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ESC First Contact Initiative Grant: Final Report

Host Institute: Laboratory for Nutrition, Obesity and Thrombotic Risk
University of Marseille/INSERM, Marseille, France

Dear ESC council members,

To begin with, I would like to thank the European Society of Cardiology (ESC) for their support through the First Contact Initiative Grant which made it possible to travel to Marseille and work at the Laboratory for Nutrition, Obesity and Thrombotic Risk. This training was key to my current research project and enabled us to further study the role of SLC44A2 in venous thromboembolism (VTE) pathophysiology.

VTE is a multifactorial disease that affects millions of people worldwide and is the third leading cause of cardiovascular mortality in the industrialized world. There is a strong underlying genetic component, however, the currently identified genetic risk factors only account for approximately 5% of VTE heritability. This suggests that there are additional genetic determinants which contribute to the disease that have yet to be identified. As such, a large meta-analysis of twelve combined genome wide association studies (GWAS) aimed to identify novel small nucleotide polymorphisms (SNPs) related to VTE susceptibility (Germain et al 2015). One of these SNPs was found in the *SLC44A2* gene which was of interest, as SLC44A2 has not been previously linked to hemostasis pathways and may be a unique contributor to VTE.

The function of SLC44A2 is relatively unknown, although it has been implicated in auto-immune hearing loss. It was within this research framework that a murine *Slc44a2* deficient line (KO) was developed at the University of Michigan, and of which, we were fortunate to receive. Our goal was to study the response of the KO line in several models of venous thrombosis, and in particular, the stenosis model, which is considered to be the most representative model of deep vein thrombosis (DVT). The stenosis model is a surgical procedure in which the inferior vena cava (IVC) is partially ligated, creating a 90% reduction in blood flow and ultimately results in the formation of a thrombus below the ligature. An investigator at the University of Marseille is an expert at performing this model and is also interested in the role of SLC44A2 in disease, which formed the foundation for this research exchange.

During my training in Marseille, I learned the basics of surgery and following several trials, I was able to successfully perform the procedure and ligate the IVC. This was quite a steep learning curve as I had no previous experience performing surgery which involves several considerations such as anaesthesia, aseptic environment and tools as well as minor techniques such as suturing and the administration of analgesia. Moreover, the method itself is delicate, since venous tissue is easy to disrupt and break, lending to major blood loss and an unsuccessful treatment. I cannot thank my trainer enough for their patience and skill as an educator in teaching me this technique. Upon my return to the Leiden University Medical Center, I have ordered the tools necessary to perform the surgery in house and am currently in the process of setting it up, which will aid in the ongoing research of several lines within the department.

In addition to learning the stenosis model itself, we also carried out a pilot study. This included isolation of the formed thrombi following 48 hours, careful measurement using callipers and proper storage in OCT compound, followed by lessons on making cryosections for immunohistological examination. Subsequently, I learned their protocols for immunostaining and was able to quantify several components of the thrombus including neutrophils and markers of NETosis. We also collected blood for cell counts, plasma Von Willebrand Factor (VWF) antigen measurement and extracellular plasma DNA levels.

Remarkably, we detected an altered response by the KO group as they exhibited less severity of thrombosis with smaller thrombi formed on average. Further histological characterization of the thrombi themselves has also determined that the thrombi composition is different between the groups. These results are encouraging and suggest that SLC44A2 does play a role in VTE and further investigation is warranted. In this regard, we are continuing our collaboration to delineate the mechanism underlying these observations and have follow up studies planned for the summer.

In closing, as part of the ESC First Contact Initiative grant, I was able to travel to the University of Marseille to learn a difficult surgical procedure which can now be used within my host institute and also to perform a pilot study to obtain preliminary results with the model. Additionally, this experience was a crucial and exciting step in my career development as a junior researcher as it fostered the expansion of my professional network, generating new collaborations for future research, and also helped me to achieve my personal goal of becoming proficient in murine models of thrombosis.

I again thank the ESC research council for making this opportunity possible, in addition to the research group for hosting me and the staff at the University of Marseille who assisted in my training.

Sincerely,

Chrissta X. Maracle