Synopsis no.: *S2.41*

Preliminary title: A case-control analyses of the DNA Methylation signature of tobacco, alcohol and stimulant use and its role in shaping individual risk to psychotic disorders.

Contact info for the person(s) proposing the synopsis

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Publication category: EWAS core paper on the main drugs of abuse (tobacco, stimulants, alcohol) signature on DNA Methylation

Working and writing group: Genetic group WP and John Mill EWAS team, Exeter

Work Packages involved: WP2 and Cardiff team

Partners involved from whom candidate co-authors (*additional to working and writing group*) should be nominated:, M Di Forti, Robin Murray, Craig Morgan, Mick O'Donovan, Alex Richards, Diego Quattrone, , Jim van Os and Bart Rutten

Objectives (scientific background, hypothesis, methods, and expected results):

Gurillo et al. collated evidence in a systematic review and meta-analysis to show an overall increase in the odds of first-episode psychosis in cigarette smokers versus non-smokers in case control and prospective studies, and show that daily smokers developed psychotic illness approximately 1 year earlier than non-smokers. In another study Kendler et al. use Swedish registry data to make similar conclusions, also showing that this association is dose dependent, withstands adjustment for a range of epidemiological confounders, and is not explained by smoking initiation during a prodromal phase of illness.

The Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC) described 108 separate genetic loci associated with an increased risk of

schizophrenia. One of these is located in a cluster of genes—*CHRNA5*, *CHRNA3*, and *CHRNB5*— on chromosome 15, and this region is associated with both early age at onset of smoking and heavy smoking. This implies that such variants increase the risk of both smoking and schizophrenia, or that smoking lies on the causal pathway - the genetic association between *CHRNA5-A3-B4* variants and schizophrenia might be mediated by increased smoking. Furthermore, environmental exposures, such as tobacco use, can have an important impact on the epigenetic mechanisms that regulate key processes in the brain, a number of which been implicated in the pathogenesis of schizophrenia. DNA methylation is strongly associated with smoking in a distinct set of loci, and some of these loci are located in characterized genes where the potential molecular pathway in response to smoking is relatively well understood. No data have looked at the epigenetic signature for tobacco use in individuals with psychosis.

Moreover, to assess the potential harmful tobacco signature on DNA Methylation it is important to identify and adequately control for other prevalent substance of abuse such as alcohol and stimulants. Nestler et al, have published extensively on the cocaine and amphetamine signature on epigenetics markers such as Histone acetylation/methylation both in brain tissue as in peripheral blood. Less consistent findings are available on the EWAS signature left by both alcohol and stimulants.

This proposed work will be the first to examine the impact of tobacco, stimulants and alcohol on EWAS data comparing healthy controls to first episode psychosis patients, accounting for the background noise of the psychotropic medication prescribed.

Our proposed work will provide data necessary for all the other EWAS EUGEI WP2 studies that will be able to carry out their analyses controlling for the tobacco and drug of abuse signatures.

OBJECTIVE 1: Investigate tobacco, stimulants and alcohol EWAS signature and their association with risk of psychotic disorders

OBJECTIVE 2: Specific to tobacco: use GWAS data and the polygenic risk score (PRS) for smoking and its overlap with the PRS for schizophrenia to try and explain variance between cases/ controls.

Combine the overlapping data on genetic correlation between PRS for SCZ and PRS for smoking to target EWAS data that might explain expected differences in the tobacco signature between first episode psychosis users and controls, to increase understanding of how tobacco use and genes interact in conditioning a possible risk for psychotic disorders.

OBJECTIVE 3: Provide a well characterised tobacco, stimulant and alcohol DNA methylation signature scores/profile to be controlled for in other proposed analyses using the WP2 EWAS data.

Data needed for the study: WP2 EWAS data. 2. GWAS WP2 data. 3. Basic Socio-demographics from social scale questionnaire (i.e. age, gender, ethnicity, level of education…). 4. All data from the EU GEI CEQ cannabis and other drugs, tobacco and Alcohol use data. 5. Medication history data.

Plan of analysis:

EWAS data will be generated with the Illumina Infinium Met 450K (CpGs) array. In collaboration with John Mill's team and Richard Dobson's BRC bioinformatics team, we will analyse the EWAS data using an IoPPN in-house pipeline, designed to: 1. Control for cell type heterogeneity DNA-Met profile (blood cells subtypes); 2. Integrate the data on exposure to different levels of stimulants and alcohol use with the two dimensional biological sets of data from GWAS and EWAS. We will calculate the tobacco score using the existing algorithm used by John Mill's group. The public Gene Expression Omnibus (GEO) datasets will be used for validation of the methylomic findings. The GEO data include DNA methylation and expression profiling of whole blood in SZ patients and healthy subjects. This will be the largest analysis performed combining DNA Methylation and GWAS data to investigate the role of common drugs of abuse use in psychotic disorders. All laboratory methods are well established in

Prof Mill's group, as part of their involvement in the NIH Epigenomics Roadmap Initiative. The large sample size will provide sufficient power to be able to group cases and controls for level of use to the individual group of substance, controlling for the tobacco scoring and the cannabis signature derived from the already approved EWAS and cannabis synopsis.
Other analyses/methods: NA
Involvement of external Parties (non EU-GEI): Dr Harriet Quigley (BRC Fellow)
IPR check:
Timeframe: 6 months after release of EWAS data
Additional comments