SEP modifies the relation between methylation of nervous-system related loci and risk of PTSD in an urban community-based sample

Monica Uddin[1], Sandro Galea[2], Shun Chiao Chang[3,] Karestan C. Koenen[3], Derek E. Wildman[4], Allison E. Aiello[1].

[1]Department of Epidemiology, University of Michigan School of Public Health; [2]Department of Epidemiology, Mailman School of Public Health, Columbia University; [3]Departments of Society, Human Development, and Health and Epidemiology, Harvard School of Public Health, Boston; [4] Center for Molecular Medicine and Genetics & Department of Obstetrics and Gynecology, Wayne State University School of Medicine.

Background:

Low socioeconomic position (SEP) has previously been linked to a number of stress-sensitive negative health outcomes, including poor mental health. The biologic mechanisms linking SEP and mental health, however, remain poorly understood. Recent work confirms that epigenetic signatures—molecular marks that regulate gene function independent of DNA sequence—can be modified by social exposures. In particular, studies focusing on DNA methylation suggest that social exposures may influence DNA methylation in a manner salient to mental health; however, studies explicitly testing this hypothesis have yet to be reported. We therefore sought to investigate whether a social exposure—specifically SEP, measured as educational attainment—modifies genomic methylation profiles to predict post-traumatic stress disorder and PTS symptom severity in a uniformly trauma exposed cohort.

Methods:

Data source

Our analyses are based on a subsample of 100 persons who had been exposed to one or more potentially traumatic events (PTEs) and who were participants in wave 1 of an ongoing longitudinal study, the Detroit Neighborhood Health Study (DNHS). The DNHS is a survey-based investigation of mental health correlates in population-representative cohort of adult Detroit residents. At wave 1, 1,547 participants were surveyed, 501 of whom also provided venipuncture specimens. For this work, 100 of these 501 participants were selected midway through the wave 1 sample collection effort in order to conduct pilot testing of epigenetic profiles associated with mental illness in community-based settings. For the analyses described below, methylation data were obtained as described in (Uddin et al., 2010). Briefly, bisulfite conversion of previously extracted, whole blood-derived genomic DNA from 100 individuals was performed on 1ug of each sample using the EZ-96 DNA Methylation^{TM} Kit from Zymo Research (Orange CA) and following the manufacturer's recommended protocol. Bisulfite converted DNA was subsequently assessed for methylation status at 27,578 CpG loci covering more than 14,000 genes using the humanmethylation27 (HM27) DNA Analysis BeadChip by Illumina (San Diego, CA). The resulting data were background normalized using Ilumina's BeadStudio software and exported for subsequent analysis using the R package v2.9.0 and SAS software v9.2.

Mental health assessment and other survey-based variables

Presence or absence of lifetime post-traumatic stress disorder (PTSD) was assessed according to DSM-IV criteria. The diagnosis of PTSD was evaluated using the PTSD checklist (PCL-C), a 17-item self-report measure of post-traumatic symptoms based on the DSM-IV criteria. Additional questions about duration, timing, and impairment or disability due to the symptoms were also assessed. Participants rated each of the 17 PTSD symptoms on a scale indicating the degree to which the respondent had been bothered by a particular symptom as a result of the worst trauma from 1 (not at all) to 5 (extremely). The PTS symptom severity measure was then defined as the sum score, which can range from 17 to 85. Presence/absence of additional comborbid disorders (depression and generalized anxiety disorder) was also assessed. Additional survey-based variables included in this study were: race, sex, and age; whether a participant had ever smoked or ever drank; and socioeconomic position (SEP), assessed via participants' reported highest level of educational attainment and dichotomized according to more than high school (high SEP) or high school or less (low SEP). High SEP was used as the referent group in the analyses described below.

Analytic methods

In order to ensure our results were robust to quantitative and qualitative assessments of PTSD, we modeled a discrete and a continuous outcome. Presence/absence of lifetime PTSD was modeled using logistic regression. PTSD symptom severity was modeled using the general linear model. The severity measure was log-transformed for normality. We assessed main effects of each outcome across all CpG sites on the array using the methylation beta value and SEP as predictors, controlling for demographic characteristics (age, race, sex), behavioral characteristics (smoking, depression, GAD, medication use), and peripheral blood mononuclear cell (PBMC) count. Continuous variables such as age, PBMC count, and methylation beta values were centered to the mean. Following main effect analyses, we assessed the presence/absence of methylation x SEP interactions across all CpG sites on the array by including an interaction term in the main effects model. In the main effects model, effect estimates for gene methylation value and SEP were accepted as significant if $p<0.01$. In the interaction models, interaction terms were accepted as significant if $p<0.01$.

Functional analyses of genes showing (1) significant methylation effects in main effect models (2) significant SEP effects in main effect models and (3) significiant methylation x SEP interactions in interaction models were conducted for each of the two outcomes. Analyses were performed using the functional annotation clustering (FAC) tool in DAVID, which clusters similar annotations based on the co-occurrence of particular gene sets. Clusters were identified by selecting the overrepresented annotation that conveyed the broadest biological meaning within each FAC.

Preliminary results

The distribution of the demographic, behavioral and other study characteristics are presented in Table 1.

In main effect models assessing lifetime PTSD, significant coefficients were obtained for methylation beta values in 118 CpG sites, corresponding to 116 unique

genes. In main effect models assessing PTS symptom severity, significant coefficients were obtained for methylation beta values in 80 CpG sites, corresponding to 79 unique genes. Significant coefficient estimates for SEP were obtained for only a few CpG sites in main effect models assessing lifetime PTSD (n=1) and PTS symptom severity (n=6), preventing analysis with the FAC tool. Results for the top three FACs for genes showing significant methylation beta value coefficients are summarized in Table 2 for both outcomes. Two of the three FACs (secreted, response to nutrient) were comprised by very similar annotations for both lifetime PTSD and PTS symptom severity, although their rank order differed slightly.

In interaction models assessing lifetime PTSD, significant methylation x SEP interaction coefficients were obtained for 119 CpG sites, corresponding to 119 unique genes. In main effect models assessing PTS symptom severity, significant methylation x SEP interaction coefficients were obtained for 55 CpG sites, corresponding to 55 unique genes. Results for the top 3 FACs for genes showing significant methylation x SEP interaction terms are summarized in Table 3 for both outcomes. Both outcomes showed evidence of SEP effect modification characterized by FACs relating to the nervous system; however, in contrast to the main effect results, the specific annotations comprising these FACs differed between PTSD and PTS symptom severity: the top three FACs for PTS symptom severity were comprised of annotations relating to synapse (e.g. postsynaptic cell membrane, synapse part), GTPase regulator activity and oxidation reduction (oxioreductase, mitochondrion). In contrast, the top three FACs for PTSD were comprised of annotations relating to hippocampus development, forebrain development, and RNA transport.

Preliminary conclusions

These results demonstrate that SEP modifies the relation between methylation and risk of PTSD in genes predominantly related to nervous system function. This pattern was observed for both dichotomous and continuous measures of PTSD, suggesting that the observed effect modification by SEP is consistent across qualitative and quantitative assessments of this disorder. The identification of hippocampus-related annotations in the PTSD-based analyses is especially noteworthy, in light of work indicating that this brain region is especially sensitive to stress and environmental enrichment. Taken together, results from both PTSD- and PTS symptom severity analyses help to shed light on how social exposures, such as relative deprivation measured here by SEP, may interact with epigenotype to predict risk of traumatic stress-related outcomes.