To the ESC Council on Basic Cardiovascular Science
First Contact Initiative Grant

Recipient:
Charlotte FARAH, PhD

Current Institution:
Group of Prof. Jean-Luc Balligand
Pole of Pharmacology and Therapeutics (FATH)
Institute of Experimental and Clinical Research (IREC)
Catholic University of Louvain,
Brussels, Belgium

Host Institution:
Group of Prof. Jolanda van der Velden
Department of Physiology
Amsterdam Cardiovascular Sciences (ACS)
VU University Medical Center (VUmc)
Amsterdam, the Netherlands

Dear Council Members,

I would like to kindly thank the ESC Council on Basic Cardiovascular Science for awarding me the 2017 FCIG funding. It offered me a privileged opportunity to acquire a new advanced and specific technical skill, in a leading laboratory internationally recognized for its expertise in the field. To join the Prof. J. van der Velden's group for 5 consecutive weeks was a rewarding experience, as such for the training and the acquisition of the experimentations than for the scientific personal enrichment and my inclusion in the European network of basic cardiovascular sciences research.

For my personal research career, having learned the evaluation of the sarcomeric properties on permeabilized cardiomyocyte allows me to complete and improve my cardiac physiologist profile, extending my expertise of the cardiac function evaluation to the sarcomeric level. Moreover, this collaboration reinforced my specialization field on the Ca^{2+} signaling alterations that underlie dysfunction of the contractile apparatus in cardiac diseases.

Finally, the establishment of a stable collaboration between the FATH laboratory (UCLouvain University, Brussels, Belgium) and the ACS laboratory (VUmc, Amsterdam, Netherlands), initiated by the FCIG funding, has allowed the elaboration of an innovative research project combining the complementary expertise and knowledge transfer of both laboratories.

Your sincerely,

Charlotte Farah

ESC FIRST CONTACT INITIATIVE GRANT 2017 – REPORT.

1. Scientific background of the basic research scientific project

Heart failure with preserved ejection fraction (HFpEF) is primarily characterized by a diastolic dysfunction despite the maintenance of a normal systolic function (ejection fraction >50%). Among the morphological and physiological alterations related to the aetiology of the disease, the increase of myofilament Ca^{2+} sensitization, responsible for an altered cardiomyocytes relaxation, has been demonstrated as a key trigger of diastolic dysfunction. This mainly results from a hypo-phosphorylation of sarcomeric protein troponin I (TnI), myosin binding protein C (MyBPC) and titin (Hamdani *et al*., Cardiovasc Res 2013; Circ. Heart Fail. 2013; Rosas *et al*., Circ. Heart Fail. 2015), associated with a reduction of protein kinase G (PKG) activity (Heerebeek *et al*., Circulation 2012). Impairment of myocardial relaxation in HFpEF is also induced by an increase of left ventricular stiffness, interstitial fibrosis and capillary rarefaction (Mohammed *et al*., Circulation 2015), linked to the development of left ventricular concentric hypertrophy remodeling.

Otherwise, β3-adrenergic receptors (β3-AR) are coupled to the NOS/cyclic GMP and downstream PKG signaling, which regulates diastolic function especially through the decrease of myofilament Ca^{2+} sensitivity by TnI phosphorylation (Lee *et al*., BRC 2010; Cawley *et al*., A.J.P. Heart Circ. Physiol. 2011) and the modulation of myocardial stiffness by titin phosphorylation (Krüger *et al*., Circ. Res. 2009). Intriguingly, Balligand and co-workers showed in a transgenic mouse model with cardiac myocytes-specific human β3-AR expression that stimulation of β3-AR signaling inhibits cardiac hypertrophic remodeling in response to neuro-hormonal or hemodynamic stress through a NOS/PKG mediated mechanism (Belge *et al*., Circulation 2014), and prevents cardiac fibrosis (Hermida *et al*., Eur. Heart J. 2018). Thus, we propose that activation of a "relaxation" pathway *via* the stimulation of β3-AR may provide benefit on diastolic dysfunction in HFpEF by lowering myofilament Ca^{2+} sensitivity. Further, we expect that β3-AR stimulation will prevent or delay hypertrophic remodeling.

2. First Contact Initiative Grant application

To successfully complete the proposed research project, a key point of the study is the exploration of the sarcomere Ca^{2+} sensitivity and intrinsic properties of myofilaments contractility and relaxation (i.e. force generation, diastolic stiffness, cross-bridge interaction on isolated permeabilized cardiomyocytes), performed in collaboration with the group of Prof. J. van der Velden, internationally recognized for its expertise in the field.

The FCIG offered the opportunity to **i)** establish the collaboration between the Prof. J. van der Velden's group (Physiology Department, Amsterdam Cardiovascular Sciences (ACS), VU Medical Center - Amsterdam, Netherlands) and Prof. J-L. Balligand's group (Pole of Pharmacology and Therapeutics (FATH), Institute of Experimental and Clinical Research, Catholic University of Louvain, Brussels, Belgium), **ii)** allow me to train for and perform the experimental assessment of myofilaments Ca^{2+} sensitivity and intrinsic properties of myofilaments contractility and relaxation on isolated permeabilized cardiomyocytes within the ACS laboratory.

3. First Contact Initiative Grant funding

The FCIG funding was used to support my transport costs and accommodation for 5 full weeks of experimentation in the ACS laboratory in Amsterdam from 2017.11.06 to 2017.12.08.

4. Scientific results outcomes of the First Contact Initiative Grant

Material and methods. The evaluation of myofilaments intrinsic properties and Ca^{2+} sensitivity is performed on isolated permeabilized cardiomyocytes from frozen cardiac tissue. Briefly, left ventricular frozen sample is crushed in Triton X100 (0.5%) containing solution, allowing to isolate single cardiomyocytes with membrane permeabilization. Thus, owing to the permeabilized membrane, the evaluation of the mechanical properties of the "skinned" cell mainly reflects the intrinsic properties of myofilaments, depending on sarcomeric proteins structure and interactions (i.e. especially titin stiffness and actin-myosin cross-bridges). Once obtained, the permeabilized cardiomyocyte is glued to a force transducer and a needle associated-stretching piezoelectric motor. Sarcomeric passive tension is assessed by measuring the opposition force developed by the cell in response to sarcomere length stretching from 1.8µm to 2.2µm in a relaxing solution, and myofilament Ca^{2+} sensitivity is assessed by measuring the force produced, at sarcomere length of 2.2µm, depending of various Ca^{2+} concentrations (from pCa 6 to 4.5, with pCa = -log ($[Ca^{2+}]$). The normalization of the force developed at each pCa to the maximal force allows to obtain a relative force – pCa curve. The pCa value corresponding to the half of maximal force (= pCa50) reflects the calcium sensitivity of myofilament. An increase of myofilament Ca^{2+} sensitivity is thus reflected by a left-shift of the relative force – pCa curve, while a decrease of myofilament Ca^{2+} sensitivity is reflected by a right-shift of the curve.

Results. As part of the FCIG funding, the aim was to characterize the basal intrinsic myofilaments properties of cardiomyocytes isolated from a transgenic mouse model expressing the human β3-AR targeted on cardiac myocytes (hβ3-AR TG**),** compared to myocytes isolated from homologous wild type (WT) mice. The evaluation of the sarcomeric passive tension (Fig. 1A), sarcomere contractile force (Fig. 1B) and myofilaments Ca^{2+} sensitivity (Fig. 2C) did not reveal any difference between hβ3-AR TG mice and the WT mice in baseline condition. Such results are in accordance with the similar *in vivo* cardiac function and morphology assessed by transthoracic echocardiography between the two groups (data not shown).

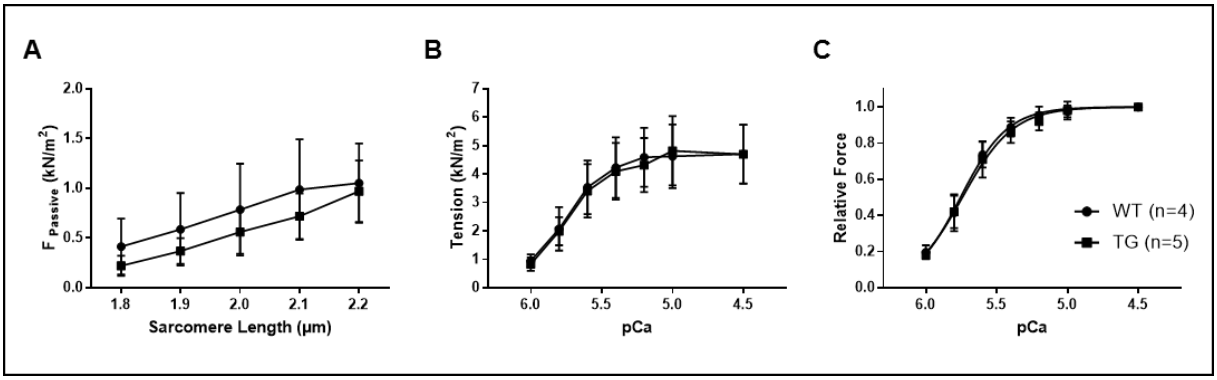

Figure 1: Evaluation of myofilaments intrinsic properties in membrane permeabilized cardiomyocytes from hβ3-AR TG mice (TG) and homologous wild type (WT) mice in baseline. **A**: Assessment of myofilaments passive tension measured at sarcomere length (SL) from 1.8 to 2.2 µm. **B**: Assessment of absolute force–Ca^{2+} relation at SL 2.2 µm. **C**: Evaluation of the relative force-Ca^{2+} relation at SL 2.2 µm, indicating no difference in the myofilaments sensitivity to $[Ca^{2+}]$ between groups. Experimentations were performed on n=4 WT mice (15 cells/group) and n=5 TG mice (18 cells/group). Results are expressed as mean ± s.d.

Perspectives. The perspective of the project, initiated by the FCIG funding, is to investigate if stimulation of the β3-AR pathway (hβ3-AR TG mice) could prevent and/or delay the alterations of myofilaments intrinsic properties associated with the HFpEF-like phenotype, obtained in mice by the combination of surgical transverse aortic constriction and DOCA pellet implantation (Methawasin *et al*., Circulation 2016).