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Concept Paper Form

Provisional Paper Title: A trans-omic investigation of convergent evidence for biological pathways associated with adult Attention Deficit Hyperactivity Disorder Symptoms
Proposing Author: Klaasjan G. Ouwens
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P.I. Sponsor: Terrie Moffitt is sponsor of the project, PhD advisor is Dorret I. Boomsma (if the proposing author is a student or colleague of an original PI)
Today's Date: 1/11/2020

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

Objective of the study:
We will investigate if combining DNA, RNA, and methylation data and their associations with ADHD symptoms can give us a deeper understanding of the etiology of ADHD, their associated biological pathways and their interplay on different omics-levels.

Data analysis methods:
In short, we will combine association statistics between ADHD and variation in genetic variations, gene expression levels and methylation levels. To ensure comparability, we will aggregate association statistics to a per-gene P-value using FUMA (Watanabe et al., 2017). In addition, we will ensure there is no sample overlap between the three studies. To avoid sample overlap, we request access to per-cohort data of a recent Moffitt & Caspi study (Van Dongen et al., 2019), since we want to exclude participants of NTR.

DNA To study overlap of gene expression signal with previously identified loci from GWAS, we will consider the two most recent large GWA studies. The most recent and largest study by Demontis et al. is the first GWAS for ADHD diagnoses to find loci that are genome-wide associated with ADHD diagnosis. This study was done in 20,183 cases and 35,191 controls. We combine these data with largest GWAS for childhood ADHD symptoms, measured in 17,666 children on a continuous scale (Middeldorp et al., 2016), with people from NTR excluded. We will re-run the EAGLE meta-analysis excluding all NTR participants and subsequently repeat the MA, now including participants from PGC/iPsych and EAGLE without NTR. We will use a modified sample size-based weighting method as described by Demontis et al., (2018) to account for heritability, genetic correlation and measurement scale of dichotomous-based GWAS results of clinically ascertained ADHD-cases from PGC and continuous ADHD symptom scores in population based cohorts in

EAGLE. We will use FUMA to calculate gene-based P-values based on the SNP-based summary statistics from our meta-analysis.
RNA Associations between gene expression and number of ADHD symptoms are tested for each genomic site under a generalized estimation equation (gee) model accounting for relatedness between participants. Age, sex, body-mass index (BMI) and current smoking status will be included as covariates. The NTR has analyzable gene expression data for 1730 persons from RNA sequencing (Ouwens et al, 2019). For 1333 participants phenotype data for ADHD is available from survey studies. Associations between gene expression and number of ADHD symptoms will be tested for each genomic site under a generalized estimation equation (gee) model accounting for relatedness between participants. Age, sex, body-mass index (BMI) and current smoking status will be included as covariates.
Methylation For methylation-based ADHD-associations, we would like to use results from the Dunedin Study (N=800), and the Environmental Risk Longitudinal Twin Study (E-risk, N=1631), as it is the most prominent study in the field. By excluding the NTR-cohort, we will ensure there is no sample overlap with DNA- and RNA-based samples. We will use FUMA software (Watanabe et al., 2017) for calculation of enriched pathways and compare these to enriched biological pathways from other data.

We will convert all results to per-gene values using MAGMA software (De Leeuw et al., 2015) where needed. All enrichment tests will be performed using FUMA software .

Variables needed at which ages:
Only permission is needed to analyze the EWAS results that were uploaded separately from the NTR results.

NOTE FROM TEMI: THE KEY THING HERE IS THAT THIS PROJECT WILL USE ONLY RESULTS ALREADY CALCULATED AT DUKE BY KAREN SUGDEN AND ALREADY UPLOADED OR THE PURPOSE OF JENNY VAN DONGEN'S PAPER THAT WS PREVISOULY APPROVED BY US. NO NEW INFORMATION IS BEING SENT. THE NETHERLANDS TEAM WILL JUST USE THE SAME INFORMATION TO PREPARE A DIFFERENT PAPER.

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

This study will provide novel insight into trans-omics convergent evidence for biological pathways associated with ADHD symptoms.

References cited:

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- Middeldorp, C. M., Hammerschlag, A. R., Ouwens, K. G., Groen-Blokhuis, M. M., Pourcain, B. S., Greven, C. U., ... & Vilor-Tejedor, N. (2016). A genome-wide association meta-analysis of attention-deficit/hyperactivity disorder symptoms in population-based pediatric cohorts. *Journal of the American Academy of Child & Adolescent Psychiatry*, *55*(10), 896-905.
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Data Security Agreement

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☒	I am current on Human Subjects Training (CITI (www.citiprogram.org) or equivalent)
☒	My project is covered by the Duke ethics committee OR I have /will obtain ethical approval from my home institution.
☒	I will treat all data as "restricted" and store in a secure fashion. My computer or laptop is: a) encrypted (recommended programs are FileVault2 for Macs, and Bitlocker for Windows machines) Yes, bitlocker b) password-protected Yes c) configured to lock-out after 15 minutes of inactivity AND Yes d) has an antivirus client installed as well as being patched regularly. Yes
☒	I will not "sync" the data to a mobile device.
☒	In the event that my laptop with data on it is lost, stolen or hacked, I will immediately contact Moffitt or Caspi.
☒	I will not share the data with anyone, including my students or other collaborators not specifically listed on this concept paper.

☒	I will not post data online or submit the data file to a journal for them to post. *Some journals are now requesting the data file as part of the manuscript submission process. Study participants have not given informed consent for unrestricted open access, so we have a managed-access process. Speak to Temi or Avshalom for strategies for achieving compliance with data-sharing policies of journals.*
☒	I will delete all data files from my computer after the project is complete. Collaborators and trainees may not take a data file away from the office. This data remains the property of the Study and cannot be used for further analyses without an approved concept paper for new analyses.

Signature: Click or tap here to enter text.