E-Risk Study Concept Paper template

Provisional Paper Title: Methylome-wide association study of antidepressant exposure implicates mitochondrial metabolism
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Today's Date: 1/16/2024
Please indicate if you will require an E-Risk independent reproducibility check: ☐

Please describe your proposal in 2-3 pages with sufficient detail for helpful review.

Background & objective of the study:

Objective: To identify epigenetic variation, specifically DNA methylation (DNAm) changes, associated with antidepressant treatment.

Objective reasoning: Understanding antidepressant mechanisms can advance our understanding of Major Depressive Disorder and help design more effective and tolerated treatments.

Background:

Major Depressive Disorder (MDD) is a common and debilitating condition predominantly characterised by persistent sadness and a loss of interest in previously enjoyed activities[1]. MDD is predicted to become the leading cause of disability worldwide by 2030[2], partly due to the limitations of current treatments[3]. Although antidepressants have been linked to an improvement in MDD symptoms and are highly prescribed[4], they are ineffective in a high proportion of cases, with an estimated 40% of those presenting with MDD developing treatment resistant depression[5]. Furthermore, many treatments are commonly accompanied by undesirable side effects, including weight changes, dizziness, fatigue and sexual dysfunction[3]. There is a need for more effective and well-tolerated antidepressant treatments and to target existing treatments to those most likely to respond. Advances are hampered by poor mechanistic understanding of both MDD itself[1] and how currently prescribed antidepressants lead to therapeutic effects[6].

Barbu *et al* (2022) performed a methylome-wide association study (MWAS) of self-reported antidepressant exposure in a subset of participants in Generation Scotland (GS, n = 6, 428) and the Netherlands Twin Register (NTR, N = 2, 449)[7] and identified altered DNAm nearby genes involved in the innate immune response in those exposed to antidepressants[7]. However, the analysis of self-report measures may be unreliable due to memory biases, poor understanding of the medication nosology, and intentional non-disclosure[8]. This study uses a new release of data from Generation Scotland to update the methylome-wide association study of self-reported antidepressant exposure (n = 16, 536). Furthermore, this study also performs an MWAS using prescription-derived measures, to examine the similarities and differences between prescription and self-reported evidence. A methylation risk score (MRS) was trained using Generation Scotland which can be calculated and tested for association with antidepressant exposure in independent

cohorts to assess the robustness of our results. We propose to replicate part of these findings using the available data within the E-Risk cohort.

Significance of the study (for theory, research methods or clinical practice):

This study aims to investigate the association of antidepressant medication and DNA methylation, using a large sample within Generation Scotland. Currently, it is debated how antidepressants have their antidepressant effects. This study will shed light on how DNAm changes associated with antidepressant use which may be informative for assessing the efficacy but also the side effect profile of the antidepressants. Antidepressants are one of most widely prescribed medications worldwide, often being taken for long periods with undesirable side effects. Understanding more about their function may help in guiding clinical practices whilst also gaining insight into major depressive disorder itself.

Data analysis methods:

Generation Scotland Discovery analysis: Methylome-wide association studies were performed on two measures of antidepressant (AD) exposure; self-reported and prescription-derived evidence. Self-reported AD exposure was measured using questionnaires and coded as 1 ('yes currently taking'), and 0 ('no not currently taking'). Prescription derived measures were defined as someone being in an active treatment period at the time of DNAm measurement (evidence from prescription dispensing) and those not taking ADs were classed as those with no AD prescriptions.

MWAS models were conducted using the Mixed-linear-model Omics-based Analysis (MOA) model implemented in the OSCA software[12]. The AD exposure phenotypes were regressed against a genetics relatedness matrix (GRM) using the Best Linear Unbiased Prediction (BLUP) tool in GCTA. The residuals were then taken forward into the EWAS analysis.

AD exposure residual (GRM) ~ DNAm + age + sex + lymphocyte cell proportions (aggregated (CD8T, CD4T, NK and B cell proportions) + monocyte cell proportions + AHRR probe methylation levels + Batch. We also ran this model on MDD cases only, to assess the potential confounding by MDD. Functional follow-up and pathway analysis (using FUMA and SynGO) was conducted on the significant CpG sites identified by this analysis.

> **Aim 1- CpG look-up in E-Risk:** *(Chloe will conduct)*
>
> For the 7 significant CpGs identified in our self-report MWAS analysis, we would look up the distribution of the methylation at these probes in those self-reporting exposed (n=46) and not exposed (n=2020) to antidepressants in the past 2 weeks in E-Risk.

MRS analysis:

Training in GS: We then generate a methylation risk score of self-reported antidepressant exposure, using a LASSO model. We first regressed our AD GRM residuals against all covariates included in our MWAS models (age, sex, Batch, AHRR, and lymphocyte and monocyte cell proportions). We then fit a big lasso model:

AD exposure residuals (GRM + all covars) ~ DNAm

And extracted all the features (CpGs) with non-zero weights in the model and their effect estimates for the calculation of methylation risk scores.

> **Aim 2- Testing in E-Risk:** Methylation risk scores (MRS) will be calculated for external cohorts, including E-Risk *(Chloe will calculate for E-Risk)*. MRS are calculated as a

weighted sum of the non-zero coefficients from the big lasso model. The MRS will then be tested for association *(by Eleanor)* with actual measured AD exposure phenotype using a generalised linear mixed model, within the binomial family, including all covariates in the previous models (Monocyte and lymphocyte cell proportions, AHRR probe, sex, batch as a random effect). In related and twin cohorts, additional covarying for family structure will be required (including familyID as a random effect).

Antidepressant exposure ~ MRS + age + lymphocyte cell proportions + monocyte cell proportions + AHRR probe M vals (standardised) + sex + (1 |Batch) + (1|Family).

The effect estimate, standard error and significance of AD MRS ~ AD exposure will be assessed alongside a McFaddens pseudo R^2, Nagelkerke's R2 (where applicable) and the ROC curves and the AUC.

Demographics of the AD sample in E-Risk will also be assessed to identify if there is any strong confounding (e.g BMI, smoking status).

Variables needed and at which ages:

FAMILYID (ID Family)

ATWINID (ID Twin 1)

BTWINID (ID Twin 2)

SAMPSEX (Sex of twins)

ZYGOSITY (Zygosity of twins)

RORDERP5 (Random order variable)

Age 18:

BMIE18 (BMI - P18 – Elder)

BMIY18 (BMI - P18 – Younger)

SMKPKYRE18 (Smoking - pack years, ages 12 to 18)

SMKCNUME18 (Smoking - current number of cigarettes)

Depression

DXMDEE18 Major depressive episode, dsm4 - P18 - Elder

DXMDEY18 Major depressive episode, dsm4 - P18 – Younge

Antidepressant exposure

Antidepressants18_E

Antidepressants18_Y

DNA Methylation data

Illumina 450K DNA methylation data of selected probes (212 probes for the MRS calculation and AHRR probes) from peripheral blood at age-18 + related variables (probes, batch number, methylation array control probe principal components, chipID etc, cell type composition estimates) for both elder and younger twin.

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