Synopsis no.: *S2.37*

Preliminary title: Using the EWAS cannabis signature to investigate the biological mechanism of cannabis associated psychosis.

Contact info for the person(s) proposing the synopsis

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Publication category: Secondary to the EUGEI WP2 EWAS general Core-Paper

Working and writing group: cannabis group, genetic group and John Mill EWAS team, Exeter

Work Packages involved: **WP2 and the 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):

Lifestyle choices and environmental exposures can have an important impact on the epigenetic mechanisms that regulate key processes in the brain, processes that have been implicated in the pathogenesis of psychosis. Moreover, our collaborator, Mill published the first genome-wide analysis of DNA methylation differences in blood between MZ twins discordant for psychosis, showing considerable disease-associated variation and epigenetic disruption to biological pathways. Part of the discordance may result from differential exposure to an environmental factor (e.g. drug use) inducing epigenetic changes, which increase individual risk. Changes in DNA methylation and gene expression profiles have been found to occur in response to illicit drug use. Indeed, and consistent with animal data, regular cannabis users show higher levels of CB1 mRNA expression and promoter methylation status in peripheral blood cells than non-users. Thus, cannabis use may induce DNA methylation changes in the promoter region of the CB1 receptor gene leading to a disruption of the Endocannabinoid system. No data have yet explored the signature of cannabis use on EWAS data.

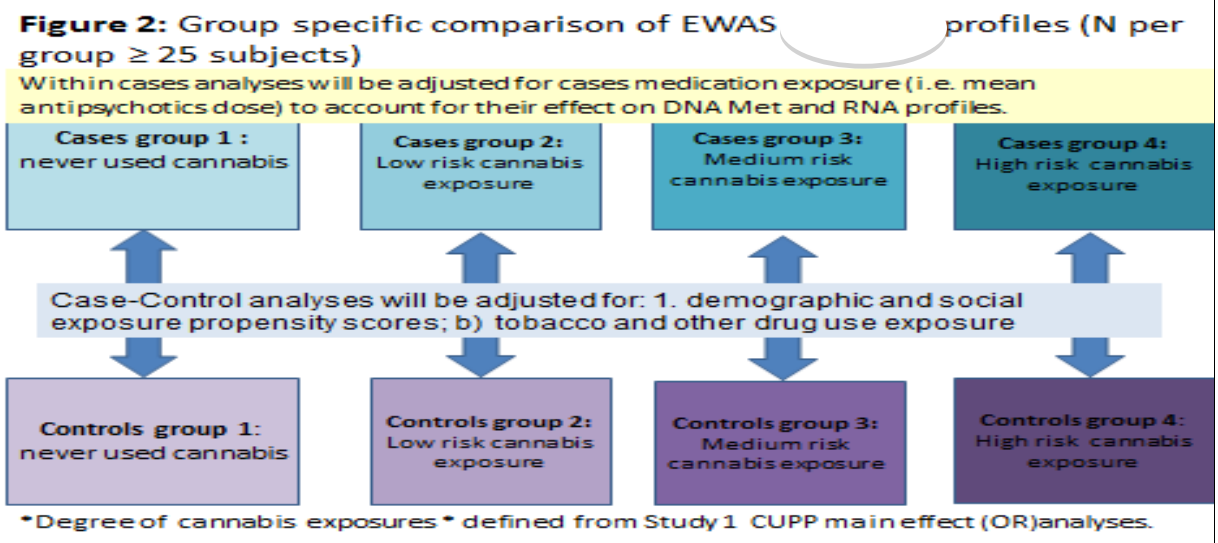
Objectives: a) Examine variations in genome wide DNA methylation and expression RNA associated with differences in the pattern of cannabis use between cases and controls. b) Thus, to increase understanding of how cannabis use and genes interact in conditioning risk for psychotic disorders.

Hypotheses to be tested : a) Differences in DNA methylation across the genome will be associated with differences in pattern of cannabis use between cases and controls. b) I expect these differences when combined with the genome wide expression RNA

data to involve disruption of the expression of genetic variants regulating biological pathways and systems (i.e. Endocannabinoid, Dopamine, AKT, GABA, Glutamate, ∆FosB) with a plausible role in increasing the susceptibility to psychotic disorders when cannabis exposure occurs.

Data needed for the study: 1. 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, I will analyse the EWAS data using an 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 cannabis use with the two dimensional biological sets of data from GWAS and EWAS. The public Gene Expression Omnibus (GEO) datasets will be used for validation of the methylomic. 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 cannabis 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 as in **Figure 2.** This will both increase the power of the analyses and overcome potential pitfalls of epigenetic studies that, lacking information on environmental exposures, cannot disentangle the effects of potential confounding factors from the causal main effect.



Other analyses/methods: not yet planned
Involvement of external Parties (non EU-GEI): Prof Cathryn Lewis and Chloe Wong, Richard Dobson's BRC bioinformatics team,SGDP, IoPPN. John Mill and Emma Dempster Exeter, Epigenetic team.
IPR check:
Timeframe: 6 month from the delivery of the EWAS WP2 data
Additional comments: