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FACTSHEET N°3

May 2016

EXPOsOMICS has received funding from the European Union's Seventh Framework Program under grant agreement No. 308610

SEVENTH FRAMEWORK PROGRAMME

This issue of the factsheet features two main achievements of the project. Piscina is one of the first studies to have been completed in the project, and suggestive associations between metabolomics, transcriptomics, proteomics and exposure to water disinfectants are shown. The next steps will involve better annotation of the signals, pathway analysis, cross-omics and the study of the relationship of omic features with outcomes such as genotoxicity.

The second story in this factsheet is a new method developed in Maastricht to extract good quality RNA from blood stored in liquid nitrogen for many years, like in the large cohort studies we are currently using for our environmental epidemiological investigations. This new method is certainly of interest for all epidemiologists and laboratory scientists working in similar projects.

Paolo Vineis, Imperial College London

The PISCINA Study

WATER CONTAMINATION

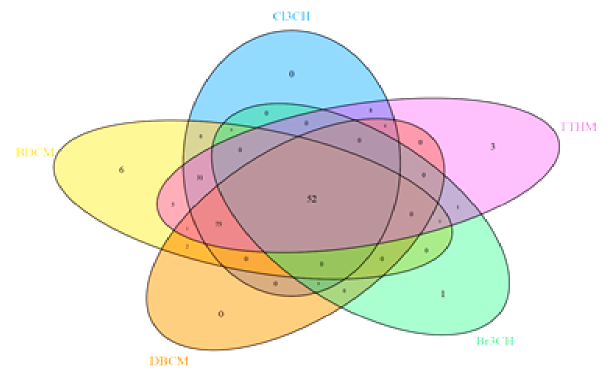
- Cross– over study—indoor chlorinated pool
- Subjects: 120 volunteers (18-40y non-smokers, non-professional swimmers) swam for 40 minutes;
- Untargeted metabolomics of blood samples (n=60) collected before and 2h after swimming was conducted using a UHPLC-QTOF mass spectrometer operated in ESI positive mode.
- Whole genome expression analyses using Agilent 8x60K Whole Human Genome microarray technology performed on blood samples collected before and 2h after swimming (n=41)

Karin van Veldhoven, Imperial College London

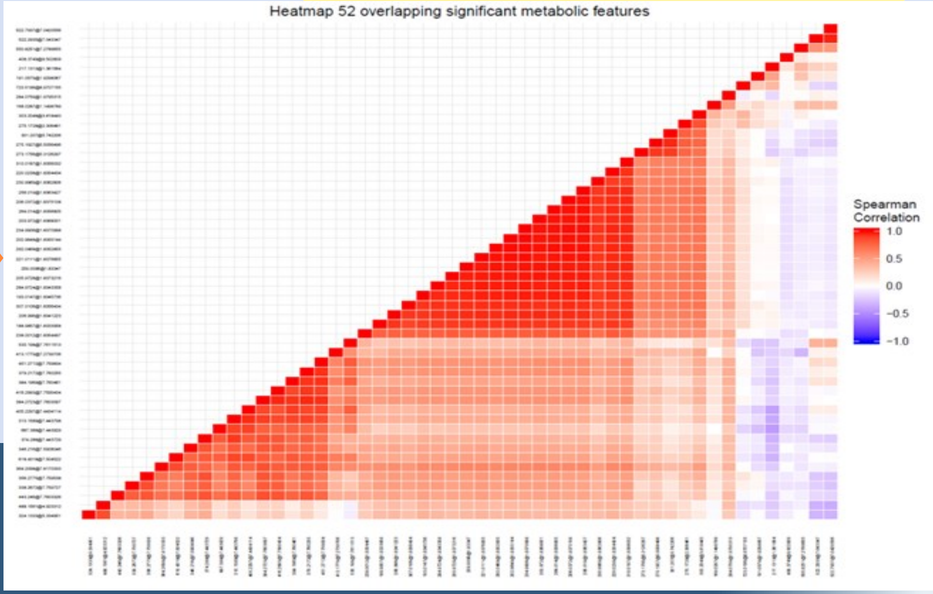
PISCINA Metabolomics

Confounder adjusted MultiVariate Normal (MVN) regression models (one for each exposure) resulted in 150-250 metabolomic features the level of which was significantly associated with DBP exposure. After stability analyses (subsampling of the population over 1000 iterations) fewer features were found to be stable for each exposure (see table).

Cl3CH	BDCM	DBCM	Br3CH	TTHM
166	172	130	54	176



The Venn diagram shows the overlap of the levels of 52 stable features found to be associated with all exposures .


Heatmap 52 overlapping significant metabolic features

High correlation among some of the 52 overlapping features, not among others.

Annotation is needed before pathway analyses can be performed.

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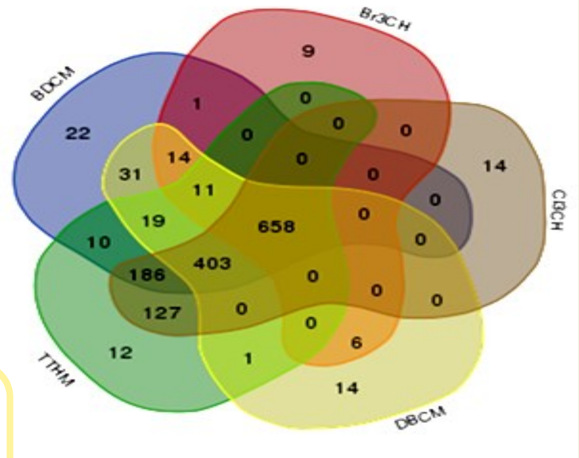
PISCINA Transcriptomics

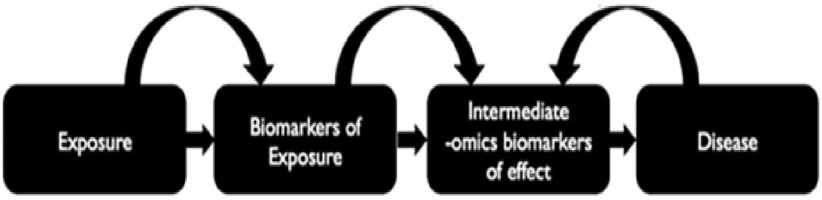
Confounder adjusted MultiVariate Normal (MVN) regression models (one for each exposure) resulted in roughly 750-1650 transcripts the expression levels of which were significantly associated with DBP exposure.

Almudena Espin Perez, Maastricht University

	Cl3CH	BDCM	DBCM	Br3CH	TTHM
Bonf <0.05	1608	1546	1298	758	1647
FDR < 0.05	8438	8241	7907	7395	8496



The Venn diagram shows the overlap between the transcripts per exposure. Levels of 1,526 genes were significantly associated with at least one exposure. Levels of 1,427 transcripts were significantly associated (bonf <0.05) to TTHM.



When outcome variables will become available (measure of genotoxicity, mutagenicity, respiratory biomarker CC16) the meet-in-the-middle principle can be applied for both metabolomic and transcriptomic data.

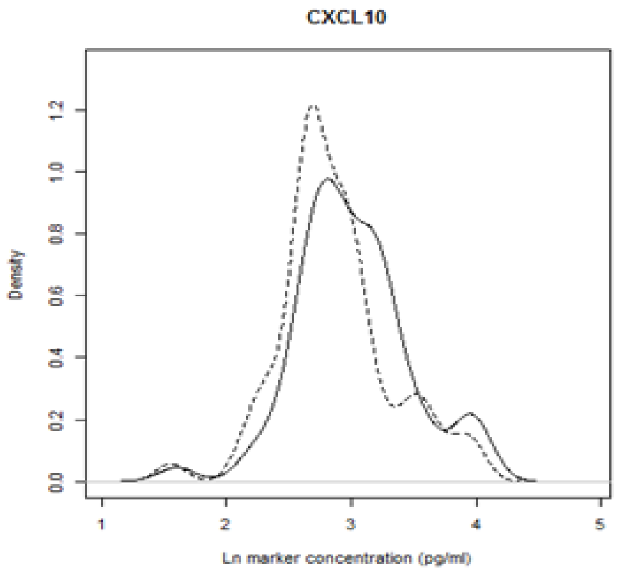


Figure 1. Density distribution of CXCL10 concentration (natural log transformed) before (continuous line) and after (dashed line) swimming.

PISCINA Proteomics

The hypothesis used here is that acute changes in the immune system in people that have been exposed to disinfectants and disinfection by-products play a role in the development of allergic health effects.

To study this hypothesis the levels of 12 markers of the immune system in serum were assessed before and after swimming . Significant associations were observed between the change in total trihalomethanes and a decrease in CCL11, CCL22, CXCL10, CRP, and IL-8, while we observed an increase in IL-1RA. See Figure 1 for an example.

The study indicates that short-term exposure to disinfection by-products induces perturbations of the immune response through acute alterations of patterns of cytokine and chemokine secretion. Although the analyses were corrected for a potential influence of physical activity among other factors, residual confounding might still remain.

Jelle Vlaanderen, IRAS Utrecht

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Interesting Method for Lab Colleagues

Methodology for isolation of transcriptomics-quality RNA in the absence of RNA stabiliser

Soterios Kyrtopoulos, National Hellenic Foundation

Jos Kleinjans, Maastricht University

The application of omics technologies in epidemiological studies raises certain practical issues of sample suitability, especially in relation to RNA quality for transcriptomics analysis, requiring that care be taken for blood samples to be collected and stored in the presence of RNA preservative. However, millions of human biosamples currently in cold storage in older biobanks were collected and processed by methods that did not anticipate the demands of omics technologies. In the context of the European project EnviroGenomarkers (http://www.envirogenomarkers.net), the utility of blood-derived biobank samples for omics-based analyses was explored (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3620742).

In this context, a video demonstrating methodology for the isolation of transcriptomics-quality RNA from buffy coats stored in biobanks in the absence of RNA stabilizer was published online, and can be accessed by following the link http://ehp.niehs.nih.gov/wp-content/uploads/121/4/ehp.1205657.s002.mp4

Thank you to the following contributors :

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