Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA18211

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<u>Details of the STSM</u>

Title: Investigating the link between birth mode and child DNA methylation over the life course using ALSPAC data

Start and end date: 19/03/2023 to 26/03/2023

<u>Description of the work carried out during the STSM</u>

In working group 4 (WG4) we discussed and developed a detailed model to examine how birth mode may affect child DNA methylation across childhood and adolescence. As Dr. Jonathan Turner's lab at the Luxembourg Institute of Health has access to data from the large cohort study ALSPAC (including epigenetic data in ARIES), this STSM aimed to conduct the analyses for the research project planned within WG4 to answer the question whether different birth modes, specifically instrumental vaginal birth, emergency c-section, and elective c-section compared to non-instrumental vaginal birth, differentially affect the offspring's DNA-methylation.

During the STSM, I met with Dr. Jonathan Turner and his PhD student Cyrielle Holuka daily to discuss the data as well as the next analysis steps. I worked particularly closely with Cyrielle on the data, benefitting immensely from her far-reaching expertise in running epigenome-wide association studies and working with the ALSPAC and ARIES data. I really had the opportunity to learn a lot during my STSM and expand my horizon. I also enjoyed learning about the day-to-day running of the lab and research group in Luxembourg, where I got to attend the institute's bimonthly research talks as well as the research group's weekly update meeting.

A major task during the STSM revolved around the variables needed for the analyses. First, we had to check whether the variables we needed had been delivered and were available for analysis. This was the case for almost all the variables we needed, except for the variable on who had an instrumental vaginal birth. Second, for the available variables I identified the variables we had selected to include in our analyses and made sure they represented the question we thought they would and whether the answer format was correct. Then, we had to decide how to code them, i.e., which categories to use, and then merge them with the epigenetic data.

I really benefitted from my prior experience with large cohort study data from working with the DREAM study in Dresden. It was very interesting to see similar issues with such large data sets and great number of variables. At the same time, I felt that my experience helped me grasp better the complexity of the data set and contribute valuably to statistical discussions with Cyrielle and Jon.

During the STSM I also had the chance to become more familiar with the statistical program R which is used as the standard statistical software at LIH. Jon and Cyrielle provided me with reading on the R packages used in the analysis of epigentic data, such as DMRcate, and I conducted several online searches to solve coding problems that we encountered while running the analyses.

Taken together, a lot of my STSM was spent working on the data and deciding which statistical package and method was most suited to answering our research questions and figuring out how we can best put the analysis plan we had prepared into practice with the help of Cyrielle. As Jon pointed out, no plan survives contact with the data, and this rang true also in our case, with some adjustments having to be made after working with the data. As we did not find a significant effect of birth mode on child DNA methylation at 7 and 15 years in the ARIES cohort, we were unfortunately not able to carry out follow up moderator analyses with breastfeeding and mother-child bonding as we had originally planned. Nevertheless, our results are interesting and add important insights on the epigenetic effectsof birth mode.

Description of the STSM main achievements and planned follow-up activities

The primary aim of this STSM was to execute the analysis plan developed within WG4 to investigate the effect of birth mode on offspring DNA methylation from birth to adolescence. During my time in Luxembourg, we managed to write a detailed R script documenting the entire analysis process. We calculated the descriptive statistics for our participants and managed to compare them between participants of ARIES and ALSPAC to see whether participants differed significantly regarding the variables of interest to our study. In a second step, we managed to run epigenome wide association studies looking at the effect of birth mode on offspring DNA methylation at birth (in cord blood), at 7 years (peripheral blood), and at 15 years (peripheral blood). Here the results showed that only in cord blood did the offspring show differential methylation on two CpG sites and three differentially methylated regions. As no differentially methylated CpGs were found at 7 years and at 15 years of age, we were not able to conduct the follow-up analyses looking at whether the effect of birth mode on childhood and adolescence DNA methylation at these CpG sites depended on the duration of breastfeeding or mother-child bonding. While this was not possible, our results are meaningful in that they show that within the ARIES cohort birth mode did not seem to affect offspring DNA methylation in the long-term. As one variable we had planned to include in the analyses (namely whether the vaginal birth was instrumental or not) was not delivered in time, we were unable to check whether the results changed when taking this variable into account. We plan to add these analyses afterwards by organising online meetings with Cyrielle and Jon to further collaborate on this exciting research project.

Moreover, we are planning to publish these findings in a scientific journal. It will be important to discuss these results in terms of the limitations present in the ARIES cohort (e.g., that the number of individuals with an elective or emergency caesarean section was very small) and in terms of their meaning within the available literature. I have prepared a powerpoint presentation of the results we obtained during the STSM and hope to present this to working group 4 at the next meeting. As we have been invited to submit the findings to a journal as part of a special issue, we hope to prepare these findings in a publishable format as soon as possible. For this goal we plan to closely collaborate with Jon and Cyrielle to publish these interesting findings.