ENVIRONMENTAL PROGRAMMING OF RESPIRATORY ALLERGY: UTILITY OF A CHILD'S SPIT EPIGENOME

Sabine A.S. Langie[1,2], Matthieu Moisse[3], Katarzyna Szarc vel Szic[4], Ellen Van Der Plas[1], Gudrun Koppen[1], Sylvie Remy[1], Guy Van Camp[5], Diether Lambrechts[3]; Greet Schoeters[1,4], Wim Vanden Berghe[4] and Patrick De Boever[1,6]

[1]Environmental Risk and Health unit, Flemish Institute of Technological Research (VITO), Mol, Belgium; [2]Theoretical Physics, Hasselt University, Diepenbeek, Belgium; [3]VIB Vesalius Research Center, KU Leuven, Leuven, Belgium; [4]Department of Biomedical Sciences, University of Antwerp, Wilrijk, Belgium; [5]Laboratory of Cancer Research and Clinical Oncology, Center for Medical Genetics, University of Antwerp, Edegem, Belgium; [6]Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium

sabine.langie@vito.be

Epigenetic DNA methylation changes can be part of the underlying molecular mechanisms leading to complex diseases. Early life exposures like parental lifestyle and exposure to chemicals can alter DNA methylation patterns, and thereby predispose the child to develop respiratory allergy (RA) later in life. Longitudinal birth cohorts are instrumental to study disease development, but DNA biomarker research is hampered because blood sampling is kept to a minimum for practical and ethical reasons. Saliva is a non-invasive and convenient source of DNA that can be used for biomarker research. In this study, we aimed at discovery and confirmation of differential methylation regions (DMR) in saliva of children with RA when comparing to controls.

Saliva samples collected in the two independent longitudinal birth cohorts (Flanders Environment and Health Surveys FLEHS1 & FLEHS2) were analysed using Illumina Methylation 450K BeadChips. A statistical analysis pipeline was developed in R to identify genome-wide differential methylation. We identified 27 DMRs in saliva from 11y old allergic children (self-reported/doctor's diagnosed RA, Phadiatop IgE $\geq$ 0.35 kU/L; N=26) vs. controls (no self-reported/diagnosed RA, Phadiatop IgE< 0.35 kU/L; N=20) in the FLEHS1 cohort. A set of 8 DMRs was selected for further validation by iPLEX MassArray analysis. First, iPLEX analysis was performed in the same 46 FLEHS1 samples that were previously analysed on the 450K methylation arrays, to allow technical validation. iPLEX results correlated significantly with the 450K methylation array data ($P<0.0001$), though iPLEX analysis confirmed 5 of the 8 identified DMRs in the FLEHS1 study.

Aiming for biological confirmation, we studied these DMRs in an independent birth cohort FLEHS2. Due to a lack of blood samples to measure IgE levels in the FLEHS2 cohort, cases and controls were identified as: 1) cases = doctor's diagnosed/self-reported RA symptoms ever (N=19); and 2) controls = no self-reported/diagnosed RA (N=20). When studying the 8 DMRs by means of iPLEX analysis in the FLEHS2 cohort, only a DMR in the *GLI2* gene showed a statistically significant difference in methylation between RA cases and controls. GLI2 has a regulating role in IL4 signalling and can modulate T-helper differentiation and allergic disease, and might thus be an interesting DNA methylation marker to study for further biomarker development.

Interestingly, the RA-related hypermethylation in *GLI2* correlated significantly with life time exposures towards air pollution markers PM_{10}, NO_2 and O_3. Using the statistical framework developed by Valeri and VanderWeele (*Psychol Methods, 2013*), *GLI2* hypermethylation was observed to partially mediate the effects of PM_{10}, NO_2 and O_3 on RA.

This project is providing novel insights in the molecular mechanisms that may predispose children to RA development. We are among the first to show the utility of saliva to identify DNA methylation marks in children that are relevant for RA.