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Examining the timing of trauma, PTSD onset, and DNA methylation: A multicohort investigation of candidate CpG sites

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ABSTRACT

Epigenome-wide association studies (EWAS) have identified cytosine-phosphate-guanine sites (CpGs) associated with trauma and PTSD. However, prior studies have largely focused on cross-sectional associations, and it remains unclear whether epigenetic profiles associated with traumatic stress might predispose individuals to develop PTSD or might change in response to PTSD. In this study, we characterized associations between traumatic stress and three CpGs identified in previous EWAS of PTSD (cg05575921 and cg26703534 in *AHRR*, and cg19534438 in *G0S2*) using two cohorts—the Dunedin Multidisciplinary Health and Development Study ($n = 846$) and the Post-Deployment Mental Health Study (PDMH; $n = 2309$). We first replicated cross-sectional associations, and then investigated the time course linking PTSD, trauma, and DNA methylation. In cross-sectional analyses, we found trauma and PTSD were consistently associated with hypomethylation at cg05575921—including at age 26, 38, and 45 in the Dunedin Study—whereas neither cg26703534 nor cg19534438 showed consistent associations. These results were robust to multiple methods of accounting for tobacco exposure, which is notable given associations between smoking and changes in *AHRR*. In the Dunedin Study, we also found that people who developed PTSD between age 26 and 45 showed greater hypomethylation at cg05575921 over the same period, however, people with hypomethylation at cg05575921 at age 26 were no more likely to develop future PTSD. Hypomethylation at cg05575921 was also associated with childhood adversity in the Dunedin Study and a past diagnosis of PTSD in the PDMH. Our findings suggest traumatic stress is associated with cg05575921 in the *AHRR* gene, and that trauma and PTSD may be associated with changes in cg05575921.

1. Introduction

Traumatic stress has been linked to poorer cardiovascular functioning (Bourassa et al., 2020, 2021a; Schneider and Schwerdtfeger, 2020), higher levels of systemic inflammation (Bourassa et al., 2021b; Plantinga et al., 2013; Sumner et al., 2020), and accelerated biological

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aging (Bourassa et al., 2023, 2024a; Wolf et al., 2016;Wolf et al., 2024). Epigenetic characteristics are another physiological mechanism that index how genetic profiles are expressed and can change in response to environmental influences. The ability of epigenetics to modulate gene expression based on lived experiences offers a potential pathway for understanding how traumatic stress may impact health. New approaches to quantify the epigenome using DNA methylation (DNAm) have been integrated into existing research cohorts focused on mental health, which has provided the opportunity to link trauma and post-traumatic stress disorder (PTSD) to epigenetic changes that might be relevant to physiology (Bourassa et al., 2024b; Reed et al., 2022). However, longitudinal evidence linking trauma and PTSD to changes in DNAm is currently lacking, which has made determining the temporal ordering of traumatic stress and DNAm challenging, particularly given different possible directions of association. For example, epigenetic profiles could predispose individuals to develop PTSD (Katrinli et al., 2022). Alternatively, the experience of trauma and PTSD could change people's epigenetic characteristics.

Epigenome-wide association studies (EWAS) have linked traumatic stress to DNAm. Across a series of studies from 2018 to 2022, the Psychiatric Genomics Consortium PTSD Workgroup found 31 cytosine-phosphate-guanine (CpG) sites associated with PTSD (Logue et al., 2020; Rutten et al., 2018; Smith et al., 2020; Snijders et al., 2020; Uddin et al., 2018). In 2022, Katrinli and colleagues (2022) assessed these 31 associations and found that three met nominal significance: cg05575921 and cg26703534 in the aryl hydrocarbon receptor repressor gene (*AHRR*), and cg19534438 in the G0/G1 Switch 2 gene (*G0S2*). *AHRR* assists in regulating the activity of the aryl hydrocarbon receptor, a receptor involved in the body's response to environmental toxins and linked to immune system regulation, including T cell differentiation (Goudot et al., 2017; Julliard et al., 2014; Wheeler et al., 2017). Epigenetic changes at *AHRR* are influenced by exposure to polycyclic aromatic hydrocarbons and can come from a variety of air pollutants—most notably, tobacco smoking (Bojesen et al., 2017; Grieshober et al., 2020), though it is notable that PTSD is associated with CpGs in *AHRR* among people who have never smoked (Smith et al., 2020). The G0/G1 Switch 2 gene (*G0S2*), linked to cg19534438, is associated with changes in cortisol and fear response systems related to the amygdala and hippocampus (Daskalakis et al., 2014; Stimson et al., 2017), as well as lipid metabolism regulation (Zhang et al., 2017) associated with obesity, diabetes, and aging (Li et al., 2016). These candidate CpG sites represent plausible mechanisms through which traumatic stress could influence physiology and health, though the longitudinal evidence to support this possibility is currently lacking.

1.1. Present study

We used data from 3155 participants in two well-characterized cohorts—the Dunedin Multidisciplinary Health and Development Study ($n = 846$) and the Post Deployment Mental Health Study (PDMH; $n = 2309$)—to investigate associations between trauma, PTSD, and methylation in three candidate CpGs—cg05575921, cg26703534, and cg19534438. Given established associations with smoking, particularly CpGs associated with *AHRR*, we conducted a series of analyses adjusting for multiple measures of smoking. We also investigated the timing of traumatic stress and methylation characteristics using longitudinal data in the Dunedin Study and data on past PTSD in the PDMH.

2. Methods

2.1. Participants and study design

The Dunedin Study (Poulton et al., 2022) included 420 women (49.6 %) and 426 men (50.4 %) born in the same 1-year period (1972–1973). The PDMH (Brancu et al., 2017) was comprised 1818 men (78.7 %) and 491 women veterans (21.3 %) who averaged 37.4 years old ($SD = 10.1$ years) and included 1109 non-Hispanic Black veterans (47.0 %) and 1200 non-Hispanic White (53.0 %) veterans.

Dunedin Longitudinal Study. The Dunedin Study is a longitudinal investigation of health and behavior in a birth cohort followed until age 45. The cohort comprised all individuals born between April 1, 1972, and March 31, 1973, in Dunedin, New Zealand, who were eligible based on residence in the province of Otago and participation in the first assessment at 3 years of age (Poulton et la., 2023). Participants (Supplemental Fig. 1). Study participants are primarily of New Zealand European ethnicity; 8.6 % reported Māori ethnicity at age 45. Assessments were performed at fourteen visits from birth to age 45. For this study, 846 participants met criteria for inclusion by having DNAm assessed at least twice from ages 26 to 45. Informed consent was obtained from participants and protocols were approved by the Health and Disability Ethics Committee at the New Zealand Ministry of Health and Duke University Institutional Review Board.

Post Deployment Mental Health Study. The Post Deployment Mental Health (PDMH; Brancu et al., 2017) is a multi-site study of Afghanistan and Iraq-era veterans. The Veterans Integrated Service Networks 6 (VISN 6) Mental Illness Research, Education, and Clinical Center (MIRECC) PDMH started enrolling participants in 2005 to facilitate mental health research on veterans from the post-9/11 period. A subset of the PDMH data ($n = 545$) was used for a prior meta-analysis of DNAm and PTSD (Logue et al., 2020; Smith et al., 2020), to which 1764 new participants were added. The current study included participants who had DNAm data available and comprised the two major self-reported racial/ethnic groups (Bourassa et al., 2024b), resulting in a final sample of 2309 veterans.

2.2. Measures

DNA Methylation. In the Dunedin Study, DNAm values were derived using whole blood samples collected at age 26, 38, and 45. As described in prior studies (Belsky et al., 2022), the Illumina 450k Array was used at ages 26 and 38, and the Illumina EPIC 850k Array was used at age 45 (Illumina Inc, San Diego, CA). Methylation assays were run by the Molecular Genomics Shared Resource at Duke Molecular Physiology Institute Duke University (USA). Normalization was performed using methylumi Bioconductor package (Davis et al., 2021) and probes were excluded if they had a detection p-value >0.05 in at least 1 % of the samples. Data were processed using the dye bias normalization approach to eliminate systematic differences across the arrays. Principle components were created to account for technical variation and were used to create residualized estimates for each CpG at each age.

In the PDMH Study, methylation scores were derived using whole blood samples collected at the study baseline. As described previously (Bourassa et al., 2024a), DNAm values were assayed using either the Infinium HumanMethylation450 or MethylationEPIC Beadchip (Illumina Inc, San Diego, CA). Quality control (QC) was performed using the minfi (Aryee et al., 2014) and ChAMP (Morris et al., 2014) R packages. Probe QC and data normalization was performed within each batch using the R package wateRmelon using the dasen approach (Pidsley et al., 2013) and batch and chip adjustments were accomplished using ComBat in the R package sva (Leek et al., 2012).

Posttraumatic stress disorder (PTSD). In the Dunedin Study, participants were assessed to determine whether they met criteria for PTSD at age 26, 32, 38, and 45 using the Diagnostic Interview Schedule according to then current versions of *DSM-IV* (ages 26, 32, and 38; Robins et al., 1995) and *DSM-5* (age 45). Participants were first asked whether they experienced a Criterion A trauma and were interviewed on their symptoms related to the traumatic experience that had most affected them. Measures of trauma exposure were used in concert with PTSD status to create three categories for the "PTSD and trauma" combined measure, in which PTSD was coded as 2, trauma exposure, no PTSD was coded as 1, and no trauma exposure or PTSD was coded as 0 at each age.

In the PDMH Study, PTSD was assessed using *DSM-IV* structured interviews (First et al., 1994) and PTSD symptoms from the Davidson Trauma Scale (DTS; Davidson et al., 2002), a 17-item self-report scale. Veterans were categorized as having current PTSD if they received a diagnosis from the diagnostic interview or had a score of 35 or more on the DTS, a validated clinical cutoff for post-9/11 veterans (McDonald et al., 2009). In addition, total scores for the DTS assessed self-reported PTSD symptoms, with higher scores representing more symptoms. Finally, the PDMH *DSM-IV* interviews also assessed the presence of PTSD in the past (Bourassa et al., 2024a).

Trauma exposure. In Dunedin, age at which index traumas occurred were assessed using participants' recall of trauma at ages 38 and 45. Age at which traumas were experienced was not reported at age 26. The age of index trauma which used to code whether participants had been exposed to trauma (coded 1) or not (coded 0) at age 26, 38, and 45.

In the PDMH, trauma exposure was assessed using self-reports from the Traumatic Life Events Questionnaire (TLEQ; Kubany et al., 2000). The TLEQ assesses whether respondents have experienced 22 categories of potentially traumatic events across the lifespan, which were summed to create an index of traumatic event types experienced across the lifespan, with higher scores representing more trauma.

Childhood adversity. Records from the first 15 years of life for the Dunedin Study members were reviewed by four independent raters to yield a prospective measure comprising 10 adverse childhood experiences (ACEs), including five types of child harm and five types of household dysfunction (Felitti, 2002). Inter-rater agreement across all ACEs averaged a kappa of 0.79 (range = 0.76-0.82) and counts greater than four were recoded to four.

Study covariates. ***Demographics.*** Dunedin and PDMH participants self-reported their sex. PDMH participants also reported their race/ethnicity and age which were confirmed using sex chromosomes and ancestry estimates derived from genetic data.

Smoking methylation scores. In both studies, we used an established method to derive DNAm smoking scores for study participants (Joehanes et al., 2016; Sugden et al., 2019).

Smoking behavior. Smoking was also assessed in both studies using self-report. Participants were categorized as either never smokers, past smokers, or current smokers.

Pack years. Self-reported duration and amount of smoking was used to calculate pack years (20 cigarettes a day for 1 year = 1 pack-year) in both studies.

White blood cell count proportions. We used the FlowSorted.Blood.450k and FlowSorted.Blood.EPIC packages to estimate cell count proportions (Houseman et al., 2015) for six major types of white blood cells, which were used as covariates in all models.

2.3. Data analysis

We first tested the associations between trauma, PTSD, and the three candidate CpGs using multiple regression models. All models controlled for smoking methylation scores, and were also run when controlling for self-reported smoking status, pack years, and stratifying by current smoking status. After these initial models, conducted a series of additional analyses for CpGs that had consistent associations with trauma and PTSD. First, we examined the timing of PTSD onset and change in methylation in the Dunedin study. Second, we tested the association between adverse childhood experiences and methylation at age 26 in the Dunedin Study. Third, we examined associations between lifetime PTSD status and methylation in the PDMH by accounting for past PTSD diagnostic status. Finally, we examined the associations in the PDMH while stratifying by race and ethnicity. All models were run in MPLUS version 8.3 (Muthén and Muthén, 1998) and adjusted for estimated white blood cell count proportions and demographics. Models used full maximum likelihood estimation to account for missing data, and as a result the full samples were used for analyses within each cohort.

3. Results

3.1. Associations in the Dunedin Study

People with a history of trauma and PTSD showed hypomethylation at cg05575921 at ages 26, 38, and 45 (Fig. 1) when assessed using the combined measures of trauma and PTSD. Trauma exposure and PTSD diagnostic status and were also associated with hypomethylation at cg05575921 at all three ages when using the dichotomized measures individually, with the exception of PTSD at age 38. In contrast, cg26703534 or cg19534438 were not consistently associated with PTSD or trauma exposure (Table 1).

3.2. Associations in the PDMH study

Veterans with a current PTSD diagnosis, more self-reported PTSD symptoms, and a greater trauma burden showed significant hypomethylation at cg05575921 (Fig. 1). Neither cg26703534 nor cg19534438 were consistently associated with PTSD or trauma exposure (Table 1).

3.3. Associations when using additional methods to account for tobacco smoke exposure

We used three additional approaches to account for tobacco exposure. First, we ran models controlling for self-reported smoking status (never smoker, past smoker, current smoker) rather than smoking methylation scores, which are highly sensitive to changes in cg05575921 (44–45). The results for PTSD largely replicated across both cohorts, though trauma exposure was no longer associated with cg05575921 (Supplemental Table 1). Second, we stratified the samples and examined associations between trauma, PTSD, and cg05575921 separately in non-smokers and current smokers, while also controlling for smoking methylation scores to account for lifetime tobacco exposure. Associations between trauma, PTSD, and cg05575921 were observed primarily among non-smokers (Supplemental Table 2), providing additional evidence that the associations observed in this study were not primarily driven by differences in cg05575921 associated with smoking behavior. Finally, we ran our models when controlling for pack years in both studies. Associations for the combined measure of trauma and PTSD with cg05575921 remained significant at age 26 in Dunedin, but not at age 38 or 45, whereas associations for trauma and PTSD became slightly larger in magnitude in the PDMH (Supplemental Table 3).

3.4. Longitudinal associations for cg05575921 in the dunedin and PDMH studies

PTSD onset and change in cg05575921 in the Dunedin Study. We used the longitudinal data available in the Dunedin Study to investigate whether PTSD onset between age 26 and 45 was associated with change in cg05575921 over the same period. For these analyses, the main predictors were the presence or absence of a PTSD diagnosis prior to age 26 ($n = 73$ with PTSD diagnosis by age 26) and the presence or absence of a PTSD diagnosis from age 26 to 45 ($n = 93$ with a PTSD diagnosis between age 26 and 45); models also controlled for covariates and age 26 cg05575921 levels. PTSD onset was associated with a decrease in cg05575921 methylation from age 26 to age 45 (Table 2). However, PTSD onset was not associated with change in cg05575921 from age 26 to 38, possibly due to reduced power from the smaller number of people who had a new diagnosis of PTSD from age 26 to 38 ($n = 68$ from age 26 to 38; $n = 93$ with PTSD diagnoses from age 26 to 45).

cg05575921 at age 26 and subsequent PTSD onset in the Dunedin Study. We also examined whether cg05575921 methylation levels at age 26 might predict the subsequent onset of PTSD in the Dunedin Study. Models controlled for self-reported smoking status, rather than smoking methylation scores, to reduce multicollinearity. In these models,

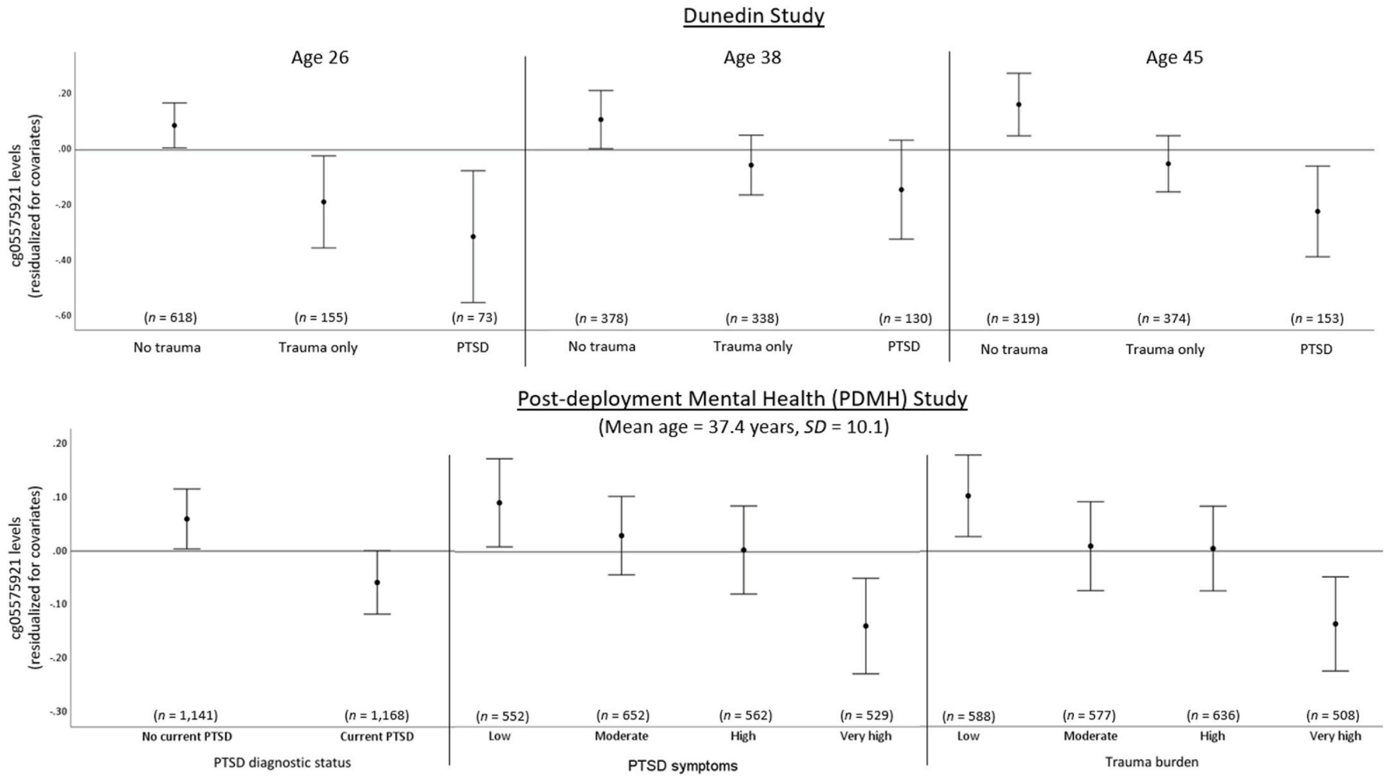

Fig. 1. Levels of cg05575921 by trauma and PTSD across ages in the Dunedin Study and different measures in the PDMH study. Dunedin categories include lifetime exposure to trauma and PTSD, and the PDMH categories include PTSD diagnostic status and symptoms, as well as trauma burden, which were binned into quartiles for visualization purposes only. The Y-axis represents cg05575921 levels that have been residualized for sex, white blood cell proportions, smoking methylation scores and technical PCs in the Dunedin Study, and sex, age, race/ethnicity, white blood cell proportions, and smoking methylation scores in the PDMH study.

Table 1
Association of Trauma and PTSD with Three Candidate CpG sites.

	cg05575921		cg26703534		cg19534438	
	β	95 % CI	β	95 % CI	β	95 % CI
Dunedin (age 26)						
PTSD and trauma	−0.09**	[-0.13, −0.05]	0.03	[-0.03, 0.09]	−0.06*	[-0.12, −0.00]
PTSD diagnosis	−0.22**	[-0.37, −0.07]	0.11	[-0.16, 0.33]	−0.13	[-0.31, 0.04]
Trauma exposure	−0.18**	[-0.29, −0.08]	0.02	[-0.16, 0.18]	−0.13*	[-0.25, −0.00]
Dunedin (age 38)						
PTSD and trauma	−0.06**	[-0.10, −0.02]	−0.03	[-0.09, 0.03]	−0.00	[-0.07, 0.07]
PTSD diagnosis	−0.11	[-0.22, 0.01]	0.00	[-0.16, 0.13]	0.03	[-0.15, 0.21]
Trauma exposure	−0.10*	[-0.18, −0.02]	−0.07	[-0.18, 0.04]	−0.02	[-0.15, 0.11]
Dunedin (age 45)						
PTSD and trauma	−0.08**	[-0.13, −0.04]	−0.03	[-0.09, 0.03]	0.02	[-0.06, 0.07]
PTSD diagnosis	−0.16**	[-0.26, −0.06]	−0.06	[-0.20, 0.08]	−0.06	[-0.24, 0.11]
Trauma exposure	−0.13**	[-0.21, −0.05]	−0.07	[-0.15, 0.01]	0.09	[-0.05, 0.22]
PDMH study (average age = 37.4)						
PTSD diagnosis	−0.09**	[-0.14, −0.05]	−0.09**	[-0.16, −0.04]	−0.04	[-0.11, 0.02]
PTSD symptoms	−0.06**	[-0.08, −0.03]	−0.05**	[-0.09, −0.02]	−0.01	[-0.05, 0.02]
Trauma exposure	−0.04**	[-0.06, −0.01]	−0.02	[-0.05, 0.02]	0.04*	[0.00, 0.08]

Note: Dunedin $N = 846$, PDMH $N = 2309$. The Dunedin Study included 420 women (49.6 %) and 426 men (50.4 %) born in the same 1-year period (1972–1973) assessed at three occasions. The PDMH was comprised 1818 men (78.7 %) and 491 women veterans (21.3 %) who averaged 37.4 years old (SD = 10.1 years), and included 1109 non-Hispanic Black veterans (47.0 %) and 1200 non-Hispanic White (53.0 %) veterans. PTSD and trauma is coded as 0 = no trauma, no PTSD, 1 = trauma exposure, no PTSD, and 2 = trauma exposure and PTSD in Dunedin. Trauma exposure assessed lifetime exposure (0 = no, 1 = yes) at each age in Dunedin, and the count of trauma categories experienced in the PDMH. All models control for white blood cell count proportions, methylation smoking scores and gender; PDMH models also control for age and race/ethnicity. Gender coded 0 = men, 1 = women; PDMH race/ethnicity coded 0 = non-Hispanic Black, 1 = non-Hispanic White; CI = confidence interval.
$^*p < .05$. $^{**}p < .01$.

levels of cg05575921 at age 26 did not predict the onset of PTSD from age 26 to 45 ($OR = 0.79$, 95 % CI [0.55, 1.15], $p = .219$) or age 26 to 38 ($OR = 0.72$, 95 % CI [0.47, 1.13], $p = .155$).

Childhood adversity in the Dunedin Study. We also examined associations between adverse childhood experiences (ACEs) prior to age 18 and cg05575921 at age 26 in the Dunedin Study. People who

Table 2
Associations Between Change in cg05575921 and Onset of PTSD in the Dunedin Study.

N = 846	Model 1		Model 2	
	β	95 % CI	β	95 % CI
Predicting age 45 cg05575921				
PTSD between age 26 and 38	−0.19**	[-0.32, −0.07]	−0.18**	[-0.29, −0.07]
PTSD prior to age 26	−0.13	[-0.27, 0.01]	0.00	[-0.12, 0.13]
Sex	−0.05	[-0.14, 0.03]	−0.04	[-0.11, 0.03]
Smoking methylation score	−0.85**	[-0.87, −0.82]	−0.60**	[-0.64, −0.55]
Age 26 cg05575921			0.37**	[0.32, 0.42]
Predicting age 38 cg05575921				
PTSD between age 26 and 38	−0.09	[-0.24, 0.06]	−0.08	[-0.21, 0.04]
PTSD prior to age 26	0.17*	[-0.32, −0.03]	−0.02	[-0.13, 0.10]
Sex	−0.02	[-0.11, 0.06]	−0.01	[-0.08, 0.06]
Smoking methylation score	−0.83**	[-0.85, −0.80]	−0.46**	[-0.51, −0.41]
Age 26 cg05575921			0.50**	[0.46, 0.55]

Note: Estimates for Model 1 and Model 2 are identical with the exception of the inclusion of Age 26 values for cg05575921 as a predictor in Model 2. As such, Model 1 is predicting levels of cg05575921 at ages 38 and 45, whereas Model 2 is a residualized regression assessing change in cg05575921 while accounting for age 26 cg05575921. All models included white blood cell proportion counts as covariates.
*$p < .05$. **$p < .01$.

experienced more ACEs had significantly lower methylation at cg05575921 at age 26 (β = −0.07, 95 % CI [−0.11, −0.02], $p = .005$; Fig. 2). When included in the same model, both ACEs (β = −0.06, 95 % CI [−0.11, −0.02], $p = .007$) and a PTSD diagnosis prior to age 26 (β = −0.20, 95 % CI [−0.35, −0.05], $p = .009$) were uniquely associated with hypomethylation in cg05575921 at age 26.

Past PTSD in the PDMH Study. A subset of veterans in the PDMH study did not have current PTSD, but met criteria for PTSD in the past ($n = 124$). We tested cg05575921 levels when including veterans with past PTSD among those with current PTSD, resulting in a measure of lifetime PTSD that aligned the PTSD diagnoses in the Dunedin Study. Veterans with a history of PTSD showed hypomethylation in cg05575921 compared to veterans without a history of PTSD (β = −0.11, 95 % CI [−0.15, −0.08], $p < .001$), matching well with findings in the Dunedin Study (Fig. 3).

Stratifying by race and ethnicity in the PDMH Study. We examined whether associations for cg05575921 would replicate within the two major race/ethnicity groups in the PDMH, specifically non-Hispanic

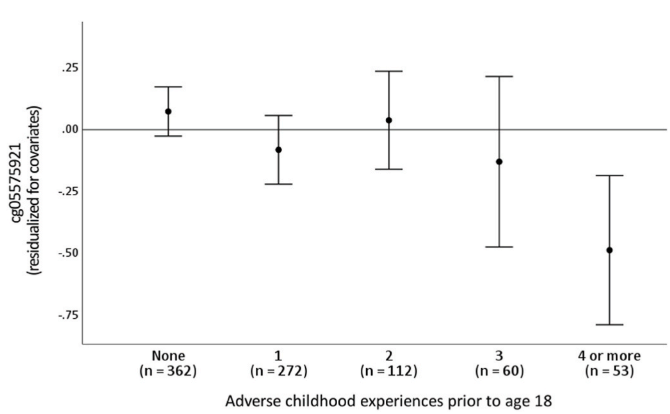

Fig. 2. Levels of cg05575921 methylation by number of adverse childhood experiences in the Dunedin Study. The Y-axis represents cg05575921 levels that have been residualized for sex, white blood cell proportions, technical PCs, and smoking methylation scores.

Fig. 3. Levels of cg05575921 by PTSD lifetime PTSD diagnostic status in the PDMH study. The Y-axis represents cg05575921 levels that have been residualized for sex, age, race/ethnicity, white blood cell proportions, and smoking methylation scores in the PDMH study.

Black veterans and non-Hispanic White veterans. Veterans with current PTSD and more self-reported PTSD symptoms showed hypomethylation at cg05575921 in both non-Hispanic Black and non-Hispanic White veteran subsamples (Supplemental Table 4), whereas trauma was associated with hypomethylation in cg05575921 among non-Hispanic White veterans, but not among non-Hispanic Black veterans.

4. Discussion

People who experienced trauma and developed PTSD showed hypomethylation at cg05575921 in *AHRR* across two independent cohorts, including 846 New Zealanders assessed at age 26, 38, and 45, and 2309 U.S. military veterans. We did not observe consistent associations for cg26703534 or cg19534438. Our results replicated the cross-sectional association between cg05575921 and traumatic stress (Katrinli et al., 2022; Smith et al., 2020) when accounting for smoking methylation scores, self-reported smoking, pack years (though associations at age 38 and 45 were attenuated in the Dunedin Study), and replicated in non-smokers when stratified by current smoking status. The results, particularly those in the Dunedin study, suggest a stepwise pattern where individuals with PTSD have the lowest levels of methylation in cg05575921, followed by those people who experienced a trauma but did not develop PTSD, and with individuals who have no experienced a trauma having the highest levels of cg05575921 (Fig. 1).

When examining longitudinal change, we found that the onset of PTSD was associated with greater hypomethylation of cg05575921 in the Dunedin Study over time. Notably, the reverse was not true—cg05575921 levels did not predict future onset of PTSD. These results support the hypothesis that the onset of PTSD is associated with changes in cg05575921, rather than the alternative that PTSD might be more likely to develop among people with hypomethylation in cg05575921. Our findings extend prior findings for cg05575921 and provide new insight into the links between the timing of traumatic stress and changes to the epigenome.

Our findings for cg05575921 are notable due to replication across two independent cohorts. The DNAm data generation for the Dunedin Study and PDMH differed in the specific methods used to account for technical variation, and both studies included multiple generations of methylation chips. We believe these differences in approach and technology are an important strength of our findings. Prior studies investigating traumatic stress and methylation using EWAS, such as those conducted by the PTSD-PCG, have largely aimed to standardize the DNAm data generation pipelines across cohorts. Standardization has the advantage of increasing reliability, which is important to maximize power for exploratory approaches. However, it is equally critical to test whether associations replicate across different methylation chips, different study teams, and different DNAm data generation pipelines. In doing so, our findings provide new evidence of generalization, which is an essential step on the potential translational pathway from EWAS to clinical applications. Said simply, if the association between a given CpG site—such as cg05575921—and traumatic stress is reliable and large enough in magnitude to have clinical utility, we would expect it to replicate across cohorts, regardless of technical variation from methylation chips, data generation pipelines, or the methods employed by the team deriving DNAm values.

The consistency of our findings linking traumatic stress and cg05575921 also extends to the populations sampled and the measurement of PTSD, trauma, and smoking. In terms of samples, the two cohorts had different compositions. The Dunedin Study included New Zealanders from a civilian birth cohort, who differed from the post-9/11 U.S. veterans in the PDMH in terms of their countries of residence, demographic characteristics, and life experiences. In terms of demographics, the Dunedin Study cohort is predominantly comprised of self-identified NZ-European individuals. The PDMH study, in contrast, is comprised of roughly half non-Hispanic Black and non-Hispanic White veterans, with largely African and European ancestry (Bourassa et al., 2024b; Brancu et al., 2017), respectively. Our results showed consistent associations not just across cohorts, but also within the non-Hispanic Black and non-Hispanic White veterans assessed independently in the PDMH, as well as across multiple ages in the Dunedin Study. In terms of measurement, there were differences in how trauma and PTSD were assessed. For the Dunedin Study, trauma and PTSD were measured independently using dichotomous measures for lifetime exposure, as well as a combined measure capturing both lifetime trauma exposure and PTSD diagnostic status. For the PDMH study, measures included both current PTSD status and symptoms, lifetime trauma burden, and past PTSD status. Regardless of the sample and measurement across cohorts, traumatic stress was associated with hypomethylation at cg05575921. Similarly, we found ACEs experienced prior to age 18 were associated with later hypomethylation at cg05575921 at age 26.

These associations were largely consistent when using several methods to account for smoking (smoking methylation scores, self-reported smoking, pack years) and when stratifying by current smoking. It is challenging to disentangle the timing and contribution of PTSD and smoking to changes in cg05575921, as smoking is both a predictor of future PTSD risk and a sequela of PTSD onset (Bourassa and Sbarra, 2024). For example, individuals who smoke are more likely to develop PTSD, and those with PTSD are also more likely to begin or continue smoking (Breslau et al., 2004; Koenen et al., 2006; Kearns et al., 2018). Given the typical onset of smoking during adolescence and early adulthood, determining the exact timing and causal pathway linking PTSD and smoking to changes in cg05575921 will likely require creative methodological approaches, such as twin studies or longitudinal samples extending from childhood into adulthood, when PTSD and smoking onsets typically occur.

Our findings are also noteworthy as we were able to investigate the time course of traumatic stress and cg05575921 across multiple assessments in the Dunedin Study. We found people who had a diagnosis of PTSD between age 26 and 45 showed increased hypomethylation in cg05575921 over that same period. Given that we did not find evidence that cg05575921 levels at age 26 predicted future PTSD onset, our results suggest the experience of traumatic stress occurs prior to hypomethylation in cg05575921. However, the relatively small number of individuals who developed PTSD between age 26 and 45 (as might be expected given the base rate of PTSD in non-military, non-clinical samples) highlights the importance of replicating this finding in other longitudinal studies of traumatic stress and methylation.

These results highlight an epigenetic marker (cg05575921) that is associated with traumatic stress and may have relevance to future health. Prior studies have linked cg05575921 to a number of negative health outcomes, including increased risk for lung disease (Bojesen et al., 2017), cancer (Jacobsen et al., 2022; Philibert et al., 2022), cardiovascular disease (Fernández-Sanlés et al., 2021; Langsted et al., 2020), and mortality (Bojesen et al., 2017), though other studies have failed to replicate these findings when controlling for smoking (Grieshober et al., 2020). As might be expected given the strong association between smoking and cg05575921, the negative health outcomes associated with cg05575921 are often attributed to downstream health consequences of exposure to tobacco smoke. However, it is unlikely smoking behavior fully explains the links between PTSD, cg05575921 and health. For example, PTSD is linked to cg05575921 among people who never smoked (Smith et al., 2020) and cg05575921 predicts myocardial infarction among former smokers (Langsted et al., 2020). The associations between cg05575921 and later health suggest changes in cg05575921 could be a biological intermediary linking trauma and PTSD to downstream changes in physiology and increased risk for poor health. For example *AHRR* is theorized to play a role in modulating immune responding (Brandstätter et al., 2016; Gutiérrez-Vásquez and Quintana, 2018) and vascular function (Reynolds et al., 2015), though future study is needed to empirically test the possible physiological pathways through which *AHRR* could link PTSD to health.

Our findings should be interpreted in the context of several limitations. First, these results are correlational and other variables could explain the association between PTSD and cg05575921. Although we controlled for smoking in three ways and stratified results by current smoking, it remains possible that unmeasured exposure to tobacco smoke better explain our results. Second, although we believe the variation in methodology between the two studies is a strength—with replication across cohorts, measures, and methods—it is possible these methodological variations obscured differences in how trauma and PTSD are linked to DNAm in each cohort. Third, our study used a confirmatory approach by investigating associations for CpGs identified by prior EWAS. It is possible that other CpGs might be associated with traumatic stress, but were not identified in previous EWAS due to a lack of statistical power. Future studies would benefit from investigating using an EWAS framework in larger samples. Finally, our cross-sectional models did not account for the time since the experience of the index traumas related to the development of PTSD, or when PTSD symptoms onset. Future studies would benefit from providing additional timeline regarding trauma, PTSD, and changes in DNAm.

5. Conclusions

We investigated associations between trauma, PTSD, and three candidate CpGs. We found that trauma and PTSD were consistently associated with hypomethylation at cg05575921 across two independent samples—a birth cohort of 846 New Zealanders followed to age 45 (the Dunedin Study) and a cohort of 2309 post-9/11 United States military veterans (the PDMH). Neither cg26703534 nor cg19534438 showed consistent associations across cohorts. We examined the timing of DNAm changes at cg05575921 and found that the experience of adversity and PTSD occurred prior to hypomethylation at cg05575921 in the Dunedin Study. In total, our findings provide evidence that hypomethylation at cg05575921 may be a mechanism through which traumatic stress could influence downstream physiology and health.

CRediT authorship contribution statement

Kyle J. Bourassa: Investigation, Formal analysis, Conceptualization, Writing – original draft, Funding acquisition, Data curation. **Melanie E. Garrett:** Writing – review & editing, Formal analysis, Software. **Lauren Hair:** Project administration, Writing – review & editing, Data curation. **Michelle Dennis:** Writing – review & editing, Data curation, Project administration. **Karen Sugden:** Supervision, Formal analysis, Writing – review & editing, Funding acquisition, Conceptualization. **Benjamin Williams:** Project administration, Conceptualization, Writing – review & editing, Methodology. **Madeline H. Meier:** Writing – review &

editing, Investigation, Methodology. **Renate Houts:** Writing – review & editing, Formal analysis, Supervision, Conceptualization. **Jean C. Beckham:** Supervision, Investigation, Conceptualization, Writing – review & editing, Project administration, Funding acquisition, VA Mid Atlantic MIRECC Workgroup, Resources, Methodology, Writing – review & editing, Project administration, Funding acquisition. **Allison E. Ashley-Koch:** Writing – review & editing, Funding acquisition, Conceptualization, Supervision, Formal analysis. **Reremoana F. Theodore:** Writing – review & editing, Methodology, Project administration, Data curation. **Richie G. Poulton:** Methodology, Project administration, Funding acquisition. **Terrie E. Moffitt:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization, Supervision, Methodology, Data curation. **Avshalom Caspi:** Writing – review & editing, Project administration, Funding acquisition, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors have no outside interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2025.07.026.

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