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European Society of Cardiology The European Heart House 2035 Route des Colles, Les Templiers B.P. 179, 06903 Sophia Antipolis, France

Object: "ESC First Contact Initiative Grant" outcome report

To whom it may concern,

My name is María Galán and I was awarded with the "ESC First Contact Initiative Grant" in March of the present year. First of all I would like to thank the ESC council of basic science for allowing me to broaden my horizon in cardiovascular research and to set up a close collaboration with the host laboratory to further develop a research line focused on cardiovascular remodeling and fibrosis, processes deeply involved in heart failure.

I am writing to report the outcome of my grant as a researcher of the Sant Pau Biomedical Research Institute at the Hospital de la Santa Creu i Sant Pau in Barcelona. I have used the grant to visit Dr. Christoph Maack's Lab from October 12th to October 30th, 2016, in the Klinik für Innere Medizin at the Universitäts klinikum des Saarlandes \Box in Homburg, Germany. Dr. Maack has a long-standing expertise in the mechanisms of mitochondrial function and dysfunction in heart failure and in particular, the mechanisms that lead to oxidative stress in this disease. In Dr. Maack's laboratory they manage techniques that cover fluorescence microscopy coupled to the patch-clamp technique or electrical field stimulation and also the analysis of force generation in single cardiac myocytes.

This report describes briefly the guidelines of the research program we achieved. During my stay I got acquainted with the experimental procedures to measure mitochondrial activity and reactive oxygen species generation in myocytes. To achieve this aim I proceeded with the isolation of mitochondria from freshly isolated hearts of adult mice and measured mitochondrial respiration and activity through the

monitoring of Ca^{2+} concentration, NADPH and H_2O_2 production by fluorescent recordings.

To monitor Ca^{2+} mitochondrial concentration, freshly isolated myocytes were loaded with the cell-permeable Ca^{2+} indicator rhod-2-acetoxymethyl esther (rhod-2M, Invitrogen), which locates primarily to mitochondria. Also, the cardiomyocytes were loaded with the H_2O_2 -sensitive 5-(-6)-choromethyl-2′,7′-dichlorohydrofluorescent diacetate (CM-DCFDA, Invitrogen), which also locates to mitochondria. The autofluorescence of NAD(P)H was monitored in parallel. Additionally, assays of field stimulation in failing (stimulation with isoproterenol) and nonfailing myocytes to record H_2O_2 formation by CM-DCF fluorescence were performed.

The techniques that I have learnt will be implemented at my current institution, not only in cells from heart tissue but also in primary cultures of vascular cells. During my stay we have set the basis for future collaborations to run a study about mitochondrial stress in ventricular myocytes of a transgenic murine model overexpressing human lysyl oxidase (LOX) in heart, an enzyme highly expressed in failing hearts from patients. We agreed to achieve future experiments together using these transgenic animals, bred and housed in our animal quarters, with the purpose of studying how LOX transgenesis affects the cardiomyocyte and mitochondrial function under pathological conditions such as cardiac hypertrophy and heart failure after Ang II infusion or aortic banding. I already tested the deleterious effects of LOX overexpression on cardiac function in preclinical studies and my long-term aim is to detect novel molecular markers for a better prognosis of the evolution of patients suffering from hypertensive cardiomyopathy in a panel of cardiac tissue samples and plasma of patients who suffered from heart transplantation. This prospective study, in cooperation with Dr. Maack's research group, will allow us to determine the expression profile of proteins related with extracellular matrix disturbances and mitochondrial stress when compared with samples from healthy donors.

In summary, the First Initiative Contact grant has led me to establish contact with a successful European research group specialized in the pathophysiology of heart failure and has allowed me to take new challenges in the field. Thus, I would like to express my most sincere thanks to the ESC for giving me this great opportunity.

Yours faithfully,

Dr. María Galán