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First Contact Initiative Grant Report

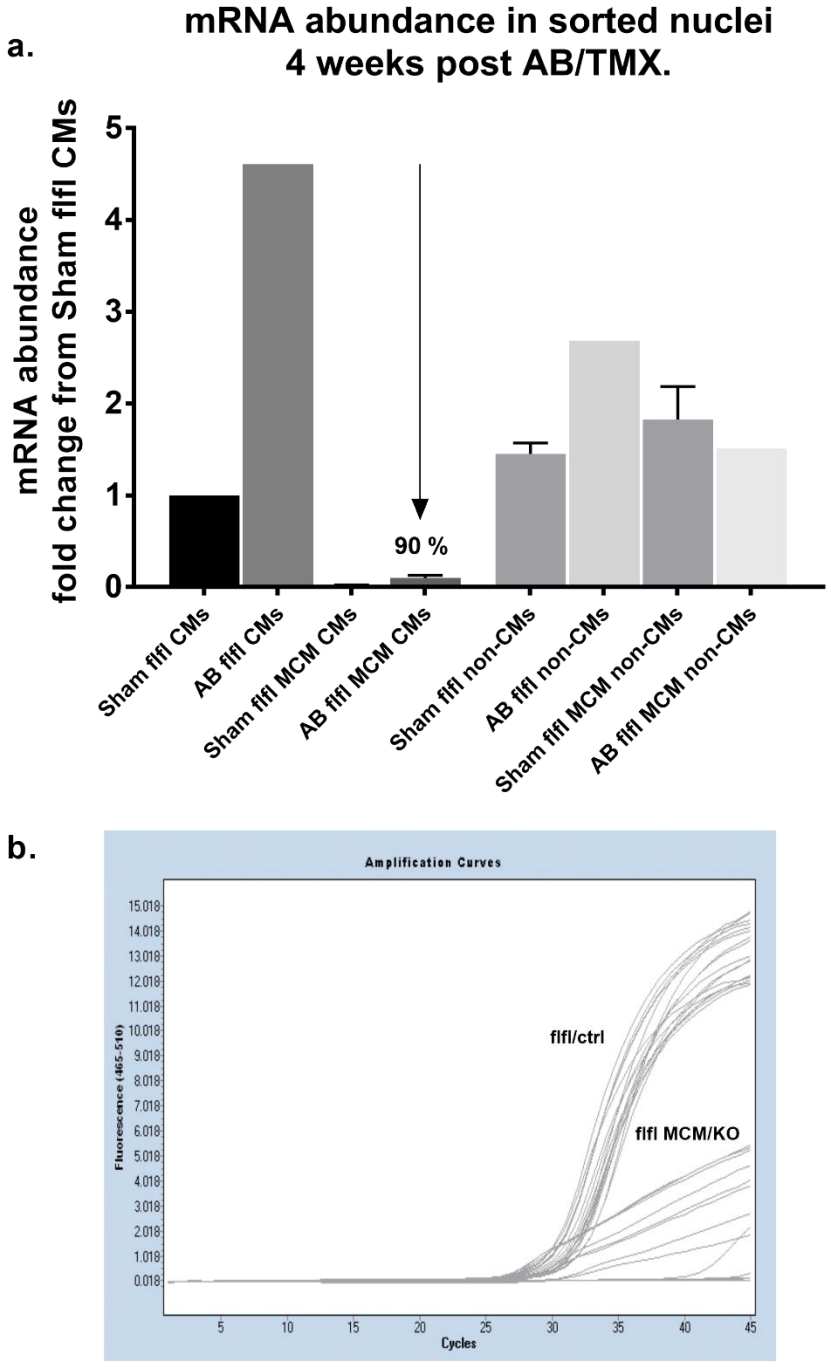
I would like to thank the European Society of Cardiology for awarding me with this grant in autumn 2016.

It provided me with the opportunity to visit the laboratory of Ivar Sjaastad at the Institute for Experimental Medical Research at Oslo University. Under the supervision of Jan Magnus Aronsen, I had the opportunity to learn from the extensive expertise and skills in the host laboratory. This included being trained in the surgical technique of ascending aortic banding in small rodents along with intrathoracic injections for AAV overexpression in vivo studies and tamoxifen injections for inducible Cre recombinase activation to drive inactivation of target gene with loxP sites.

αMHC-MCM mice with loxP sites flanking a target gene of interest, for which a role in cardiac pathological and ageing-associated remodelling has been indicted by preliminary data in our home laboratory, had been breeding over the last year in preparation for these key cardiac-specific knockout experiments. Importantly in the field of experimental cardiology, the host laboratory have been involved in the optimisation and validation of using a single tamoxifen injection to avoid acute cardiomyopathy that has been identified in mice undergoing a series of 3 – 5 tamoxifen injections for similar experiments (Koitabashi *et al.*, 2009, Hougen *et al.*, 2010, Bersell *et al.*, 2010). I learnt from the host lab the preparation of tamoxifen and single injection which resulted in successful inactivation of the gene, with less than 90 % of the target gene mRNA remaining by harvesting at 4 weeks post tamoxifen and isolating cardiac myocyte RNA (Figure).

I had the opportunity to learn from the expertise and technology in the host laboratory in cardiac imaging techniques. In particular, I was trained how to acquire and analyse a number of different measurements of cardiac function and physiology by 2D echocardiography using the Vevo 2100 system and Vevo LAB analysis software. This included 2D echocardiography long and short axis of the left ventricle (LV), mitral valve, pulmonary artery and LV tissue Doppler as well as assessment of the degree of constriction through measuring with colour Doppler flow through the aortic arch, pre- and post-constriction site.

These skills were used to test the cardiac function in the aforementioned cardiac specific αMHC-MCM knockout mice to test the effect of pressure-overload induced stress (aortic banding, performed in the host laboratory during the visit).



Cardiac myocyte-specific knockout of an epigenetic modifier with a single tamoxifen injection in alpha-MerCreMer mice with loxP sites within the gene to be inactivated. RT-qPCR data measuring mRNA abundance for the gene inactivated, showing less than 10 % of mRNA remaining in sham and AB operated flfl MCM mouse CMs compared with sham-operated no MCM littermates that were also tamoxifen injected. Quantified normalized RT-qPCR data for sham and AB flfl and flfl MCM CMs and non-CMs (a) and real time quantitative PCR amplification curves (b).

In addition, I was trained in intrathoracic injection of an AAV expressing the same gene that was knocked out from a minimal hTnnt2 promotor in a serotype 9 structure, optimal for cardiac expression (Bish *et al.,* 2008, Werfel *et al.,* 2014). The skills that I have gained thanks to the ESC FCI grant will also be used to test gain-of-function of the target gene for completion of the ongoing study back at the home institution. The data thus far implicates a novel molecular pathway linking pressure-overload associated stress with changes in epigenetic signature to bring about gene expression changes inciting maladaptive remodelling. Similar alterations as observed in rodents have also been validated in human LV hypertrophy. These data implicates the molecular axis investigated as a potential targetable pathway for the treatment of cardiovascular disease – which is biggest burden on human health in the developed world, and increasingly in developing countries.

From the results we have generated during the ESC FCI grant period, as well as experiments planned using the skills and techniques acquired for future experiments back at my home institution, a significant publication in the field of molecular cardiology is expected to be generated. The ESC and this grant will be acknowledged on this as a means by which this work was made possible. A further and anticipated outcome of this award is that it has strengthened collaboration and interactions between the home and host laboratories through which we will undertake a number of projects going forward.

Yours sincerely,

Emma Louise Robinson

References

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