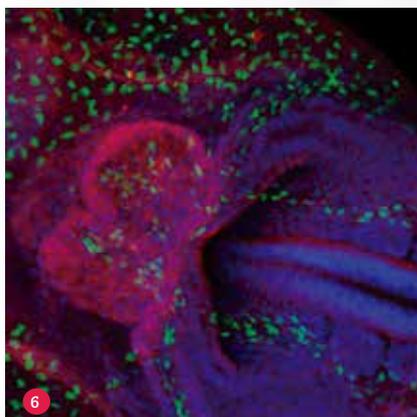
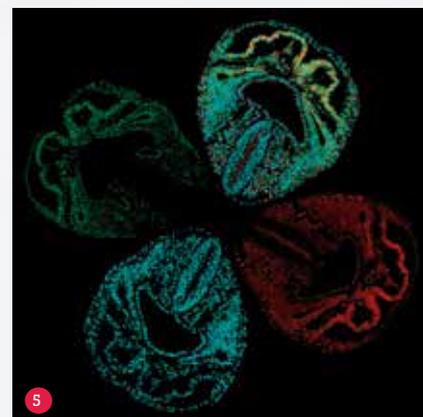
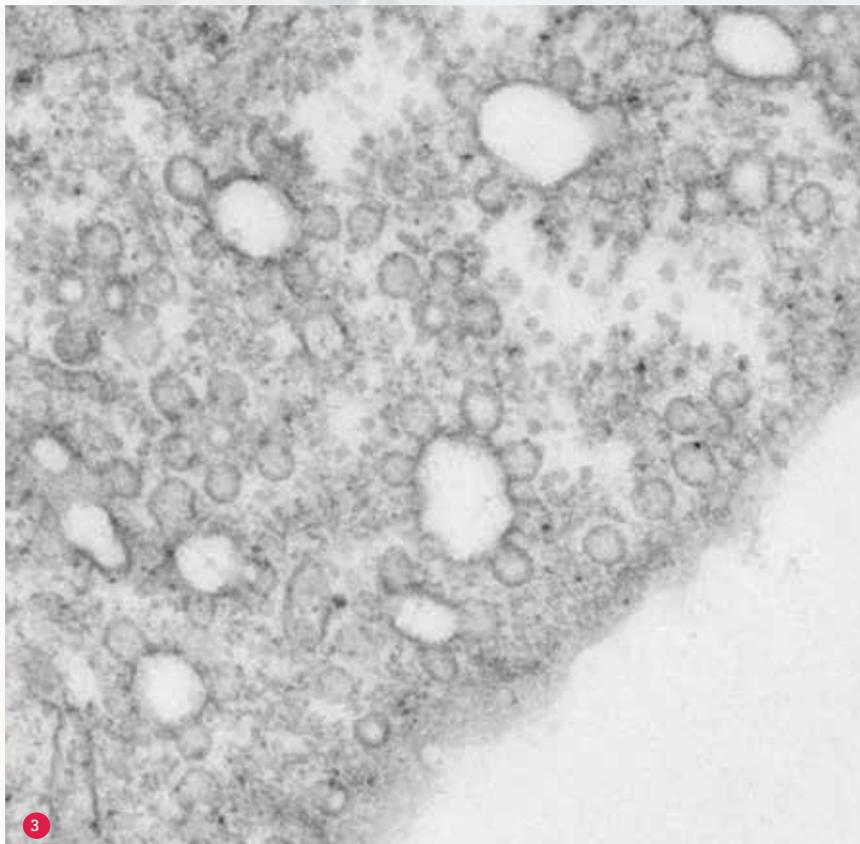
TECHNICAL UNITS

- [Microscopy](#)
- [Advanced Imaging](#)
- [Cellomics](#)



RESEARCH GROUPS

- [Jorge Alegre-Cebollada](#)
- [Rui Benedito](#)
- [Héctor Bueno](#)
- [Miguel Ángel del Pozo](#)
- [Andrés Hidalgo](#)
- [Nadia Mercader](#)
- [Carlos Pérez Medina](#)
- [Miguel Torres](#)



MYOCARDIAL PATHOPHYSIOLOGY

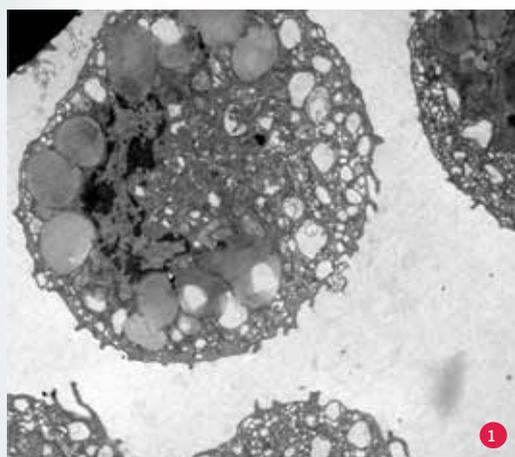
Coordinator: **David Sancho**

The Myocardial Pathophysiology Area (MPA) brings together scientists from multiple disciplines. MPA groups work on several topics: the oxidative phosphorylation system, the role of nuclear receptors in lipid metabolism and inflammatory responses, metabolic syndrome and stress kinases, immunobiology, inherited cardiomyopathies, cardiac arrhythmias, cardiomyocyte electrophysiology, molecular regulation of heart failure, and translational cardiovascular imaging and therapy. Our research in these areas produced several significant advances in 2019:

- (1) Development of an innovative and translational approach to identify specific atrial areas as targets for atrial fibrillation ablation.
- (2) Establishment of an educational intervention for instilling healthy behaviors in children living in diverse and socioeconomically disadvantaged communities.
- (3) New findings on how nuclear receptors control tissue resident macrophage identity by regulating chromatin accessibility and the transcription of canonical macrophage genes, and how macrophages communicate with endothelial cells to promote cardiac repair and remodeling.
- (4) The use of adeno-associated virus-mediated gene transfer in mice, proteomics, and CRISPR/Cas9 gene editing in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to investigate the emerging role of Na_v1.5-Kir2.1 macromolecular ion channel complexes in the mechanisms of sudden cardiac death in inheritable diseases.
- (5) Studies of the role of RNA-binding proteins in the adult heart and the molecular mechanisms underlying the development of arrhythmogenic cardiomyopathy (AC) type 5.
- (6) Work on the role of the OxPhos system in health and disease, highlighting the role of mitochondrial ROS in OxPhos system adaptation to cellular metabolic requirements.
- (7) New advances in deciphering the mechanisms involved in Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) and Long QT Syndrome type 8.
- (8) Analysis of how host-microbiota interactions regulate the immune gut barrier, showing that microbiota dysbiosis and translocation might regulate low-grade systemic inflammation underlying metabolic syndrome and cardiovascular disease.
- (9) Studies of p38gamma showed that this kinase is involved in the progression from steatosis to liver cancer and in the control of the proliferation of hepatocytes after stress. In addition, stress in adipose tissue is an important predisposing factor for liver cancer development. Interestingly, testosterone increases adipose tissue stress, reflecting the higher incidence of liver cancer in men than women.

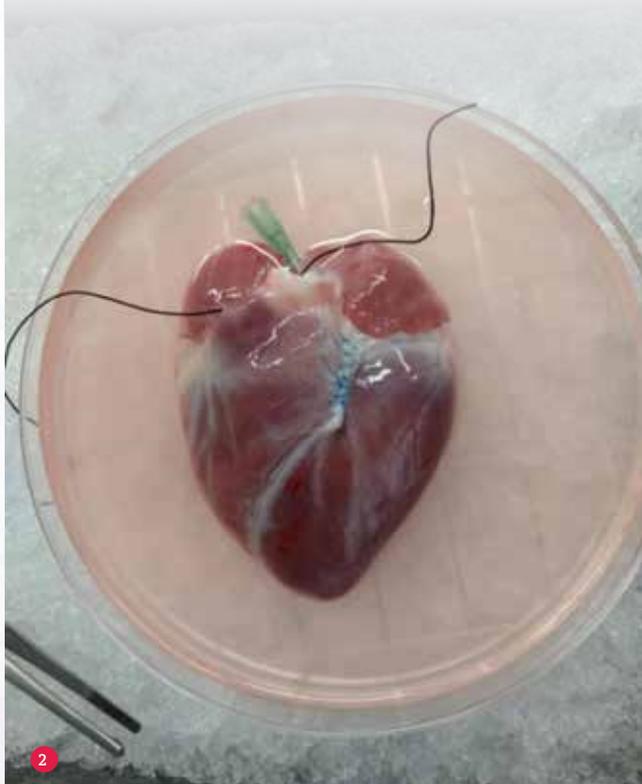
TECHNICAL UNITS

- [Transgenesis](#)
- [Pluripotent Cell Technology](#)
- [Comparative Medicine](#)
- [Viral Vectors](#)
- [Clinical Trial Coordination](#)

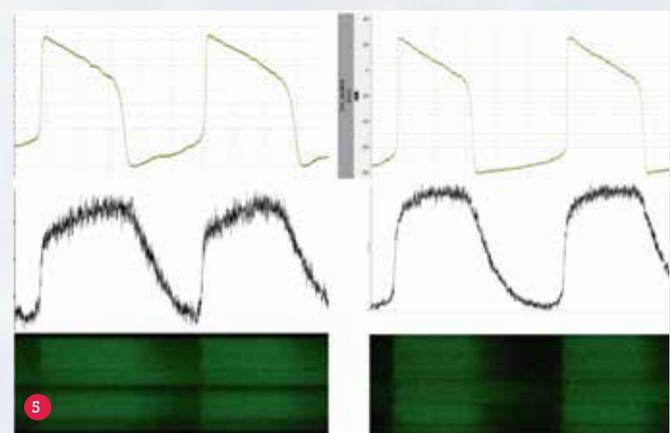
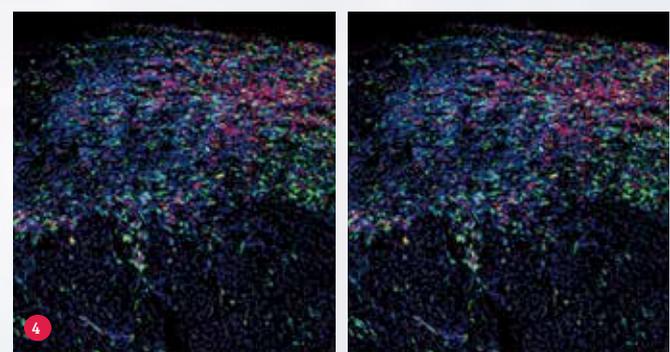
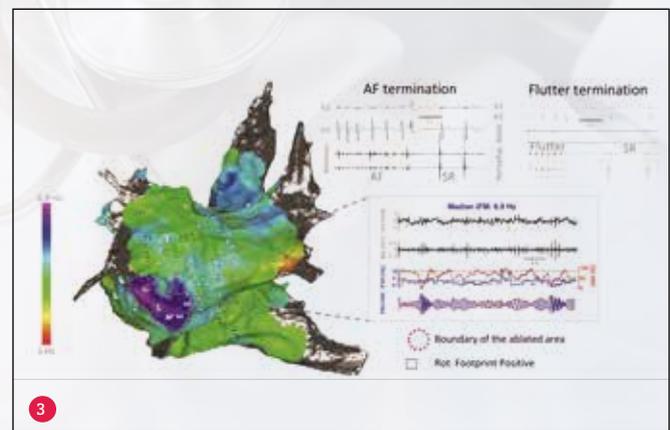


RESEARCH GROUPS

- [Juan A. Bernal](#)
- [José Antonio Enriquez](#)
- [Rodrigo Fernández](#)
- [David Filgueiras](#)
- [Borja Ibáñez](#)
- [José Jalife](#)
- [Enrique Lara-Pezzi](#)
- [Silvia Priori](#)
- [Mercedes Ricote](#)
- [Guadalupe Sabio](#)
- [David Sancho](#)



The MPA's core technical units give support to all CNIC scientists: 1) The **Transgenesis Unit** provides services in mouse strain rederivation, production of genetically modified mice, and cryopreservation of mouse strains; 2) the **Pluripotent Cell Technology Unit** has generated an isogenic hiPSC line and optimized differentiation protocols to model CVD using hiPSC-derived cardiomyocytes; 3) the **Clinical Trials Coordination Unit** continues with its mission to boost Spanish leadership in clinical trials in the cardiovascular area; 4) the **Viral Vectors Unit** provides researchers with access to state-of-the-art viral vector technology for use in preclinical studies and basic research applications; 5) the **Comparative Medicine unit** supports in vivo work in the animal facility.



2.4

CLINICAL STUDIES

The PESA-CNIC-Santander study is a long-term endeavor carried out by the CNIC in collaboration with Santander Bank. This study aims to identify the presence of atherosclerosis long before symptoms appear and to define the cues leading to its development and progression. The study, led by CNIC General Director Valentín Fuster, launched in 2010 and enrolled 4184 asymptomatic individuals between the ages of 40 and 55. Participants undergo serial (every 3 years) imaging and analytical tests, including 3D vascular ultrasound of the carotid arteries, aorta, and iliofemoral arteries to detect atherosclerotic plaques, coronary artery calcium quantification by computed tomography, and biosampling for omics analysis. A subset of 946 participants showing signs of disease are undergoing vascular 18FDG PET/MR and cardiac MR. Several CNIC clinical and basic research groups participate in PESA, which is the Center's flagship study. The PESA-CNIC-Santander study is already making seminal contributions to our understanding of the origin and progression of atherosclerosis.

In 2019, the third follow-up assessments of PESA participants were completed, and now in 2020 the new PESA-Health Initiative is expanding the scientific approaches performed in the continuing follow-up of the PESA cohort.

Adherence to treatment after an acute myocardial infarction (MI) is essential for efficient secondary prevention. Despite this, many post-MI patients abandon prescribed medication. To address this issue, CNIC researchers and FERRER laboratories developed a "polypill" including three key drugs prescribed to post-MI patients (aspirin, an ACE-inhibitor, and a statin). Having demonstrated that prescription of the CNIC Polypill significantly increases treatment adherence among post-MI patients (J Am Coll Cardiol. 2014; 64:2071-82), CNIC researchers are now leading a multinational randomized clinical trial supported by the H2020 programme. The ongoing SECURE trial (trial identifier NCT02596126) has enrolled 2500 patients soon after an MI and randomized them to standard treatment or a CNIC Polypill-based strategy. Patients will be followed up for a minimum of 2 years, and the incidence of major cardiovascular events will be assessed. Trial enrollment was completed by the end of 2019.

This trial has been extended until December 2021, and is now in the follow-up phase.



Principal investigator:
Valentín Fuster

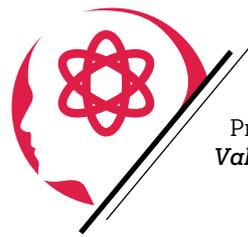
**PROGRESSION OF
EARLY SUBCLINICAL
ATHEROSCLEROSIS**
(PESA - CNIC - SANTANDER)



Principal investigator:
Valentín Fuster
Co-Principal investigator:
José M^o Castellano

**SECONDARY
PREVENTION OF
CARDIOVASCULAR
DISEASE IN THE
ELDERLY
POPULATION**
(SECURE)

Existing tools for characterizing atherosclerosis and determining the risk of its complications are inadequate. These deficiencies limit effective management across the spectrum of this disease, and therefore opportunities are lost for early, cost-effective interventions in subclinical disease, while high-risk populations with manifest disease are administered treatments almost indiscriminately. This leads to high 'numbers needed-to-treat' (NNT), unnecessary patient risk, wasted resources, and unsustainable costs for health care purchasers. In a relatively low-risk population (the PESA-CNIC cohort), we will study whether a personalized worksite-based lifestyle intervention, driven by imaging data (2D and 3D-ultrasound of carotid and ilio-femoral arteries plus coronary artery calcification) results in changes in behavior, improved control of risk factors, and reduced progression of subclinical atherosclerosis plaque burden (SAPB). TANSNIP is a randomized control trial (RCT) including middle-aged bank employees from the PESA cohort stratified by SAPB (high SABP n=260; low SABP n= 590). Within each stratum, participants are randomized 1:1 to join a lifestyle program or receive standard care. The program consists of three elements: (1) 12 personalized lifestyle counseling sessions using motivational interviewing over a 30-month period, (2) a wrist-worn physical activity tracker, and (3) a sit-stand workstation. The primary outcome measure is a composite score of blood pressure (BP), physical activity, sedentary time, body weight, diet, and smoking (the adapted FUSTER-BEWAT score), measured at baseline and at 1-, 2-, and 3-year follow-up. Secondary outcomes are individual changes in lifestyle behaviors and specific changes in anthropometric measures, blood biomarkers, self-rated health, work-related outcomes (including work productivity and absenteeism), health care consumption, program process measures, and cost measures at different measurement points.



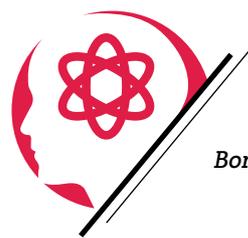
Principal investigator:
Valentín Fuster

THE TANSNIP-PESA RANDOMIZED CONTROL TRIAL (TANSNIP)

**A 30-MONTH WORKSITE-BASED
LIFESTYLE PROGRAM TO PROMOTE
CARDIOVASCULAR HEALTH IN MIDDLE
AGED BANK EMPLOYEES**

The expectation is that individual awareness of CVD risk stratification in the intervention group will lead to a reduction in the prevalence of CV risk factors related to lifestyle and an increase in physical activity compared with the control group. A second rationale is that the level of compliance with the comprehensive 3-year worksite-based lifestyle intervention will be higher among participants with a high imaging defined CV risk. The trial has been finished and primary results will be reported in Q1 2021

The prescription of beta-blockers to patients after an MI is based on evidence from trials performed in the pre-reperfusion era. While there is solid evidence for their benefit in post-MI patients with reduced ejection fraction, such evidence is lacking for patients with preserved ejection fraction. Despite this, more than 80% of post-MI patients in this category are prescribed beta-blockers for the rest of their lives. REBOOT (tREatment with Beta-blockers after myOcardial infarction withOut reduced ejection fracTion) is a multinational trial that will enroll 8600 post-MI patients with a left ventricular ejection fraction above 40%. Patients are randomized to beta-blocker therapy (type and dose decided by the attending physician) or to no treatment. The primary endpoint is the composite of all-cause death, reinfarction, or heart failure admission during 3-year follow-up. This trial is coordinated by the CNIC Clinical Trials Coordination Unit and is run in close collaboration with the Mario Negri Institute of Research in Milan. 105 hospitals across Spain and Italy participate in this large- scale project that will have a major impact on clinical practice.



Principal investigator:
Borja Ibáñez

TREATMENT WITH BETA-BLOCKERS AFTER MYOCARDIAL INFARCTION (MI) WITHOUT REDUCED EJECTION FRACTION (REBOOT)

The first patients were enrolled in October 2018, and so far 3700 patients have been recruited. It is anticipated that enrollment will be completed before the end of 2022.

There is increasing awareness of the association between atherosclerosis and cognitive function, but the mechanisms linking these processes are not fully understood. The Heart-to-Head (H2H) study is testing the hypothesis that extensive subclinical atherosclerosis is associated with subtle cognitive decline and beta-amyloid deposition in the brain. This transatlantic collaboration is framed within an agreement between the CNIC and Mount Sinai Hospital in New York and is led by CNIC General Director Valentín Fuster. In Spain, the H2H project is coordinated between the CNIC and 12 de Octubre Hospital. Other university hospitals (Fundación Jiménez Díaz, Clínico San Carlos, and Gregorio Marañón) participate in the project, which receives funding from the *Instituto de Salud Carlos III (ISCIII)* through a *Proyecto Integrado de Excelencia*. A total of 300 participants are undergoing extensive atherosclerosis phenotyping (multi-territory 3D vascular ultrasound and cardiac computed tomography) and thorough brain imaging (anatomical and functional magnetic resonance imaging and positron emission tomography (PET)-amyloid scan), as well as cognitive function testing.

Recruitment is complete, and follow-up visits are now being carried out.

The consequences of valvular heart disease on left ventricular (LV) dimensions, function, and tissue composition are important prognostic determinants. Current practice guidelines recommend surgical or percutaneous intervention in patients with significant valvular heart disease when symptoms develop or when LV remodeling or dysfunction occur. The most prevalent valvulopathies are aortic valve stenosis and mitral regurgitation. The transition from asymptomatic to symptomatic or from normal LV dimensions and function to LV dilatation/hypertrophy (LVH) and dysfunction is determined by changes in tissue composition, predominantly cardiomyocyte death, extracellular volume expansion, and fibrosis. Surgical or percutaneous intervention in severe valvular disease is currently guided by the presence of symptoms or gross anatomical and functional LV involvement (i.e. significant chamber dilatation or reduced ejection fraction). However, when these features appear, it is often too late for the intervention to restore normal cardiac function. There is therefore a need for tools able to detect myocardial involvement in valvular disease at early stages and guide treatment before overt deterioration of cardiac function.

In this project, we use a multimodality imaging approach (cardiac magnetic resonance [CMR] plus strain echocardiography) to characterize LV status in patients with significant valvular disease. We focus on 2 specific forms of valvular disease: aortic stenosis (a paradigm of LV pressure overload) and mitral regurgitation (a paradigm of LV volume overload).

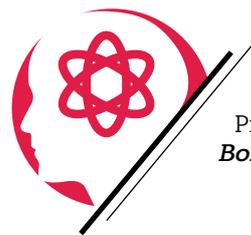
CMR is the gold standard for anatomical and functional cardiac evaluation, including detection of focal fibrosis by late gadolinium enhancement (LGE) after contrast-gadolinium administration. Recent CMR advances include parametric T1/T2 mapping, absolute myocardial perfusion quantification, calculation of extracellular volume (a surrogate of diffuse fibrosis), and tagging. Using these advances, we will obtain highly accurate tissue characterization. Focal and diffuse fibrosis will be assessed by endovenous contrast using the gadolinium contrast agent, which is in routine clinical use and has an optimal safety profile. The assessment of diffuse fibrosis will additionally require the collection of blood samples to obtain the hematocrit.



Principal investigator:
Valentín Fuster
Co- Principal Investigator:
Héctor Bueno

ATHEROBRAIN. THE HEART TO HEAD STUDY

(H2H)



Principal investigator:
Borja Ibáñez

MULTIMODAL MYOCARDIAL TISSUE CHARACTERIZATION IN PATIENTS WITH SIGNIFICANT VALVULAR DISEASE

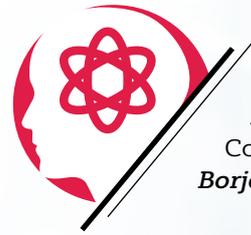
(MRVALVE)

Active deformation of the LV myocardium best assessed using the echocardiographic (Echo) strain technique, which can detect impairment of multidirectional strain (active deformation) despite preserved LV global function.

We correlate imaging data with functional data from the 6-minute walking test, which provides an objective assessment of functional exercise capacity. In addition, we use cardiac computed tomography (CT) as a noninvasive means to obtain information about the presence, location, and extent of calcium in the coronary arteries and valves; the CT-based calcium score is a widely used diagnostic and prognostic tool for patients with aortic stenosis.

By the end of 2019, 34 patients had been included, and we have now begun the follow up visits, with four of them now completed.

Pulmonary hypertension (PH) secondary to left heart disease (group PH) is the most common form of PH and currently lacks effective therapy. CNIC researchers have identified the β_3 adrenergic receptor as a novel therapeutic target for this disease in a large animal model of PH (Basic Res Cardiol. 2016;111:49). The CNIC is currently leading a phase 2 clinical trial in which group 2 PH patients are randomized to standard therapy vs standard therapy plus a β_3 selective agonist (trial identifier NCT02775539 and N^o EudraCT: 2016-002949-32). In total, 80 patients are being recruited at four Spanish hospitals and will be followed under treatment for 4 months. The study endpoints are pulmonary artery hemodynamics and the CMR profile.



Principal investigator:
Ana García-Alvarez.
Co-Principal Investigators:
Borja Ibañez and Valentín Fuster

TREATMENT WITH β_3 AGONISTS IN CHRONIC PULMONARY HYPERTENSION SECONDARY TO HEART FAILURE (SPHERE- HF)

This multicenter case-control study examines patients with takotsubo cardiomyopathy. Controls are age-, sex-, and ECG–presentation-matched AMI patients and individuals without overt CVD or acute disease.

The study includes the following substudies and techniques:

1. Psychological and neuro-psychological studies to assess personality traits, positive and negative emotions, emotional regulation, resilience, and reflection
2. EEG studies to assess the presence of neuropathophysiological responses
3. Imaging studies to determine brain areas and functional patterns related to takotsubo cardiomyopathy
4. Proteomic screening to identify molecules specifically involved in takotsubo cardiomyopathy.



Principal investigator:
Héctor Bueno

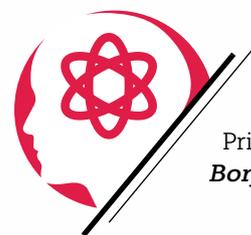
TAKOTSUBO BRAIN PROJECT. A STUDY TO IDENTIFY THE PSYCHOLOGICAL AND NEURO-PHYSIOLOGIC MECHANISMS OF THE TAKOTSUBO SYNDROME (TAKOTSUBO)

This study includes two CMR protocols:

- 1) A myocardial study, including gadolinium contrast, of cardiomyopathy and myocarditis in post-infarction patients;
- 2) Magnetic resonance coronary angiography without gadolinium contrast in patients with a clinical indication for coronary angio-CT.

The study will include 150 patients (100 in protocol 1, 50 in protocol 2) who will undergo MRI studies at Hospital Universitario Rey Juan Carlos (3Tesla MRI) and Hospital Universitario de Salamanca (1.5Tesla MRI).

This study is funded by the ISCIII (FIS-Technological Development (FIS-DTS)). During the first year, we have validated and improved the technology in the pig model and have optimized study reconstruction so that it can be completed in real time in the MRI console.



Principal investigator:
Borja Ibañez

3D ULTRA-FAST COMPREHENSIVE CARDIAC MAGNETIC RESONANCE STUDY (DASH - CMR)

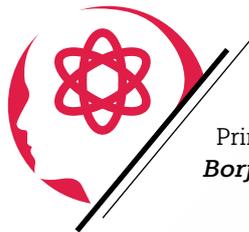
Pulmonary hypertension due to left heart disease is a pathophysiological and hemodynamic state present in a wide range of clinical conditions that affect left heart structures. Although historically the pulmonary circulation received little attention, today it is an essential part of cardiological assessment. In patients with heart failure, the most important clinical factors are the presence of pulmonary hypertension and right ventricular function. These factors are also essential for determining prognosis and must be taken into account when making some of the most important therapeutic decisions. The pathophysiological process begins passively but later transforms into a reactive process. This reactive process includes a component that can be reversed with vasodilators and another that is fixed, in which the underlying mechanism is congestive vasculopathy (essentially medial hypertrophy and pulmonary arterial intimal fibrosis). Currently no specific therapy is available for this type of pulmonary hypertension, and treatment is the same as for heart failure itself. The drugs that have been shown to be effective in pulmonary arterial hypertension have generally shown no clear effect in clinical trials. We are clinically developing a number of groups of pharmacological compounds that will enable us to make progress in this area in the near future



Principal investigator colaborator:
Hospital 12 de Octubre:
Juan F Delgado.
Co-Principal Investigator CNIC:
Borja Ibáñez

PULMONARY VASCULOPATHY IN PATIENTS WITH ADVANCED HEART FAILURE (HPIC)

Cancer patients receiving anthracyclines are at risk of developing cardiac toxicity that can lead to chronic heart failure. Early diagnosis of anthracycline-induced cardiotoxicity is vital in order to implement interventions that can ameliorate heart failure progression. Current algorithms are far from ideal because cardiotoxicity is diagnosed after there is already a cardiac motion dysfunction. Within an ongoing ERC-Consolidator project (MATRIX), we are assessing a novel, CMR-based early diagnostic test in cancer patients scheduled to undergo chemotherapy with anthracyclines. In collaboration with the Jiménez Díaz University Hospital, 100 lymphoma patients are being enrolled and will undergo serial CMR studies at the CNIC using a multiparametric approach that has been shown in a preclinical model to visualize cardiotoxicity long before the appearance of cardiac motion defects.



Principal investigator:
Borja Ibáñez

MYOCARDIAL TISSUE CHARACTERIZATION IN PATIENTS EXPOSED TO ANTHRACYCLINES FOR EARLY DIAGNOSIS OF CARDIOTOXICITY (MATRIX)