ENVIRONMENTAL-RISK (E-RISK) LONGITUDINAL TWIN STUDY
CONCEPT PAPER FORM

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Provisional Paper Title: Epigenome-wide Association Study of ADHD

Date: 13 April 2021

Objective of the study and its significance:

While research on the epigenetics of ADHD is in early stages, initial studies have suggested links between ADHD and alterations in DNA methylation. Three epigenome-wide association studies (EWAS) of ADHD symptoms in children have been published examining DNA methylation in saliva and cord blood.(1,2,3) Results were mixed, with two studies finding no significant case-control differences, and the third finding 13 loci significantly associated with ADHD symptom trajectories from age 7 to 15.(1) An EWAS of adult ADHD identified significantly differentially methylated positions (DMPs) in the Dunedin cohort, but this finding was not replicated across the other two cohorts in the meta-analysis, including the E-Risk cohort.(4) Studies are also beginning to investigate differences in DNA methylation associated with the course of ADHD: a recent study found no significant DMPs comparing persistent ADHD to controls or remitted ADHD, but found hypermethylated regions in the *APOB* and *LPAR5* genes associated with ADHD persistence.(5) Additionally, analyses have identified an association between ADHD polygenic risk score (PRS) and variable DNA methylation at a site annotated to the promoter of *GART* and *SON*;(6) further work is needed to replicate these findings.

The goal of this concept paper is to participate in a collaboration with the PGC-ADHD epigenetics working group to investigate the association of DNA methylation—specifically in blood and buccal samples—and ADHD diagnosis and symptom levels. This initiative is based on voluntary contribution of the participants to unite efforts (and data), with an overarching aim to advance the understanding of ADHD through

examination of the epigenome. The goal of the initiative is to collect and analyze as many comparable epigenetic datasets as possible under the premise that a better understanding of the epigenetics of ADHD can be achieved best by working together rather than individually.

Study aims:

Aim 1. To perform a EWAS meta-analysis on ADHD across data from several different populations.

1a. To examine the effect of phenotype heterogeneity, age and tissue-type (blood and saliva) on ADHD-associated DNA methylation.

Aim 2. To evaluate the role of variable DNA methylation associated with genetic variation in ADHD using heritability estimated for DNA methylation at specific sites across the genome derived from twin studies and known genetic risk factors for ADHD (e.g. PRS and GWAS variants). This will serve our understanding of ADHD etiology as well as aid the interpretability of EWAS as we will be able to distinguish between differences in DNA methylation due to a genotype and those influenced by other factors.

Aim 3. To investigate the longitudinal course of epigenetic change among children with and without ADHD using buccal samples collected at ages 5, 10 and 18 in E-Risk.

3a. To examine if epigenetic variation is associated with ADHD prognosis over time.

Statistical analyses:

Aim 1. For EWAS analyses, models will predict methylation in whole blood based on ADHD symptoms, age, sex, smoking, derived WBC percentages, as well as technical and cohort specific covariates (see covariate section below). Secondary analysis will further control for genetic principal components, as well as examine ADHD symptoms domains separately (inattention symptoms and hyperactivity/ impulsivity symptoms).

Aim 2. For analyses examining the role of DNA variation in methylation in whole blood, models will predict methylation based on ADHD PRS, age, sex, smoking, BMI, WBC percentages, technical and cohort specific covariates, genetic principal components, and heritability estimates from Eilis Hannon's twin study of DNAm (PLOS Genetics).

Aim 3. Longitudinal analyses examining the association between ADHD and methylation will use growth curve modeling to investigate whether ADHD is associated with level of, and change in, methylation at age 5, 10 and 18.

Covariates

Age= Age when DNA sample was collected

WBC= White blood cell percentage in the same blood sample from which DNA was extracted derived using standard methods (e.g. Houseman's reference based method))

Technical covariates + Cohort Specific covariates Includes technical (batch) covariates and other cohort-specific covariates (e.g. include 450k array row and either sample plate or principal components from the methylation data)

Smoking= Smoking status at the moment of blood sampling, 3 levels: 0=never smoked, 1=former smoker, 2=current smoker. We will also use a quantitative smoking score

derived from DNA methylation data for each individual.

Note: Because this cohort includes related individuals (i.e. twins), we will apply a statistical approach that takes the clustering of data into account (e.g. gee or linear mixed models).

Variables Needed at Which Ages (names and labels):

Study:

Age 5:
FAMILYID ID Family
ATWINID ID Twin 1
BTWINID ID Twin 2
SAMPSEX Sex of twins
ZYGOSITY Zygosity of twins
SESWQ35 Social Class Composite

INEM5 Inattention symptom count—mother
HYEM5 hyperactive/impulsive symptom count—mother
TADHDEM5 Total hyperactive/impulsive/inattention symptom count—mother
ADHDD3E5 ADHD diagnoses—new criteria

Genome-wide methylation from Phase 5 buccal (summary statistics)

Age 10:
INEM10 Inattention symptom count—mother
HYEM10 hyperactive/impulsive symptom count—mother
TADHDEM10 Total hyperactive/impulsive/inattention symptom count—mother
ADHDD3E10 ADHD diagnoses—new criteria
SE17M10 ADHD medication

Genome-wide methylation from Phase 10 buccal (summary statistics)

Age 12:
INEM12 Inattention symptom count—mother
HYEM102hyperactive/impulsive symptom count—mother
TADHDEM12 Total hyperactive/impulsive/inattention symptom count—mother
ADHDD3E12 ADHD diagnoses—new criteria
ADHDCNTE512 Number of ADHD diagnoses
ADHDANYE512 Any ADHD diagnosis age 5-12
SE17M12 ADHD medication

Genome-wide methylation from Phase 12 buccal (summary statistics)

Age 18:
DXADHD5X_18E DSM-5 ADHD Dx [incl 4 NEET & meds] – P18—ET
ADHD4CATE18 ADHD group status
SR_INSUM18E # DSM-5 Inattn symp, Max=9, 18, E-Twin
SR_HYSUM18E # DSM-5 Hyper/Imp symp, Max=9, 18, E-Twin
SR_SYMTOT18E DSM-5 Inattn/Hyper/Imp Symp, Max=18, 18 E-Twin
ser17je18 ADHD medication

SMKCURE18 Smoking daily—current

SMKCNUME18 Smoking—current (number of cigarettes)
SMKDLYE18—Ever a daily smoker
BMIE18 BMI

Genome-wide methylation from Phase 18 blood (summary statistics)
Genome-wide methylation from Phase 18 buccal (summary statistics)
WBC: White blood cell percentage in the same blood sample
Genotype PCs
ADHDPGS_Twins_Feb2020_Clumped ADHD polygenic risk score

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(6) Mooney, M.A., Ryabinin, P., Wilmot, B. *et al.* Large epigenome-wide association study of childhood ADHD identifies peripheral DNA methylation associated with disease and polygenic risk burden. *Transl Psychiatry* **10,** 8 (2020).