Research Networking Programmes

Short Visit Grant ☒ or Exchange Visit Grant ☐

(please tick the relevant box)

Scientific Report

The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.

Proposal Title: Mechanisms Underlying The Increased Risk of Atrial Fibrillation In Aldosteronism

Application Reference N°: 6475

1) Purpose of the visit

The aims of my visit at the Centre de Recherche des Cordeliers were 1) to discuss a research project focussed on the molecular mechanisms underlying atrial fibrillation (AF) and 2) to develop a collaboration between the Department of Medicine–DIMED of the University of Padua and the team headed by Prof. Frederic Jaisser.

AF is the most common arrhythmia, and some evidences suggest that the patients with hyperaldosteronism have an increased AF risk (Milliez P., 2005). The ongoing PAPPHY (Prospective Appraisal of the Prevalence of Primary aldosteronism in HYpertensive patients presenting with non valvular atrial fibrillation) Study, by exploiting a research network involving European referral centres for hypertension, is expected to clearly establish the AF prevalence in the patients with primary aldosteronism and assess the predictors in such patients (Rossi G.P., 2013). However, the molecular mechanisms underlying the increased AF risk in aldosteronism would remain unexplored. The project that I proposed to Prof. Jaisser was aimed at clarifying this issue and, in particular, at identifying the genes triggered by

aldosterone that may affect atrial contractility and remodelling, thereby favouring AF onset.

References
Milliez P. et al. J Am Coll Cardiol 2005; 19: 1243-1248
Rossi G.P. et al. J Hum Hypertens 2013; 27: 158-163

2) Description of the work carried out during the visit

During my short visit from July 7th to July 18th I discussed my proposal with Prof. Frederic Jaisser and planned some preliminary experiments to evaluate if some genes of interest are expressed in the atrial tissue. Moreover, the exchange program also allowed me to visit the laboratories and facilities at the Centre de Recherche des Cordeliers, and to interact with Prof. Jaisser's team by discussing the ongoing projects, exchanging information and taking advantages of reciprocal expertise.

Research Proposal

Some years ago Jaisser and coll. showed that MR-Cardio mice, a transgenic model with cardiomyocyte-targeted mineralocorticoid receptor (MR) over-expression, exhibited prolonged ventricular repolarisation associated with severe ventricular arrhythmias (Ouvrard-Pascaud A., 2005). More recently, by exploiting a microarray-based technique, they found that a not negligible number of genes in the left ventricles, among the 865 genes differentially regulated between MR-Cardio and wild type mice, coded for ion channels and cell-to-cell junction proteins (Messaoudi S., 2013). Moreover, the infusion of aldosterone in MR-Cardio mice, even at a dose that did not increase blood pressure, modified the gene expression of other ion channels (Messaoudi S., 2013). Taken together, these data provided evidence that both MR and aldosterone affect the gene expression of some ion channels in the ventricular tissue. Whether such genes are expressed in the atria and are regulated by aldosterone and, moreover, whether the up-regulation in the atria favours the onset of atrial fibrillation remains to be established.

The hypothesis underlying the present research proposal is that aldosterone, by affecting the expression levels of genes coding for ion channels and cell-to-cell junction proteins in the atria, induces changes in the electrical properties of the atrial chambers that in turn favour remodelling and atrial fibrillation. To test such hypothesis, the study is aimed at answering the specific questions:

- What genes are aldosterone- and/or MR-regulated in the atria?

• Do aldosterone and/or MR per se induce changes in atrial contractility and remodelling thereby predisposing to AF? Are atrial changes associated with hemodynamic ventricular abnormalities?
• Does aldosterone induce changes in ion permeability and excitability of atrial cells?

The experimental approach will include 1) transcriptome analysis of atrial tissue and real time RT-PCR as validation test; 2) protein expression analysis and also histomorphometry for cell-to-cell junctions, 3) high-resolution echocardiography, EKG and blood pressure telemetry, 3) patch-clamp experiments in myocardiocytes isolated from the atria.
Three models will be used: MR-Cardio mice, aortic coarctation-induced hypertensive rats and aldosterone-infused Wistar rats. Short-term (7 days) and long-term (4 weeks) treatments with a MR antagonist will be also planned.

Preliminary Experiments

Moving from the data obtained from Jaisser's and coll., which showed that some genes coding for ion channels are differentially regulated in the ventricles of MR-Cardio mice when compared to the wild-type mice (Messaoudi S., 2013), we have planned preliminary experiments to evaluate whether such genes are expressed in the atrial tissues and, if so, whether they are differentially regulated in the atria of MR-Cardio mice (vs. wild-type). We therefore focussed our attention on
• Kcnk1, which codes for TwiK1, two-pore domain K+ channel that regulates leak K+ conductance;
• Kcnmb4, which codes for the beta subunit of the seven trans-membrane domains BK channels;
• Trpc4 gene, which codes for TRPC4, the transient receptor potential cation channel, subfamily C, member 4.

Of interest, in the ventricular tissue, Kcnk1 gene was found to be MR-regulated (differentially expressed between MR-Cardio mice and wild type), Trcp4 was both aldosterone- and corticosterone-regulated (differentially expressed between aldosterone-treated MR-Cardio mice and untreated MR-Cardio mice, and also up-regulated by corticosterone), and Kcnmb4, which was identified after crossing the subset of MR-regulated genes with the subset of aldosterone-regulated genes in the MR-Cardio mice, was up-regulated in MR-Cardio mice and further enhanced by aldosterone administration (Messaoudi S., 2013).

Previous studies showed that all three channels (KCNK1, BK and TRP) are expressed in the cardiac tissue (Gaborit N., 2007; Poulsen A.N.,

2009; Ju Y-K., 2007), and some data suggest a possible role in the development of arrhythmias:
• Kcnk1 was found to play a role in maintaining the resting membrane potential of many cell types, and a higher expression was observed in the human atria and Purkinje fibers than in the working ventricles (Gaborit N., 2007).
• BK channels were hinted to play a role in the heart rate regulation and cardioprotection from ischemic injury (Poulsen A.N., 2009). However, no data are available on the expression of the beta subunit coded by Kcnmb4 in the atria.
• The channels coded by the Trp gene family may regulate the pacemaker firing in the heart (Ju Y-K., 2007), but data for the TRP4 channel in the atria are lacking.

References
Gaborit N. et al. J Physiol 2007; 58: 675-693
Ju Y-K et al. Circ Res. 2007; 100: 1605-1614
Messaoudi S. et al. Hypertension 2013; 61: 361.367
Ouvrard-Pascaud A. et al. Circulation 2005; 111: 3025-3033
Poulsen A.N. et al. Biochim Biophys Acta. 2009; 1788: 380-389

3) Description of the main results obtained

1. During my short visit I discussed my research proposal with Prof. Jaisser. Because the project needs a multidisciplinary approach and many efforts to pursue the specific goals, Prof. Jaisser suggested me to focus the attention not only at the experimental aims, but also on the feasibility of the project and logistic aspects. Hence, we planned some preliminary experiments and finalized some critical issues.

2.The preliminary experiments are aimed at investigating whether the genes found to be MR- and/or aldosterone-regulated in the MR-Cardio mice are also expressed in the atria and, if so, whether they are differentially regulated as in the ventricles (for details see section 2). After isolating left and right atria from the heart of wild type mice under a stereomicroscope, RNA was extracted following a standard protocol and checked for its quality. Expression levels of Kcnk1, Kcnmb4 and Trpc4 genes will be measured with real time RT-PCR.

3. Taking advantages of the expertise of Prof. Jaisser and his team, I enhaced my knowledge on the MR regulation in the cardiac tissue.

4) Future collaboration with host institution (if applicable)

At the end of the ENS@T-granted short visit Prof. Jaisser gave me the opportunity of visiting his laboratories again in August to clarify some methodologic aspects of the project and plan the next experiments. The collaboration with Prof. Jaisser's team is expected to be continued at least until the completion of the project (approximately 12-15 months).

5) **Projected publications / articles resulting or to result from the grant *(ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant)***

Publications are expected at the end of the project.

6) Other comments (if any)

I am grateful to Prof. Frederic Jaisser and all his collaborators for their kind suggestions and fruitful criticisms, which greatly helped me in revising the proposal that I originally submitted to the ENS@T for the grant application. I thank the ENS@T for giving me the opportunity of visiting one of the most renowned Research Centre of excellence in the world.