Hamburg, 13.01.2025

Report on my research stay at Imperial College London (Mayr-Lab) within the framework of the ESC First Contact Initiative Grant

Dear members of the ESC Council on Basic Cardiovascular Science,

As a recipient of the ESC First Contact Initiative Grant 2024, I had the exceptional opportunity to undertake a three-week research stay at Imperial College London in the laboratory of Prof. Manuel Mayr (Chair of Cardiovascular Proteomics).

The aim of this visit was to deepen my understanding of proteomic methods and to apply these advanced techniques to my ongoing MD-project, which focuses on the in-vitro characterisation of a ryanodine receptor 2 (RYR2) variant associated with a left ventricular non-compaction cardiomyopathy (LVNC) and sudden cardiac death (SCD) overlap syndrome.

The EFCI grant opened up the window for this unique experience, where I was able to learn cutting-edge proteomic techniques while advancing my project and foster international collaborations.

Background:

I am a 5th year medical student currently working on my dissertation at the University Medical Centre Hamburg-Eppendorf (UKE) under the supervision of Prof. Thomas Eschenhagen. My project revolves around a novel RYR2 variant (RYR2 c.5654G>A homozygous – RyR2 p.G1885E - exon37:missense) that has been identified in a large family from northern Italy. While homozygous carriers of this variant present with an overlap syndrome of LVNC and SCD, heterozygous carriers remain asymptomatic. Using patient-derived pluripotent stem cells (hiPSCs), I generated Engineered Heart Tissues (EHTs)[1] to further characterise this variant and broaden our pathophysiological understanding of misguided compaction.

So far, my experiments revealed functional impairments in EHTs with the homozygous RYR2 variant, including abnormal force development, reduced inotropic responses to isoprenaline and increased arrhythmogenicity. Notably, after several weeks in culture homozygous EHTs developed teratoma-like structures, in line with a progressive loss of strength. In histology, these abnormal structures display a marked increase in proliferation activity (Ki-67+). Importantly, this phenotype was absent in EHTs of an isogenic control line, which I generated from the affected homozygous line using the CRISPR/Cas 9 technology, indicating a causal relation with the homozygous RYR2 variant.

The findings are in line with recent research suggesting that the pathophysiology of LVNC may be less about “too little” compaction but has rather to do with “too much” proliferation[2,3]. Initial RNA sequencing provided some insights, but further molecular characterization was required to better understand this firstly described phenotype.

The main goal of my stay in Prof. Mayr's lab was to use proteomic methods to gain a better understanding of the molecular mechanisms driving these structural abnormalities. This

included learning sophisticated proteomic techniques, applying them to my EHT samples and integrating the results into my ongoing research.

Research Activities On-Site (Methods/Results):

On the very first day, I had the chance to present the latest results from my project in a group meeting and we set up a plan for the following three weeks. I immediately felt welcome and fully integrated into this cheerful and enthusiastic team.

The main steps can be broken down into the following three major areas:

- **Sample preparation:** In the first week, we tested various protein extraction methods on my EHT samples and assessed their suitability using BCA and Western blot analyses. Based on the results, we selected a two-step extraction method, which was implemented during the second week: **1. Tissue lysis buffer extraction:** Samples were incubated in a buffer containing protease and phosphatase inhibitors, vortexed, centrifuged, and the supernatants were stored at -80 °C ("cellular fraction"). **2. Guanidine hydrochloride buffer extraction:** Samples were subsequently incubated in a guanidine hydrochloride buffer to solubilize extracellular matrix components, centrifuged, and again supernatants were left at -80 °C ("extracellular fraction").

  The third week was dedicated to sample conditioning for mass spectrometry (MS): Proteins were precipitated with ethanol and deglycosylated enzymatically using the following enzymes:

  - Endo-α-N-acetylgalactosaminidase
  - β-N-acetylglucosaminidase
  - Endo-β1,4-galactosidase and β1,4-galactosidase
  - Chondroitinase ABC
  - α-2-3,6,8,9-Neuraminidase
  - N-glycosidase F in the presence of O18 water

  Subsequently, proteins were denatured, reduced with DTT, alkylated with iodoacetamide and digested with trypsin to produce peptides for MS-analysis.[4]

- **Mass spectrometry:** Alongside the sample preparation, I was introduced to the practical work with the Ultimate 3000 nanoflow HPLC and Orbitrap Fusion Lumos Tribrid mass spectrometer. This allowed me to expand my understanding of the latest advancements as well as the limitations of the Orbitrap Fusion Lumos Tribrid MS system.
- **Bioinformatic analysis:** Inseparable from mass spectrometry is the bioinformatic processing of the data obtained. Proteome data were analysed using the 'Proteome Discoverer' software (Thermo Scientific), whereby peptide identifications were achieved by comparing the experimental MS2 spectra with the theoretical spectra generated using UniProt database for human and bovine proteins. This is particularly important in the context of EHTs, as other species' proteins pose a risk of contamination due to culture conditions.

Progress in my doctoral project:

The proteomic analysis of my EHT samples provided valuable data on the molecular mechanisms driving the proliferative structures in homozygous RYR2 mutant tissues. Preliminary results indicate differential expression of structural and signalling proteins that may explain the phenotypic abnormalities observed in functional assays. These results will form the basis for further detailed analyses and a future publication.

Reflection/Conclusion

In summary, my research stay at Imperial College London as part of the ESC First Contact Initiative Grant was an invaluable experience. It enabled me to expand my technical and analytical skills, achieve significant progress in my research project, and to build valuable collaborations within the international scientific community. I am confident that the knowledge and skills I acquired during this time will contribute to my future career in translational cardiovascular research. My special thanks go to Prof. Manuel Mayr and Dr. Xiaoke Yin for their invaluable support and guidance during my stay, as well as to Prof. Thomas Eschenhagen for his mentorship and great support. I would also like to express my gratitude to the whole Mayr group for their welcoming and always supportive nature throughout my stay. Finally, I extend my heartfelt thanks to the European Society of Cardiology (ESC) for making this opportunity possible. I highly recommend this program to every young researcher seeking to expand their expertise and network.

Sincerely and with gratitude,

Julian Phillip Schlobohm

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Logos on the first page: www.imperial.ac.uk and www.uke.de