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HUMAN DEVELOPMENT, BIRTH TO DEATH

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Concept Paper Form

Provisional Paper Title: Examining the association of PTSD with DNA methylation CpG sites
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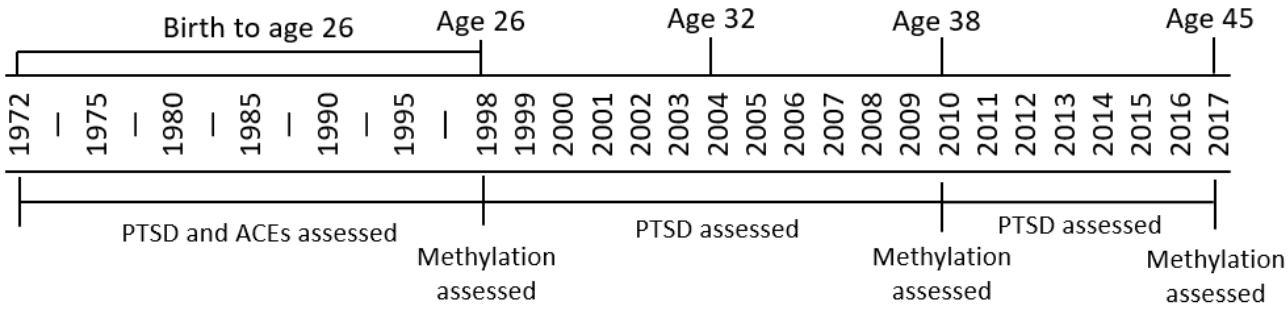
Objective of the study:

We propose to investigate the association of PTSD status and trauma exposure with DNA methylation in three candidate CpG sites—cg05575921, cg26703534, and cg19534438. These analyses will also be run in a sample of 2,309 Veterans (mean age 37.4, *SD* = 10.1) who were assessed for PTSD and have methylation data in the Post-Deployment Mental Health Study (PDMH).

People who experience trauma and adversity are at increased risk for poor health as they age[1-7]. This includes both people who experience trauma, as well as those who go on to develop posttraumatic stress disorder (PTSD)[6-8]. Much of the work linking PTSD to poor health has done so via psychosocial mechanisms, such as social support and health behaviors[9-10]. However, more recent studies have shown DNA methylation (DNAm) might be associated with exposure to trauma and PTSD. For example, people with PTSD have accelerated biological age using DNAm measures of aging[11]. Beyond epigenetic clocks, there is evidence that PTSD might be associated with changes in specific CpG sites. For example, a recent meta-analysis of 10 military and civilian samples found that CpG sites associated with AHRR expression were associated with PTSD status[12], even among non-smokers. Subsequent work with DNAm and PTSD symptoms[13] examined candidate CpGs from five previous studies of PSTD and DNAm[12,14-17] and found associations for two of these AHRR CpG sites, as well as an additional CpG site (located on G0S2). As a result, these three CpG sites (cg05575921, cg26703534, cg19534438) are strong candidate DNAm markers to examine in association with the onset of PTSD and trauma exposure. These CpG sites may also be relevant to health. For example, AHRR is linked to increased risk for morbidity and mortality[18-20], and better characterizing the associations between PTSD with DNAm could provide insight into biological mechanisms that might link PTSD and stress to poor health.



Although PTSD has been linked to DNAm in prior work[13], existing studies have generally used smaller samples that were cross sectional and comprised largely of trauma-exposed military samples. This has

limited the ability to characterize how trauma and PTSD could be associated with DNAm. The Dunedin Study is well suited to investigate epigenetic associations with trauma, having assessed PTSD across the lifespan and DNA methylation (including the three candidate CpG sites) at ages 26, 38, and 45. The Dunedin study also has detailed measurement of smoking across the lifespan, both in terms of timing and amount. Two of the candidate CpGs are associated with AHRR, which has been linked to smoking behavior in prior studies[12]. As such, it will be essential to control for smoking in the current study, particularly as a prior meta-analysis found that PTSD was more strongly associated with AHRR CpG sites among non-smokers. The Dunedin study can also complement data from a large sample of post-9/11 deployment veterans in the form of the Post-Deployment Mental Health Study (PDMH). Investigating the candidate CpG sites in Dunedin and the PDMH will help provide critical evidence as to whether observed associations with these candidate CpG site replicated in a combined sample size (*N* ~ 3,150) larger than both the prior meta-analysis[12] (*N* = 1,896) and replication sample[13] (*N* = 429) referenced above.

Data analysis methods:

Primary Analyses: We propose to investigate the association of PTSD status and trauma exposure with DNA methylation in three candidate CpG sites—cg05575921, cg26703534, and cg19534438. All models described below will also be run while controlling for smoking status.

Aim 1: We will first estimate the within occasion association between the candidate CpGs and PTSD/trauma status at age 26, 38, and 45 in the Dunedin Study. We will run two sets of models examining associations at each of the three ages. The first set of models will examine PTSD status and will compare methylation levels for people who have not had PTSD (no lifetime PTSD, scored 0) to those who had PTSD prior to that occasion (lifetime PTSD, scored 1) at each age (age 26, 38 and 45). We hypothesize that PTSD status will be associated with the three candidate CpG sites at each occasion. The second set of models will add trauma exposure without a PTSD diagnosis as a third category of comparison. In these models, people without trauma exposure (or PTSD) will serve as the comparison group (coded as 0) to people with prior trauma exposure but no PTSD (scored as 1), and people with PTSD diagnosed by that occasion (scored as 2), at each age (age 26, 38, and 45). We hypothesize the candidate CpG sites will have a stepwise association trauma exposure and PTSD.

These models will also be run in a sample of 2,309 Veterans (mean age 37.4, *SD* = 10.1) who were assessed for PTSD and have methylation data in the Post-Deployment Mental Health Study (PDMH). Models in the PDMH will be run using PTSD diagnostic status (comparing current PTSD to no current PTSD), a continuous assessment of self-reported PTSD symptoms, and a continuous measure trauma exposure (number of categories of exposure).

Aim 2: We will examine candidate CpG levels for more recent PTSD diagnosis and trauma exposure compared to people with no PTSD/trauma exposure and more distal PTSD diagnosis and trauma exposure (i.e., prior to age 26). As with Aim 1, we will run two sets of models examining associations with the candidate CpG methylation levels, specifically at age 38 and 45. The first set of models will test the association between the candidate CpG sites and PTSD diagnostic status prior to the start of the study period of interest (PTSD diagnosis by age 26 or not), as well as PTSD diagnostic status from age to 26 to age 38 (for the age 38 methylation outcomes) and age 45 (for the age 45 methylation outcomes). We hypothesize that recent PTSD status will also show stronger associations with the candidate methylation markers than PTSD prior to age 26. The second set of models will then add trauma exposure but no PTSD diagnosis as a third category of comparisons, similar to Aim 1. This will create a third category for comparison (trauma exposure) for both the period prior to age 26, and the age 26 to 38 / age 26 to 45 periods (for the analyses examining age 38 methylation and age 45 outcomes, respectively). We hypothesize the candidate CpG sites will have a stepwise association with recent trauma exposure and PTSD.

We will also run models in the PDMH examining methylation data for veterans with current, past, and no history of PTSD, as a conceptual replication of the Aim 2 models run in Dunedin. We hypothesize that veterans with past PTSD will show methylation levels in the candidate CpG sites more similar to veterans without PSTD than veterans with current PTSD.

Secondary analysis: We will compare associations between PTSD and methylation across two major ancestry groups (non-Hispanic Black veterans and non-Hispanic White veterans), as defined by self-report and ancestral genotyping, in the PDMH.

General analysis methods: Participants in Dunedin will be included in this study if they have methylation data and PTSD assessed during at least two time points from ages 26, 38, and 45. PDMH study participants will be included if they have methylation data assessed. Models will use multiple regression to compare the association between predictors and the three candidate CpG sites. All models will control for sex and the relevant methylation technical principle components (PCs). All models will be run in MPLUS[21] using full information maximum likelihood estimation[22] to account for missing data.

Variables needed at which ages:

- Methylation data at age 26, 38, and 45
    - Methylation data (3 CpG sites) identified by previous research[12-13]
        - cg05575921 (AHRR) – Reliability[23] = 0.88
        - cg26703534 (AHRR) – Reliability = 0.71
        - cg19534438 (G0S2) – Reliability = 0.16
    - Methylation control variables
        - PCs
- PTSD status
    - Current PTSD diagnostic status at age 26, 32, 38, and 45
    - Recent (since last wave) PTSD diagnostic status at age 26, 32, 38, and 45
    - Lifetime PTSD diagnostic status at age 26, 32, 38, and 45
    - Whether Study Member endorsed having experienced a trauma (from PTSD assessment) at age 26, 32, 38, and 45
    - Lifetime trauma status (any trauma present to that age) at age 26, 38, and age 45
    - Age of trauma reported (from age 38 and 45 PTSD assessment)
    - PTSD impact on life (1-5) at age 26, 32, 38, and 45
- Sex as a demographic covariate
- Smoking
    - Pack years at age 26, 32, 38, and 45
    - Occasions with smoking Dx (Cross phase)
    - Current smoking status (at 26, 32, 38, and 45)
    - Whether someone is currently smoking in the home (ages 26, 32, 38, and 45)
    - Methylation measure of smoking at age 26, 38, and 45 (if available)

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

This study will help provide new knowledge about the association between PTSD and DNAm. Although there is prior evidence linking three specific CpG sites to PTSD, this study will examine these associations in two large cohorts, one with multiple assessments of PTSD and methylation. This work would also allow for comparisons across a more diverse set of participants and could support future research on PTSD and health by providing evidence as to epigenetic mechanisms that could explain how PSTD might increase risk for poor

health.

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