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.

2.1 VASCULAR PATHOPHYSIOLOGY

Coordinator: **Almudena R. Ramiro**

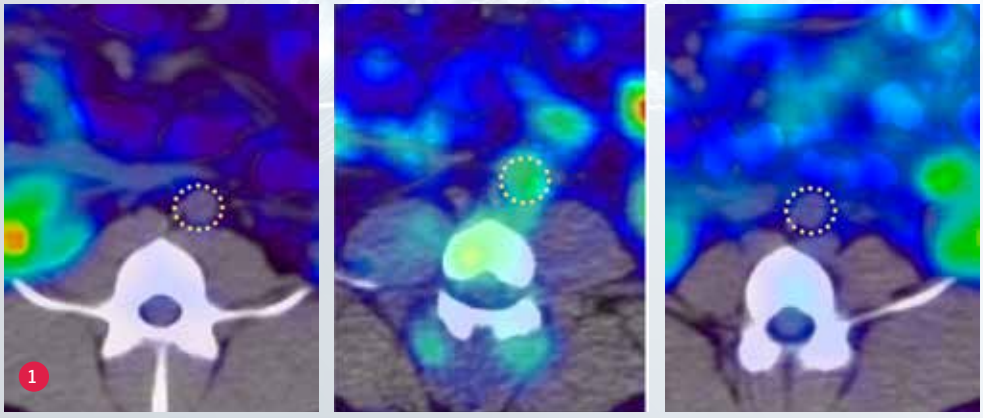
The research groups in the Vascular Pathophysiology (VP) area study the vascular system in health and disease, with a particular focus on the molecular mechanisms governing vascular biology, cardiac and muscle differentiation and regeneration, and atherosclerosis, the main underlying cause of myocardial infarction and stroke. The VP area comprises 12 research groups and 3 core Technical Units (Bioinformatics, Genomics, and Proteomics).

VP-area groups made a number of key advances in 2021. Ongoing research into the mechanisms and mediators of Marfan syndrome (MFS) aortic disease showed that increased NOS2-derived NO activates the guanylate cyclase (sGC)–protein kinase G (PRKG) pathway in MFS patients and causes elevated nitration of certain plasma proteins. This finding has identified potential biomarkers and therapeutic targets for MFS.

Another key research theme is ischemic stroke and cardiovascular risk factor-driven cognitive impairment. Work in this area last year elucidated the role of neutrophil heterogeneity and innate immune pattern recognition receptors in stroke and immunothrombosis.

Mechanisms of atherothrombotic disease are also a focus of research into Hutchinson-Gilford progeria syndrome (HGPS). This rare disorder is characterized by premature aging and death mainly from myocardial infarction, stroke, or heart failure. HGPS is caused by progerin, a mutated variant of lamin A expressed in most differentiated cells of HGPS patients. VP-area scientists have generated a mouse model to assess the reversibility of progerin-induced damage and the relative contribution of specific cell types to the disease. The findings suggest that it is never too late to treat HGPS, although benefit are much greater when progerin is targeted in mice with mild symptoms.

Atheromatous fibrous caps are produced by smooth muscle cells (SMCs) that are recruited to the subendothelial space. By using conditional mouse models, VP-area researchers have found that sequential loss and gain of Notch signaling is needed to build the cap SMC population. The shared



No atherosclerosis **Progressing atherosclerosis** **Regressing atherosclerosis**

RESEARCH GROUPS

- **Vicente Andrés**
  Molecular and Genetic Cardiovascular Pathophysiology
- **Jacob Fog Bentzon**
  Experimental Pathology of Atherosclerosis
- **José Luis de la Pompa**
  Intercellular Signaling in Cardiovascular Development and Disease
- **Valentín Fuster**
  Cardiovascular Imaging and Population Studies
- **José Javier Fuster**
  Hematovascular Pathophysiology
- **Pilar Martín**
  Regulatory Molecules of Inflammatory Processes
- **Mª Angeles Moro**
  Neurovascular Pathophysiology
- **Pura Muñoz**
  Tissue Regeneration
- **Almudena R. Ramiro**
  B Lymphocyte Biology
- **Juan Miguel Redondo**
  Gene regulation in Cardiovascular Remodelling and Inflammation
- **Francisco Sánchez-Madrid**
  Intercellular Communication in the Inflammatory Response
- **Jesús Vázquez**
  Cardiovascular Proteomics

TECHNICAL UNITS

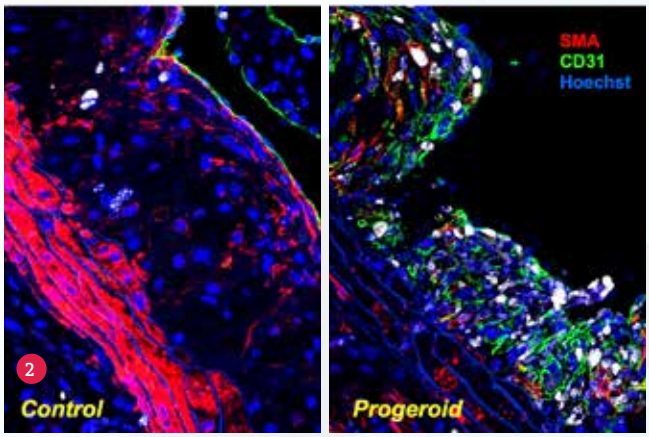
- Bioinformatics **Fátima Sánchez cabo**
- Genomics **Ana Dopazo**
- Proteomics **Juan Antonio López**

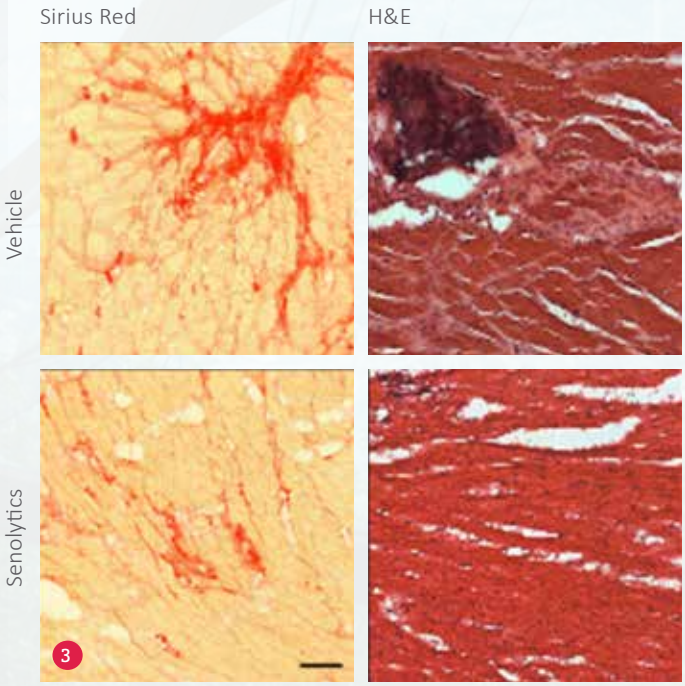
mechanisms with embryonic arterial media assembly suggest that the cap forms as a neo-media that restores the connection between endothelium and subendothelial SMCs, transiently disrupted in early atherogenesis.

Advanced quantitative high-throughput proteomics methods have provided new clues about the implication of oxidative stress in early cardiovascular disease, hypertension-induced endothelial dysfunction, and the immune response in abdominal aortic aneurysm. This analysis has identified a set of three circulating protein biomarkers, promising easy detection of subclinical atherosclerosis in individuals with low cardiovascular risk.

Other studies by VP-area scientists have shown that muscle repair relies on nuclear migration for cellular reconstruction, which provides an alternative, self-repair model for understanding the restoration of muscle architecture in health and disease.

Mutations in the G protein-coupled receptor GPR126/ADGRG6 cause human diseases, and global Gpr126 inactivation in the mouse is embryonically lethal, with mutants having thin-walled ventricles while having no effect on heart patterning and maturation. Through the generation of new genetic mouse and zebrafish models, a VP-area team has found that the placenta–heart axis accounts for



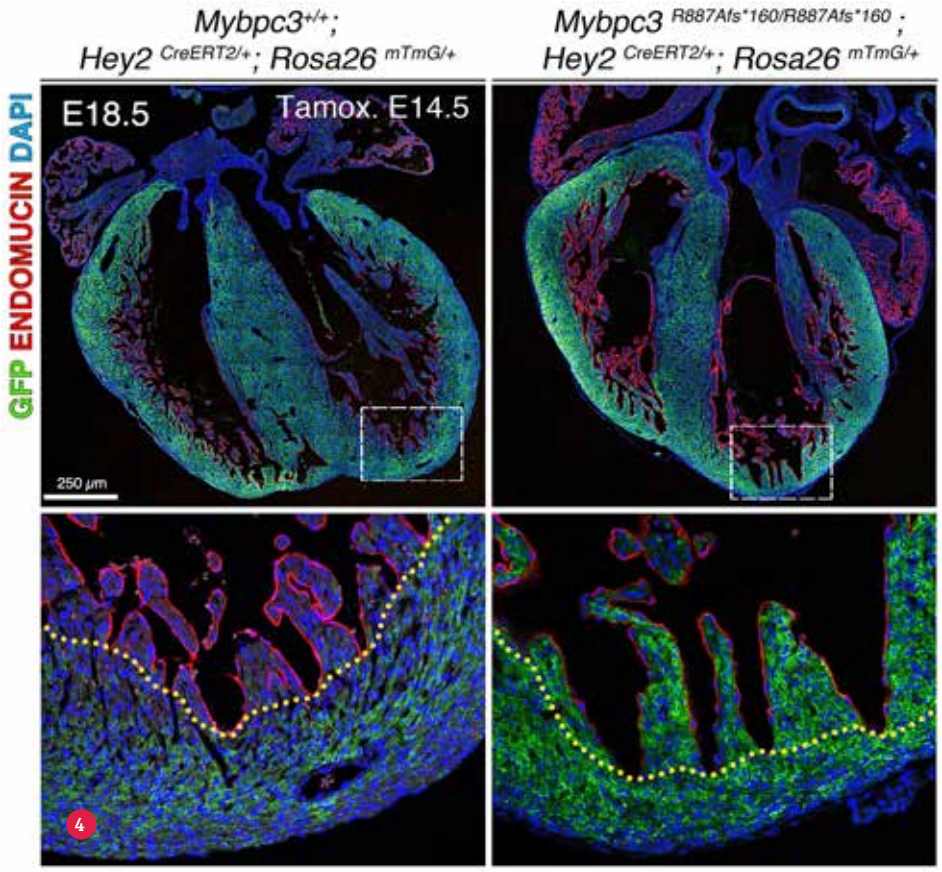


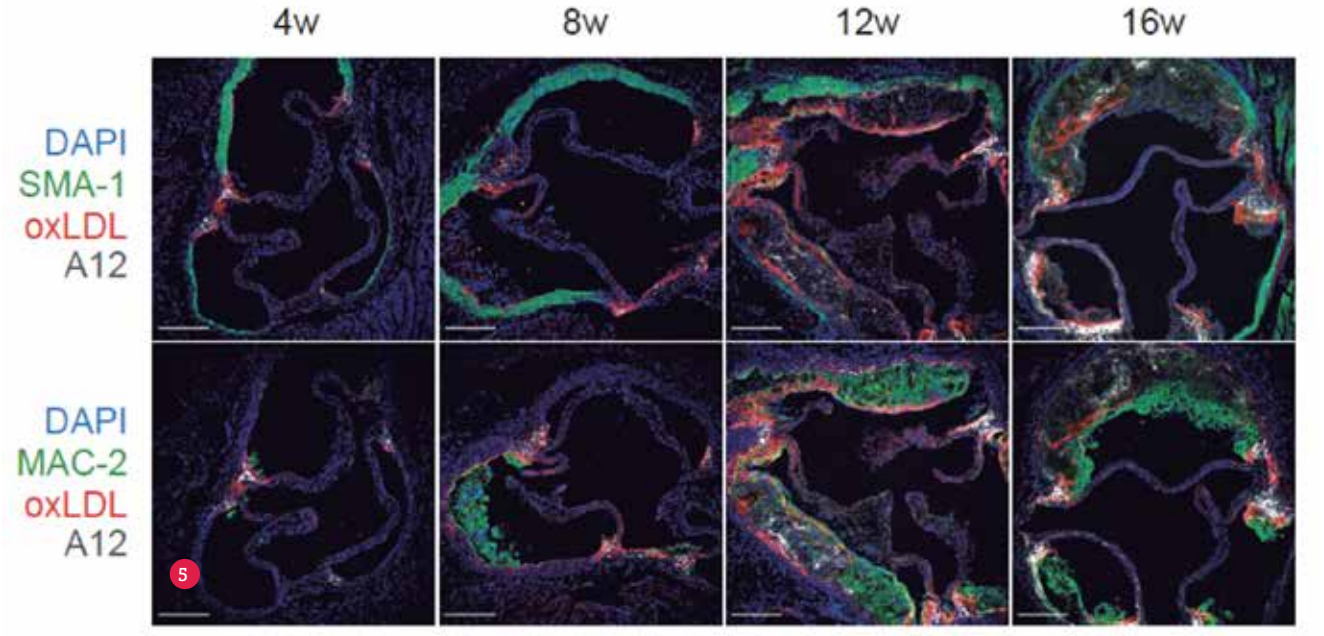
heart abnormalities secondary to placental defects in Gpr126 mutants.

High throughput repertoire analysis in atherosclerotic mice revealed a high predominance of antibodies that recognize the atheroma plaque. A deep proteomics analysis identified the target antigen of one of these autoantibodies (A12) as the mitochondrial enzyme ALDH4A1. Circulating ALDH4A1 is increased in mice and humans with atherosclerosis, and infusion of A12 antibody infusion into atherosclerosis-prone LDLR-/- mice delayed plaque formation, opening new avenues for diagnostic and therapeutic interventions in CVD.

VP-area groups maintain an active research effort on microRNAs (miRNAs). Research in this area showed that the miRNA mmu-miR-721 is synthesized by Th17 cells and is present in the plasma of mice with acute autoimmune or viral myocarditis but not in those with acute myocardial infarction. The human homolog, designated hsa-miR-Chr8:96, was identified in four independent cohorts of myocarditis patients. This miRNA thus serves as a biomarker that distinguishes between patients with myocarditis and those with myocardial infarction.

The miRNA repertoire of human T lymphocytes undergoes dynamic post-transcriptional modification in response to cell activation. These modifications regulate miRNA stability, degradation, and fate. Assessment of the transcriptomic and epigenetic changes triggered in dendritic cells during antigen-cognate synaptic interactions with T lymphocytes revealed the induction of an antipathogenic program that confers resistance and enhanced immune response to further pathogen threats.





2: MYOCARDIAL PATHOPHYSIOLOGY

Coordinator: Guadalupe Sabio

The Myocardial Pathophysiology Area (MPA) includes 10 research groups and 5 core technical units. MPA groups work on a wide range of topics: inherited cardiomyopathies, arrhythmia mechanisms and therapy, molecular regulation of heart failure, metabolism and its effect on cardiovascular disease, functional genetics of the oxidative phosphorylation system, translational cardiovascular imaging and therapy, molecular cardiology, immunobiology, cardiovascular health and imaging, and nuclear receptor signaling. Research in these areas produced several scientific advances during 2021.

José Antonio Enríquez research centers on the mammalian mitochondrial electron transport chain (MtETC) and H+-ATP synthase, which together comprise the oxidative phosphorylation (OxPhos) system. One key research focus is the genetic variability of mtDNA and the repercussions this has on whole-body metabolism, the response to drugs, predisposition to disease, healthy aging, and the borderline pathology and functional variability of mtDNA alterations. The group's work has highlighted the role of mitochondrial reactive oxygen species (ROS) in OxPhos-system adaptation to the metabolic requirements of the cell. The group has also contributed to the identification of mitochondrial Na+ as a second messenger regulating inner mitochondrial membrane fluidity and ROS production by MtETC complex III. Another key research area is the role of OxPhos in metabolic adaptation, and the group has produced a key advance in the understanding of how cells optimize and regulate their metabolic capacity by inducing structural changes in the MtETC. Surprisingly, these adaptations are especially relevant in cardiovascular disease and the immune system.

Rodrigo Fernández leads the EnIGMA (Early ImaGing Markers of unhealthy lifestyles in Adolescents) project, funded by the Fondo de Investigación Sanitaria- Instituto de Salud Carlos III. The project recruited 123 adolescents who underwent multi-territory multi-parametric CMR imaging studies at the CNIC. The first EnIGMA results will be reported soon.

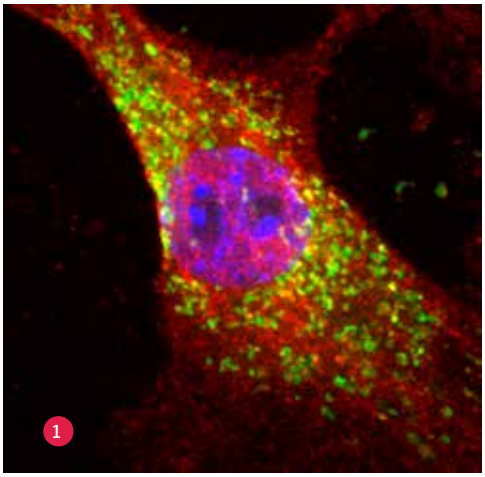
The research group led David Filgueiras has developed a novel noninvasive method to assess the relationship between mechanical and electrical activation rates during atrial fibrillation (AF), enabling the characterization of patient-specific stages of atrial remodeling as the disease progresses. The method has shown early prognostic value in rhythm control management and AF recurrences before other clinical or conventional echocardiography parameters become manifest.

The Translational Laboratory for Cardiovascular Imaging and Therapy (TLCVIT) is led by Borja Ibáñez. Continuing studies with the beta-blocker metoprolol established the use of this drug as a cardioprotective therapy against infarction and showed its utility in the treatment of critical COVID-19 patients. Research into the cardiotoxic effects of chemotherapy explored the use of remote preconditioning (RIPC) and identified microcirculation injury as an early detector of myocardial damage associated with chemotherapy drugs. The team was also central to the development of an ultrafast cardiac resonance protocol to validate a new ultrafast 3D protocol (ESSOS) for complete cardiac resonance analysis. The protocol achieves assessment of anatomy and function, as well as late enhancement to evaluate infarction, all in a single breath hold. The procedure is completed in under a minute.

José Jalife leads the Arrhythmia Research Laboratory, which investigates the role of macromolecular ion channel complexes in the molecular mechanisms of life-threatening rhythm disturbances in patients with Andersen-Tawil syndrome, Short-QT syndrome, and Duchenne muscular dystrophy. The group adopts a multidisciplinary approach, including the use of mouse models of cardiac-specific expression of mutant potassium ion channels, patient-specific iPSC-CMs, in-silico modeling of ion channel structure-function

TECHNICAL UNITS

- Comparative Medicina
- Clinical Trial Coordination **Antonio J. Quesada**
- Pluripotent Cell Technology **Giovanna Giovinazzo**
- Transgenesis **Juan De Dios Hourcade**
- Viral Vectors **Juan A. Bernal**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RESEARCH GROUPS

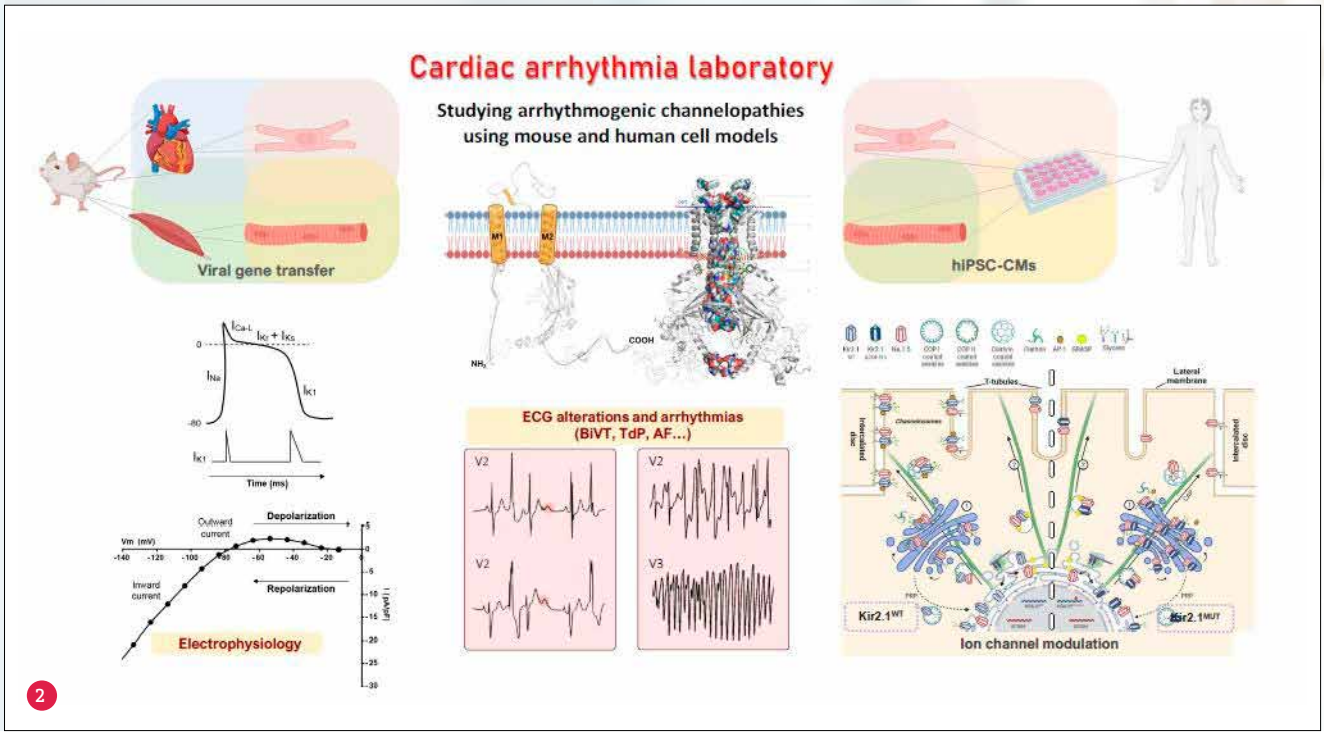
- **José Antonio Enríquez**
  Functional Genetics of the Oxidative Phosphorilation System (GENOPHOS)
- **Rodrigo Fernández**
  Cardiovascular Health and Imaging
- **David Filgueiras**
  Advanced Development in Arrhythmia Mechanisms and Therapy
- **Borja Ibáñez**
  Translational Laboratory for Cardiovascular Imaging and Therapy
- **José Jalife**
  Cardiac Arrhythmia
- **Enrique Lara-Pezzi**
  Molecular Regulation of Heart Failure
- **Silvia Priori**
  Molecular Cardiology
- **Mercedes Ricote**
  Nuclear Receptor Signaling
- **Guadalupe Sabio**
  Stress kinases in Diabetes, Cancer and Cardiovascular Disease
- **David Sancho**
  Immunobiology

relationships, transcriptomics, protein chemistry, patch-clamping, ECG recordings, intracardiac stimulation, and optical mapping. The ultimate goal is that these studies will identify novel targets for the prevention of arrhythmias and sudden cardiac death in patients suffering from these devastating diseases.

Enrique Lara-Pezzi leads a research group investigating the molecular mechanisms underlying the development of pathological cardiac hypertrophy. Their work shows that cardiac hypertrophy in mice is triggered by loss of the expression of SRSF4 (serine- and arginine-rich splicing factor 4), leading to diastolic dysfunction and abnormal repolarization. This response is due to the downregulation of the long noncoding RNA GAS5 (growth-arrest-specific 5) and consequent elevation of glucocorticoid receptor transcriptional activity. These findings may contribute to the development of new treatments for cardiac hypertrophy and myocardial pathology in patients with Cushing syndrome.

The CNIC Molecular Cardiology lab, led by Silvia G. Priori, aims to find new treatments for Timothy syndrome (also known as Long QT syndrome 8) and catecholaminergic polymorphic ventricular tachycardia, two highly lethal inherited diseases caused by mutations in key proteins that generate the heartbeat. Funded by Fundación La Caixa and a Plan Nacional grant, this project studies genetically modified pigs and mice that recapitulate these human diseases.

Mercedes Ricote's lab investigates how nuclear receptors coordinate the transcriptional landscape of cardiomyocytes in homeostasis and disease. Their work has revealed that retinoid X receptors (RXRs) are essential drivers of mitochondrial fitness and nutrient balance in perinatal and adult hearts. By integrating environmental signals, these ligand-activated transcription factors promote an appropriate epigenetic remodeling that allows the expression of key genes encoding key metabolic elements, sarcomere components, and ion channels. The team's work shows that, in addition to sustaining contractile function, RXR-controlled gene signatures are required to prevent the development of heart failure, thus establishing these nuclear receptors as therapeutic targets for treating cardiovascular disease.



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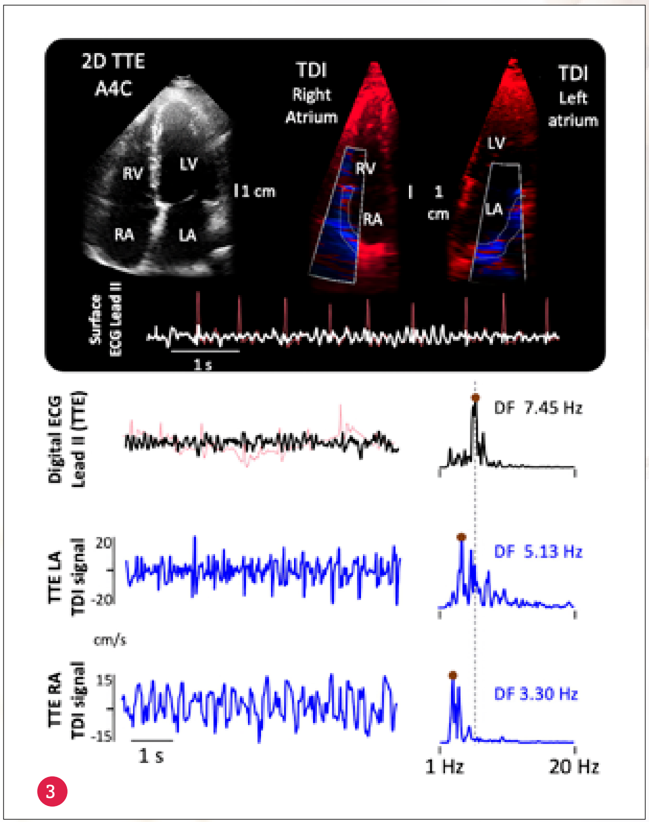
Guadalupe Sabio's lab demonstrated that p38γ/δ kinases control cardiomyocyte metabolic switching during early postnatal development. Activation of these kinases early after birth blocks glycogen production, triggering the oxidation of fatty acid by cardiomyocyte mitochondria. The teams work demonstrates that dysregulation cardiac metabolism can have whole-body metabolic consequences, showing that the heart is a key metabolic organ. These alterations in metabolic organs are due to a cardiac energetic deficit, and the studies highlights the role of heart as endocrine organ.

David Sancho's group investigates how communication between the gut microbiota and the immune system regulates the inflammatory response, and the impact this has on cardiovascular disease. The team has found that mucosal immunotherapy with polybacterial preparations can boost innate responses through a mechanism dependent on epigenetic modifications. This effect can enhance the antiviral response to diverse viruses, including influenza A, vaccinia, and SARS-CoV-2. Trained immunity can also increase vaccine immunogenicity. However, the group is also investigating potential detrimental roles of trained immunity in atherosclerosis.



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The MPA hosts five core technical units that give support to all CNIC scientists.

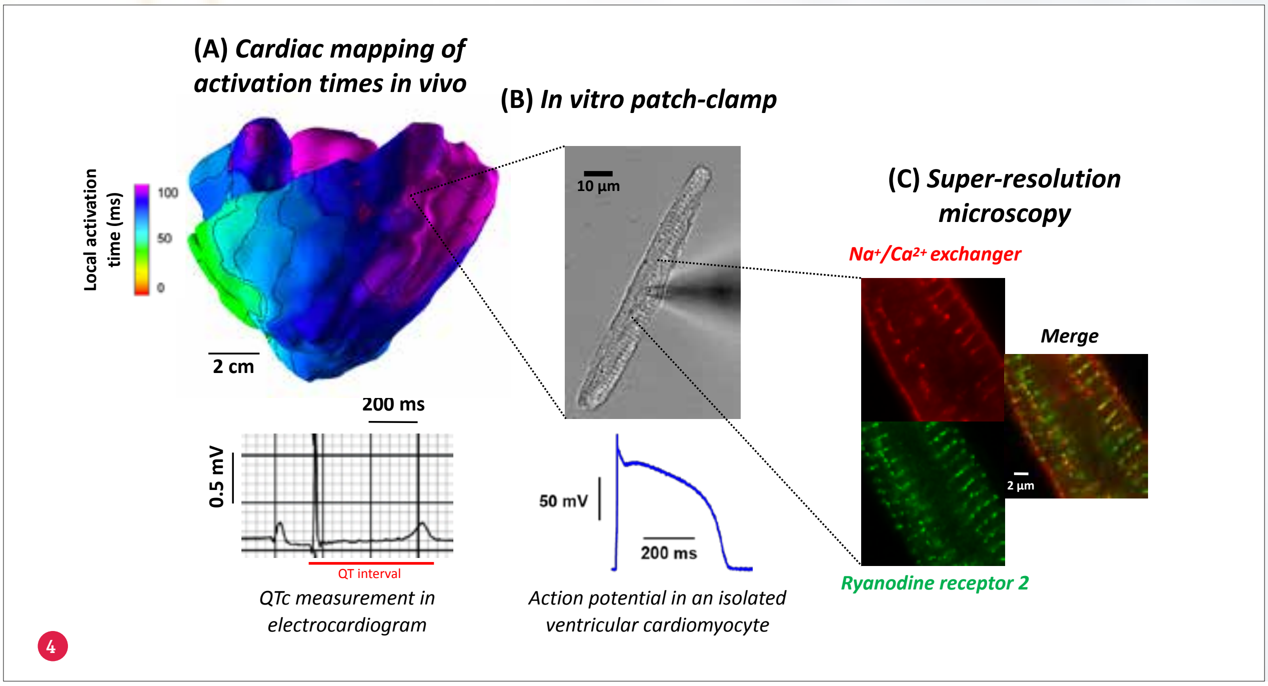
The Transgenesis Unit provides services in mouse strain rederivation, production of genetically modified mice, and cryopreservation of mouse strains. Through collaboration with J.N. Domínguez Macías and M. Torres, the unit is currently developing a microinjection model in post-implantation embryos.

The Pluripotent Cell Technology Unit (PCTUnit) provides state-of-the-art knowledge, training, and technological support in the culture and manipulation of mouse and human pluripotent cells. The PCTUnit has worked with CNIC researchers on the generation of several in vivo and in vitro models, including designing CRISPR/Cas9-based gene-editing strategies and performing the necessary experiments to obtain mouse and pig animal models and in vitro models of cardiovascular disease in hiPSCs.

The Clinical Trials Coordination Unit (CTCU) has continued to provide specialized support for clinical trials and studies carried out at the CNIC. Its ultimate goal is to boost Spanish leadership in clinical trials in the cardiovascular area. In 2021, the CTCU coordinated 11 clinical trials and studies that included more than 10,500 participants in more than 200 European hospitals.

The Viral Vectors Unit provides researchers with access to state-of-the-art viral vector technology for use in preclinical studies and basic research.

The Comparative Medicine Unit supports in vivo work in the animal facility.



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2.3 CELL AND DEVELOPMENTAL BIOLOGY

Coordinator: Jorge Alegre-Cebollada

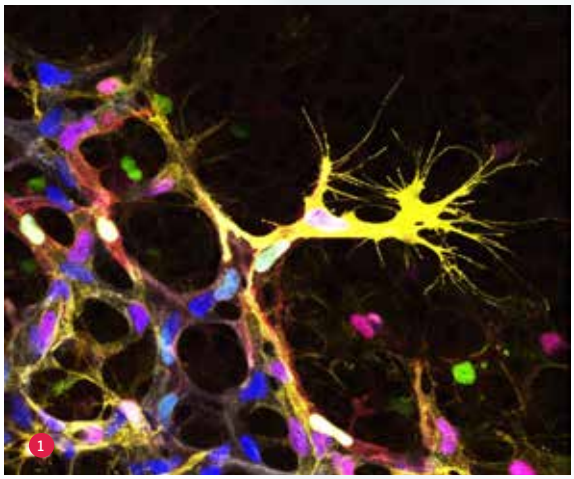
The Cell and Developmental Biology (CDB) area is a multidisciplinary forum of 8 research groups with diverse and complementary approaches to understanding cardiovascular health and disease. The CDB area strategy is to build deep knowledge of the first principles governing the function of biomolecules, cells, tissues, and organisms. Scientific activities are also grounded in the cutting-edge technology provided by the CDB-affiliated Technical Units in Flow Cytometry, Microscopy, and Advanced Imaging, together with the other CNIC Technical Units.

In 2021, CDB-area scientists reported direct targets of key transcription factors during organogenesis and identified pathways playing important roles in congenital heart malformations. This advance was grounded in the strong genetic association between congenital malformations that affect the heart and the limbs. The CDB area also reported new molecular data linking genetic variants to the development of familial cardiomyopathies, providing information useful for the genetic diagnosis of patients and their families. Other recent CDB-area findings suggest that hypertrophic cardiomyopathy can arise as the result of defective nanomechanical properties of sarcomere proteins. CDB-area scientists also reported new insights into the function of immune and vascular cells, obtained through collaborative studies that dissected the transcriptional networks responsible for the activation and programming of neutrophils at inflammatory sites. This is an important finding because neutrophils are the most abundant and potentially damaging type of leukocyte. Other neutrophil studies examined how these cells adjust to circadian patterns and highlighted their role in the pathology of COVID-19. CDB scientists also uncovered angiocrine signatures important for organ homeostasis, growth and regeneration.

Ongoing research in the CDB area includes the generation of novel animal models for in vivo multispectral and single-cell genetic barcoding and the creation of disease models of cardiomyopathy to assess the efficacy of emerging therapies. CDB-area groups are also developing methods that allow unbiased classification of cells in living tissues and are conducting mechanistic studies on how cells sense mechanical properties of the extracellular matrix and how this impacts plaque formation during atherosclerosis. Teams within the CDB area are also deepening understanding of the actions of tissue macrophages, especially in the heart, where they support mitochondrial and metabolic homeostasis.

The impact of the research conducted by the CDB area has resulted in multiple invitations to contribute expert reviews on cell and molecular mechanobiology, biomaterials, and vascular biology, as well as to give presentations at leading research institutions and conferences. In addition, two prestigious ERC-Consolidator grants to CDB scientists began in 2021 and will be active for the next five years. Other honors in 2021 included the election of CDB members to prestigious scientific societies, including the European Molecular Biology Organization, the European Vascular Biology Organization, the Swiss Academy of Medical Sciences, and the Spanish Young Academy, which add to other individual prizes for outstanding achievements awarded to CDB scientists.

CDB-area scientists actively collaborate with other leading international laboratories. For exampe, a collaborative study published in 2021 reported the potential of nicotinamide for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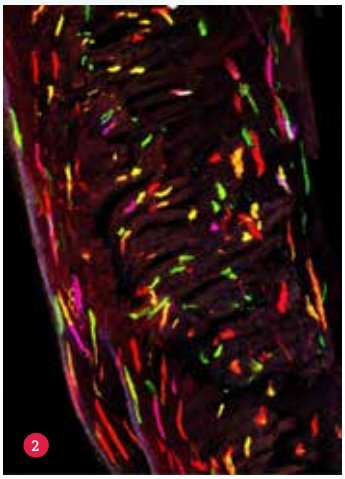
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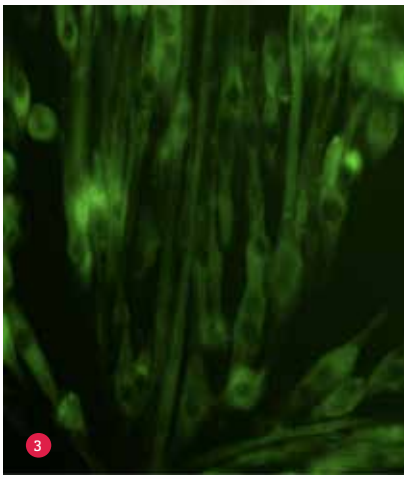
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the treatment of heart failure with preserved ejection fraction. These collaborative efforts are the basis of coordinated grants that synergize the efforts of partners to achieve a shared goal. With the support of Obra Social La Caixa Health Research program, the CDB area has built a multidisciplinary international consortium to determine in unprecedented detail how blood flow forces interplay with other risk factors in atherosclerosis. The knowledge obtained will help to identify novel biomarkers and therapeutic targets to treat and even reverse advanced disease.

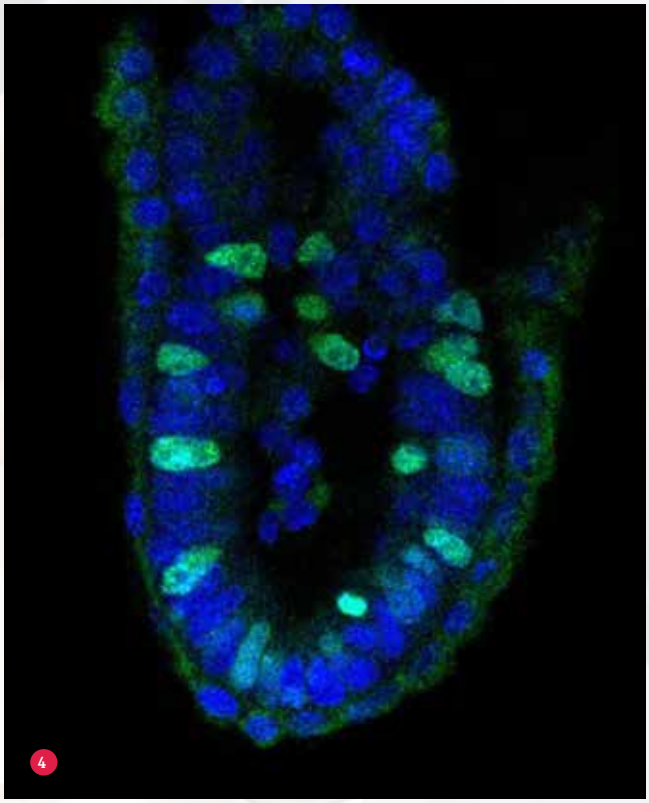
The Technical Units associated with the CDB area have continued to improve their instrumentation and know-how to keep up with ever advancing technologies. The Flow Cytometry Unit received funding for the installation of two new high-end cell-sorter flow cytometers. One of these is a spectral cell sorter that will allow the use of multiple fluorophores, greatly reducing problems derived from cell autofluorescence. This equipment will improve the unit's capacity to offer cell-sorting support to CNIC research groups. The Microscopy Unit, which is part of the National Infrastructure ReDIB network, has secured funding to launch a multiparametric non-linear optical platform, unique in Europe, for metabolic and submicrometer functional imaging in large experimental models. The Advanced Imaging Unit, also part of the National Infrastructure ReDIB network, has secured funding to improve molecular imaging equipment.

TECHNICAL UNITS

- Flow Citometry **Beatriz Álvarez**
- Imaging **Manuel Desco**
- Microscopy **Valeria Caiolfa**

RESEARCH GROUPS

- **Jorge Alegre-Cebollada**
  Molecular Mechanics of the Cardiovascular System
- **Rui Benedito**
  Molecular Genetics of Angiogenesis
- **Héctor Bueno**
  Multidisciplinary Translational Cardiovascular Research (MTCR)
- **Miguel Angel del Pozo**
  Mechanoadaptation and Caveolae Biology
- **Andrés Hidalgo**
  Imaging the Cardiovascular Inflammation and the Immune Response
- **Nadia Mercader**
  Development of the epicardium and its role during regeneration
- **Carlos Pérez-Medina**
  Nanomedicine and Molecular Imaging
- **Miguel Torres**
  Genetic Control of Organ Development and Regeneration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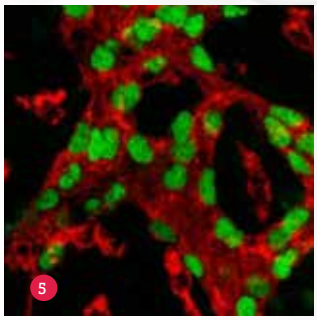
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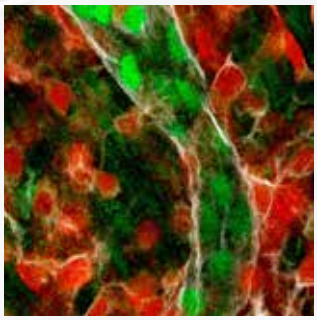

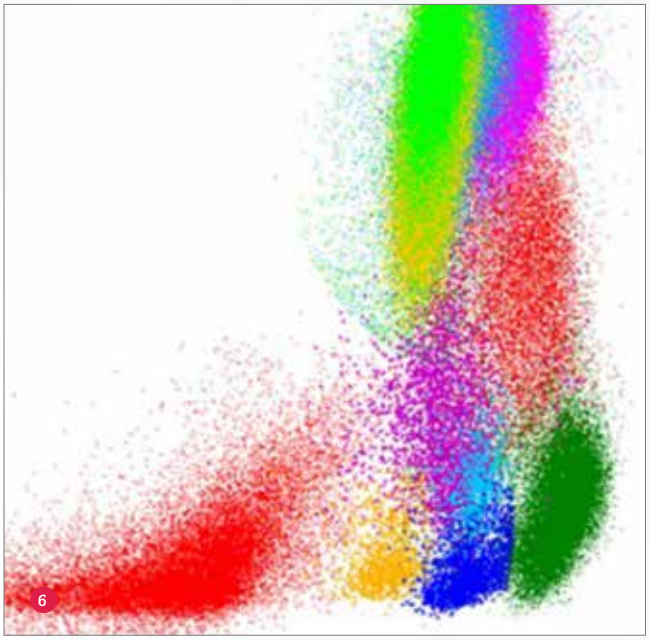
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2.4 CLINICAL STUDIES

EARLY DETECTION OF SUBCLINICAL ATHEROSCLEROSIS, PROGRESSION AND CARDIOVASCULAR HEALTH

(PESA-HEALTH-CNIC-SANTANDER STUDY)

Principal Investigator: ***Valentín Fuster***

The PESA-Health-CNIC-Santander study is the natural continuation of the long-term endeavor started in 2010 with the PESA Study, carried out by the CNIC in collaboration with Santander Bank. Within PESA-Health, the PESA participants enrolled in 2010 (4184 asymptomatic individuals between the ages of 40 and 55 years old at enrollment) are being actively followed up over an additional 10 years.

The original aim of the study was to identify the presence of subclinical atherosclerosis (SA) long before symptoms appear and to understand the cues leading to its development and progression. PESA-Health expands these objectives to new areas, such as the correlation of SA with Alzheimer's and cognitive diseases, the acquisition of somatic mutations during aging, and the correlation of these mutations with increasing cardiovascular event rates and SA progression. PESA-Health continues to take advantage of state-of-the-art imaging technologies, including 3D vascular ultrasound of the carotid arteries and aorta, coronary artery calcium quantification by computed tomography, cardiac magnetic resonance, AngioTC, PET-amyloid analysis, and biosampling for omics analysis. In addition, new state-of-the-art substudies have been added, including an investigation of the relationship between sleep apnea and SA.

PESA-Health is the CNIC's flagship study, and several CNIC clinical and basic research groups participate in it. The PESA study is already making seminal contributions to our understanding of the origin and progression of atherosclerosis.

The PESA-Health-CNIC-Santander study welcomed its first participant in February 2020, taking advantage of the follow-up of the PESA cohort to continue and expand the scientific approaches performed. By the end of 2021, 3481 participants had agreed to continue their participation, and more than 1000 participants had completed their first PESA-Health visit.

SECONDARY PREVENTION OF CARDIOVASCULAR DISEASE IN THE ELDERLY POPULATION

(SECURE)

Principal Investigator: ***Valentín Fuster***
Co-Principal Investigator: ***José Mª Castellano***
H2020 Grant# 633765

Adherence to treatment after 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 program. The SECURE trial (trial identifier NCT02596126) has enrolled 2499 patients over 65 years old in 7 European countries (Spain, Italy, Germany, Czech Republic, France, Poland, and Hungary) soon after an MI and randomized them to standard treatment or the CNIC Polypill. Patients have been followed up for a minimum of 2 years, and the incidence of major cardiovascular events is being assessed. The trial completed its follow-up phase by the end of October 2021, and the grant period came to an end in December 2021. The partners are now analyzing the data, and publication of the results is expected in 2022.

The SECURE Trial also has maintained links to other trials funded within the same H2020 call, resulting in a paper on barriers and potential solutions encountered in trials working with the elderly population (Age Ageing. 2021 Nov 10;50(6):1988-1996).

REMOTE ISCHEMIC CONDITIONING IN LYMPHOMA PATIENTS RECEIVING ANTHRACYCLINES

(RESILIENCE)

Principal Investigator: ***Borja Ibáñez***
H2020 Grant# 945118

Anthracyclines are a class of anticancer drugs that are used to treat many cancers. From the 4 million new cancer cases diagnosed in Europe every year, >3 million receive anthracyclines (alone or in combination). Very recent data show that >35% of patients receiving anthracyclines develop some form of cardiotoxicity. The trade-off between cancer and chronic heart failure (HF) places an immense psychological burden on cancer survivors, and for healthcare systems, the growing incidence of chronic HF is a devastating consequence of cancer treatment.

Remote ischemic pre-conditioning (RIPC) is a process in which brief, reversible episodes of ischemia followed by reperfusion in one region (e.g. an arm) render remote tissues and organs resistant to injury. RIPC is safe and effective, noninvasive, feasible, and inexpensive. For the heart, there is plenty of experimental evidence demonstrating that pigs undergoing 3 to 5 cycles of brief (5 min) limb ischemia followed by 5 min reperfusion have a degree of protection against subsequent induced myocardial infarction, with smaller infarcts than animals undergoing myocardial infarction without preceding cycles of RIPC. Recent evidence suggests that, to be protective, RIPC needs to be initiated before the index insult. Anthracycline-induced cardiopathy provides an ideal setting for testing this hypothesis because the chemotherapy is a planned procedure.

RESILIENCE is a multinational prospective proof-of-concept phase II, double-blind, sham-controlled, randomized controlled trial aimed at evaluating the efficacy and safety of RIPC in non-Hodgkin lymphoma (NHL) patients receiving anthracyclines. Patients scheduled to undergo ≥3 chemotherapy cycles and fulfilling all inclusion and no exclusion criteria will be enrolled and undergo baseline cardiac magnetic resonance (CMR) imaging and a high sensitivity troponin (hsTn) and NT-proBNP blood test. Patients with confirmed LVEF >40% by CMR will be randomized 1:1 to RIPC or simulated RIPC (Sham).

Nine weeks after finishing chemotherapy, a final CMR+ hsTn/NT-proBNP text will be performed. All patients will be followed up for clinical events at 12,18,30, and 42 months until the last patient undergoes the final CMR.

The RESILIENCE Trial aims to recruit 608 patients in 6 European countries (Spain, Portugal, France, Germany, The Netherlands, and Denmark) and is funded by the European Commission (Grant Agreement-945118-RESILIENCE). The grant period began in June 2021, with examination of the first patients expected in early 2022.

TREATMENT WITH BETA-BLOCKERS AFTER MYOCARDIAL INFARCTION WITHOUT REDUCED EJECTION FRACTION

(REBOOT)

Principal Investigator: ***Borja Ibáñez***

The prescription of beta-blockers to patients after a myocardial infarction (MI) is based on evidence from trials performed in the pre-reperfusion era. While there is solid evidence for the benefit of these drugs in post-MI patients with reduced ejection fraction, evidence is lacking for patients with a 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 is a multinational trial that will enroll 8600 post-MI patients with a left ventricular ejection fraction >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. In total, 77 hospitals in Spain and 29 in Italy are participating in this large-scale project, which will have a major impact on clinical practice.

The first patients were enrolled in October 2018, and 5771 had been recruited by the end of 2021. The first follow-up assessment has been completed in 84% of patients, the second follow-up in 58%, and the third follow-up in 3.5%.

THE TANSNIP-PESA RANDOMIZED CONTROL TRIAL: A 30-MONTH WORKSITE-BASED LIFESTYLE PROGRAM TO PROMOTE CARDIOVASCULAR HEALTH IN MIDDLE-AGED BANK EMPLOYEES. (TANSNIP)

Principal Investigator: ***Valentín Fuster***

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 healthcare purchasers. In a relatively low-risk population (the PESA-CNIC cohort), we are studying whether a personalized worksite-based lifestyle intervention driven by imaging data (2D and 3D-ultrasound of the carotid and iliofemoral arteries and 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 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, 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.

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 analysis is now complete, and the TANSNIP-PESA Study team has submitted an article for publication that is currently under peer-review.

ATHERO-BRAIN. THE HEART TO HEAD (H2H) STUDY

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

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 tested the hypothesis that extensive subclinical atherosclerosis is associated with subtle cognitive decline and beta-amyloid deposition in the brain. This transatlantic collaboration was framed within an agreement between the CNIC and Mount Sinai Hospital in New York and is led by CNIC General Director Valentin Fuster. In Spain, the H2H project was coordinated between the CNIC and Hospital 12 de Octubre. Other university hospitals (Fundación Jiménez Díaz, Clínico San Carlos, and Gregorio Marañón) participate in the project, which received funding from the Carlos III Institute of Health through the Proyecto Integrado de Excelencia program. A total of 300 participants underwent 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.

The study is now in the analysis phase.

MULTIMODALITY MYOCARDIAL TISSUE CHARACTERIZATION IN PATIENTS WITH SIGNIFICANT VALVULAR DISEASE (MRVALVE)

Principal Investigators: ***Borja Ibáñez***

The consequences of valvular heart disease (VHD) on left ventricular (LV) dimensions, function, and tissue composition are important determinants in clinical decision-making. Current practice guidelines recommend surgical treatment for patients with significant VHD when symptoms develop or when there is LV remodeling or dysfunction. The most prevalent valvulopathies are aortic valve stenosis (AS) and mitral regurgitation (MR). Transition from asymptomatic to symptomatic disease 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). The current therapies for severe VHD are surgery or percutaneous valve repair or replacement, and the decision to intervene is based on the presence of symptoms and/or gross anatomical and functional LV involvement, evident as significant chamber dilatation or reduced ejection fraction. When these features appear, it is often too late for interventions to fully restore heart function. There is therefore a need for tools for the early detection of myocardial involvement in patients with asymptomatic VHD, to enable appropriate intervention before overt deterioration of heart function. Cardiac magnetic resonance (CMR) is the gold standard for anatomical and functional cardiac assessment, including the detection of focal areas of fibrosis by late gadolinium enhancement (LGE) after contrast-gadolinium administration. Moreover, highly accurate tissue characterization is available with recent CMR advances such as parametric T1/T2 mapping, absolute myocardial perfusion quantification, extracellular volume calculation (a surrogate of diffuse fibrosis), and tagging. The assessment of focal and diffuse fibrosis requires endovenous contrast. We will use the contrast agent gadolinium, which has a superior safety profile and is in routine clinical use. Assessment of diffuse fibrosis also requires a blood sample for determination of the hematocrit. For the study of active deformation of the LV myocardium, the best imaging modality is strain echocardiography, which can detect impaired multidirectional strain (active deformation) even when overall LV function is preserved. We will correlate the imaging data with functional data from the 6-minute walking test, which provides an objective assessment of functional exercise capacity. The amount and extent of calcium deposition in the coronary arteries and heart valves will be assessed by cardiac computed tomography, a noninvasive method that gives the calcium score, a diagnostic and prognostic tool in AS patients. This project will use a multimodality imaging approach (CMR plus strain echocardiography) to better characterize LV status in patients with significant VHD, whether AS (a paradigm of LV pressure overload) or MR (a paradigm of LV volume overload).

So far 62 patients have been recruited, and 34 have completed the one-year follow-up visit.

ß3-AGONIST TREATMENT OF CHRONIC PULMONARY HYPERTENSION SECONDARY TO HEART FAILURE: PHASE-2 PLACEBO-CONTROLLED RANDOMIZED CLINICAL TRIAL (SPHERE- HF)

Principal Investigator: ***Ana García Álverez***
Co-Principal Investigator: ***Valentín Fuster***

Pulmonary hypertension (PH) secondary to left heart disease (group 2 PH) is the most common form of PH and currently lacks effective therapy. CNIC researchers have identified the β3 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 β3-selective agonist (trial identifier NCT02775539 and Nº EudraCT: 2016-002949-32). A total of 81 patients have been recruited in four Spanish hospitals and have been followed under treatment for 4 months. The study endpoints are pulmonary artery hemodynamics and the CMR profile.

In 2021, we completed the inclusion and follow-up of all study participants (n=81). Images from the participating hospitals were sent to the CNIC (Imaging Core Laboratory), where blinded analysis was performed of echocardiography, cardiac magnetic resonance (CMR), and computed tomography (CT) studies performed at baseline (V0) and at the end of study follow-up (V8). Results are currently being analyzed by Dr. Ana García-Álvarez's group at Hospital Clínic.

NOVEL MITOCHONDRIA-TARGETED THERAPIES FOR CANCER TREATMENT-INDUCED CARDIOTOXICITY (MATRIX)

Principal Investigator: ***Borja Ibáñez***
ERC Consolidator Grant#819775

The MATRIX Project aims to develop new and innovative treatments for cardiac toxicity associated with some cancer treatments. MATRIX will be jointly run at the CNIC and Fundación Jiménez Díaz (FJD) University Hospital within a collaborative framework established in 2015 to study myocardial diseases.

Great advances in the treatment of cancer—a disease with 4 million new diagnoses every year in Europe—sometimes come with a 'toll' to pay in the form of major adverse effects. One of the most common adverse effects is myocardial toxicity, which affects up to 25% of patients treated with the common anticancer drugs anthracyclines or trastuzumab. The cardiotoxic effects of these drugs can be very serious and condemn the cancer survivor to chronic heart failure or even death from this complication.

Cancer treatment-induced cardiotoxicity (CTiCT) can result in severe heart failure. The trade-off between cancer and chronic heart failure places an immense personal burden on patients, with physical and psychological consequences. Current therapies for CTiCT are suboptimal, featuring poor early detection algorithms and nonspecific heart failure treatments. Our recently published results and additional preliminary data indicate that CTiCT is associated with altered mitochondrial dynamics, triggering cardiomyocyte metabolic reprogramming. MATRIX adopts a holistic approach to tackling mitochondrial dysfunction in CTiCT. We propose that early-stage CTiCT could be reverted by metabolic reprogramming to shift mitochondrial substrate utilization. By refining a novel imaging-based algorithm recently developed by our group, we will achieve very early detection of myocardial damage in patients treated with commonly prescribed cancer therapies, long before clinically used parameters become abnormal. Such early detection, not available currently, is crucial for early therapeutic intervention. We also hypothesize that in end-stage CTiCT, mitochondrial dysfunction has passed a no-return point, and the failing heart will only be rescued by a strategy to replenish the myocardium with fresh healthy mitochondria. This can be achieved with the radical new therapeutic option of in-vivo mitochondrial transplant. The MATRIX project has broad translational potential, including a new therapeutic approach to a clinically relevant condition, the development of technology for early diagnosis, and advances in knowledge of basic disease mechanisms.

Patient recruitment began in 2020, and by the end of 2021 we had already hosted 31 participants.

EARLY IMAGING MARKERS OF UNHEALTHY LIFESTYLES IN ADOLESCENTS (ENIGMA)

Principal Investigator: ***Rodrigo Fernández Jiménez***
PI19/01704 ISCIII Grant

The alarming increase of unhealthy lifestyles in adolescents is a societal threat. Early and effective health promotion strategies are desperately needed, as well as noninvasive tools to detect individuals showing very early stages of subclinical disease who may benefit from more intensive prevention approaches. The main objectives of this project are 1) to identify early adverse vascular and cardiac subclinical changes in adolescents by cardiovascular magnetic resonance (CMR) and relate these changes to lifestyle patterns; 2) to assess the efficacy of a school-based intervention to promote cardiovascular health among adolescents and improve vascular and cardiac imaging parameters; and 3) to use CMR data to provide reference ranges for cardiac and vascular structure and function in adolescents.

The EnIGMA project, funded by the Fondo de Investigación Sanitaria of the Instituto de Salud Carlos III (PI19/01704), takes advantage of an already running cluster-randomized controlled trial in which we successfully recruited 24 Spanish public secondary schools (n=1326 adolescents) in 2017 to perform state-of-the-art CMR imaging of the heart and thoracic aorta in adolescents aged 15-17 years. In this trial, schools were 1:1:1 randomized to receive a short-term (2-year) educational program to promote health, a long-term (4-year) health-promotion program, or the usual curriculum (control). Participant assessment scheduled at baseline and at 2- and 4-year follow-up includes anthropometry, bioelectrical impedance, blood pressure, glucose and lipid profile, accelerometry, and the completion of lifestyle questionnaires. For the EnIGMA project, a subset of age- and sex-matched participants (n=123) have undergone a multi-territory multi-parameter CMR imaging study. This unique setting allows us to study associations between health factors, behaviors, and early imaging markers of subclinical disease.

PROSPECTIVE REGISTRY TO VALIDATE A NEW DIAGNOSTIC MARKER IN PATIENTS WITH CLINICAL SUSPECT OF MYOCARDITIS

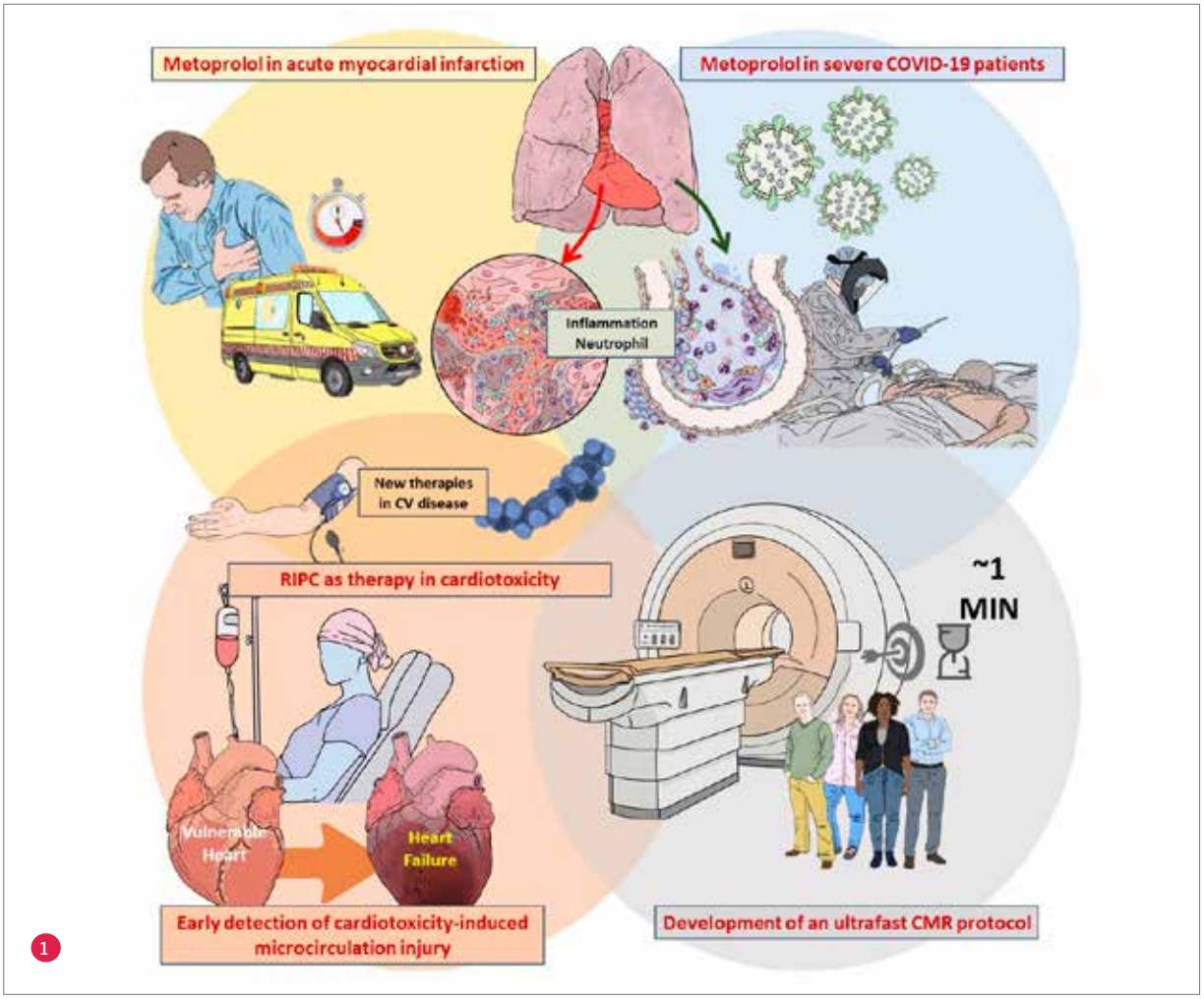
(MYOCARDITIS-CNIC)

Principal Investigator: ***Mª Pilar Martín Fernández***
Co-Principal Investigator: ***Domingo Pascual Figar***

Acute myocarditis is difficult to diagnose because of its varied clinical presentation and the lack of rapid, accessible, and accurate diagnostic methods. The nonspecific symptoms of acute myocarditis include atypical chest pain, suggesting pericarditis or angina, dyspnea, asthenia, palpitations, syncope, and even sudden death or shock. The difficulty of reaching an early diagnosis of myocarditis results from its heterogeneous presentation and the variability and lack of specificity of the findings in the usual tests (ECG, echocardiography, and laboratory tests).

Diagnosis of acute myocarditis typically requires either endomyocardial biopsy, which is invasive, or cardiovascular magnetic resonance imaging, which is not universally available, so there is a clear need for additional approaches. Dr. Martín Fernández's group has identified a novel microRNA in mice and humans with myocarditis; the team's research shows that the human homolog (hsa-miR-Chr8:96) can be used to distinguish patients with myocarditis from those with myocardial infarction (N Engl J Med. 2021 May 27;384(21):2014-2027).

In the MYOCARDITIS-CNIC Registry, run by the CNIC in collaboration with the Hospital Virgen de la Arrixaca, several Spanish hospitals (including Hospital de la Princesa and Clínica Universitaria de Navarra) will collect clinical data and biological samples from patients attending the emergency department with clinical signs of myocarditis. These data will provide valuable information on the early onset of myocarditis and will help in the validation of early clinical biomarkers.



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