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Epigenetic Signatures May Explain the Relationship between Socioeconomic Position and Risk of Mental Illness: Preliminary Findings from an Urban Community-Based Sample

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Low socioeconomic position (SEP) has previously been linked to a number of negative health indicators, including poor mental health. The biologic mechanisms linking SEP and mental health remain poorly understood. Recent work suggests that social exposures influence DNA methylation in a manner salient to mental health. We conducted a pilot investigation to assess whether SEP, measured as educational attainment, modifies the association between genomic methylation profiles and traumatic stress in a trauma–exposed sample. Results show that methylation × SEP interactions occur preferentially in genes pertaining to nervous system function, suggesting a plausible

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biological pathway by which SEP may enhance sensitivity to stress and, in turn, risk of posttraumatic stress disorder.

[Supplementary materials are available for this article. Go to the publisher's online edition of Biodemography and Social Biology *for the following free supplemental resource: Supplementary tables of full model and functional annotation clustering results.]*

Introduction

Epigenetics refers to the stable yet modifiable regulation of gene function that occurs through non-DNA-encoded mechanisms. Epigenetic mechanisms include histone modifications such as acetylation, phosphorylation, and ubiquitination, which cause structural changes to chromatin and make surrounding DNA sequences inaccessible (Tsankova et al. 2007); DNA methylation, which typically involves methylation of cytosine residues at CpG positions and often results in repression of nearby genes (Eckhardt et al. 2006); and nonprotein-coding RNAs such as micro-RNAs, which can interact with other epigenetic mechanisms to regulate epigenetic processes such as chromatin modification (Mattick et al. 2009) and DNA methylation (Havecker et al. 2010).

DNA methylation in particular has received attention as a potential epigenetic mediator and moderator of environmental exposures on health-related outcomes. Much of this interest originally stemmed from the now well-established links between environmental chemical exposures and both global and locus-specific changes in DNA methylation (reviewed in Hou et al. 2012) and has since grown as a result of the demonstrated ability of DNA methylation to change in response to aging (Bjornsson et al. 2008; Wong et al. 2010) and experiences early in life (Champagne et al. 2006; Weaver et al. 2004). Interest in the contribution of environmentally induced DNA methylation changes to mental illness has been particularly pronounced, as numerous examples exist of epigenetic associations with schizophrenia (Abdolmaleky et al. 2006; Abdolmaleky et al. 2005), suicide (McGowan et al. 2008), and bipolar disorder (Mill et al. 2008), as well as illnesses that are more closely associated with environmental conditions (Shih, Belmonte, and Zandi 2004), such as depression (Uddin, Koenen, et al. 2011) and posttraumatic stress disorder (PTSD) (Koenen et al. 2011; Smith et al. 2011; Uddin et al. 2010; Uddin, Galea, et al. 2011). Such examples, however, do not typically assess important *social* determinants that may act as environmental exposures contributing to risk for mental illness.

There is abundant literature showing that social exposures are associated with health (e.g., Due et al. 2011; Safaei 2006). Of particular interest in this regard is socioeconomic position (SEP), a fundamental determinant of health (Galea et al. 2011; Link and Phelan 1995). Two major pathways have been identified in the literature as possible routes linking SEP to health: from a material perspective, individuals of relatively higher SEP may access resources, such as health insurance, quality of food consumed, and higher education, that promote health (Lynch et al. 2000); from a psychosocial perspective, individuals of relatively lower SEP may be exposed to a greater number of stressful events and/or may be more vulnerable to the consequences of these events, ultimately resulting in poor health (McEwen and Seeman 1999). These material and psychosocial pathways are not mutually exclusive. Although the link between low SEP and a number of stress-sensitive negative health outcomes (Ahern and Galea 2006; Cairney and Krause 2005; Dowd, Aiello, and Alley 2009; Dowd et al. 2008; Evans and Kim 2007; Nicklett and Burgard 2009; Toumbourou et al. 2007; Zajacova, Dowd, and Aiello 2009), including poor mental health (Ahern and Galea 2006; Brewin, Andrews, and Valentine 2000; Cairney and Krause 2005; Nicklett and Burgard 2009), may be considered as support for the psychosocial pathway

explanation, material resources are often also associated with psychosocial processes, suggesting that it is likely a combination of the two pathways that ultimately links SEP and mental health (Kawachi, Subramanian, and Almeida-Filho 2002). Both mechanisms, however, rely on the assumption that some biological process (or processes) exists that explains how exogenous factors—in this case, SEP—ultimately manifest as endogenously determined phenotypes. It remains an open question how exposures on either pathway effect their downstream influence(s) on health.

The modifiable yet nontransient nature of epigenetic signatures makes them plausible biological candidates that account for how exogenous factors that change, like social status, are associated with poor health indicators, including poor mental health. Indeed, recent work suggests that social exposures can influence epigenetic markers in a manner salient to health in general (Borghol et al. 2012; McGuinness et al. 2012; Talens et al. 2012), and mental health in particular. One prominent example pertinent to the latter involves a large, population-based epidemiologic study of the legacy of the Dutch Hunger Winter of 1944–1945, which recently established an association between social deprivation and methylation status in the insulin-like growth factor II gene (*IGF2*) (Heijmans et al. 2008; Tobi et al. 2012), with those exposed to nutritional insufficiency during the periconception period showing lower methylation at this locus. The *IGF2* locus plays a key role in development and growth (Delaval, Wagschal, and Feil 2006), and defects in methylation at this locus are known to contribute to several imprinting-related disorders, such as Silver-Russell syndrome and Beckwith-Wiedemann syndrome (Bartholdi et al. 2009; Riccio et al. 2009). Notably, brain weight in males is positively correlated with DNA methylation at *IGF2* (Pidsley, Dempster, and Mill 2010), and, in turn, low brain weight has been associated with schizophrenia (Harrison, Freemantle and Geddes 2003), an association that emerged within the Dutch Hunger Winter cohort (Hulshoff Pol et al. 2000). Together, these observations suggest that social exposures may influence DNA methylation in a manner salient to mental health; however, studies explicitly testing this hypothesis have, to our knowledge, yet to be reported.

To address this issue, here we conduct a pilot investigation of whether SEP, measured as educational attainment, modifies DNA methylation profiles to predict traumatic stress in a trauma-exposed sample. We focus on SEP because of its strong association with a range of health indicators in the literature (Ahern and Galea 2006; Cairney and Krause 2005; Dowd, Aiello, and Alley 2009; Dowd et al. 2008; Evans and Kim 2007; Nicklett and Burgard 2009; Steptoe et al. 2010; Toumbourou et al. 2007; Zajacova, Dowd, and Aillo 2009), including those related to traumatic stress (Brewin, Andrews, and Valentine 2000; Koenen et al. 2002; Koenen et al. 2007; Kulka et al. 1990); emerging work also indicates that SEP is linked to modifiable molecular variation (Miller et al. 2009), including DNA methylation (Borghol et al. 2012; McGuinness et al. 2012). Drawing on samples from an urban, community-based cohort, the Detroit Neighborhood Health Study (DNHS), we assess methylation-SEP interactions at over 27,000 CpG sites, representing over 14,5000 genes on the HM27 BeadChip, to predict traumatic stress and characterize the biological significance of the profiles showing nominally significant methylation-SEP interactions.

Methods

Participants

Our analyses are based on a subsample of 100 persons who were exposed to one or more potentially traumatic events (PTEs) and were participants in Wave 1 of the longitudinal DNHS. The DNHS is a survey-based investigation of mental health correlates in a

population-representative cohort of adult Detroit residents. At Wave 1, 1,547 participants were surveyed; the full survey sample was representative of the Detroit population on key sociodemographic variables including age, gender, race, income, and educational attainment (Uddin et al. 2010). Respondents were also asked to provide a blood specimen by way of either venipuncture or blood spot; 612 samples were collected from consenting participants. Two-tailed chi-square tests comparing our blood sample to the full survey sample showed that our sample's sociodemographic characteristics were comparable to those of the complete sample. Similarly, we compared the 100 individuals in our final sample to the 612 consenting participants of the blood draw and found that the two samples differ only on age, with our final sample consisting of a slightly higher proportion of younger individuals (Uddin et al. 2010).

For the analyses described in the following, methylation data were obtained as described previously (Uddin et al. 2010). Briefly, bisulfite conversion of previously extracted, whole blood–derived genomic DNA from 100 individuals was performed on 1μg of each sample using the EZ-96 DNA Methylation™ 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 Illumina's BeadStudio software and exported for subsequent analysis using the R package v.2.9.0 and SAS software v.9.2. Methylation microarray data were validated in a subset of loci as previously described (Koenen et al. 2011; Uddin et al. 2010) Peripheral blood mononuclear cells (PBMCs) were isolated and quantified (Uddin et al. 2010; Uddin, Koenen, et al. 2011). Data on participants' use of over-the-counter and prescription medication, including anti-anxiety medications and antidepressants, were also collected by clinicians during in-home visits, during which the venipuncture specimens were also collected. This study was approved by the institutional review board (IRB) of the University of Michigan, and all participants provided written, informed consent.

Mental Health Assessment and Other Survey-Based Variables

Participants were administered a 40-minute telephone assessment that included questions on exposure to traumatic events; sociodemographic and behavioral characteristics; and a standardized assessment of PTSD, depression, and generalized anxiety disorder (GAD), as previously described (Uddin et al. 2010).

Briefly, presence or absence of lifetime PTSD was assessed using the PTSD checklist (PCL-C) (Weathers and Ford 1996), a 17-item self-report measure of posttraumatic stress symptoms based on DSM-IV criteria, augmented by additional questions about duration, timing, and impairment or disability resulting from the symptoms in order to identify PTSD cases that were compatible with DSM-IV criteria. Participants were initially asked to identify PTEs that they had experienced in the past from a list of 19 events that had previously been used in an earlier epidemiologic study to assess PTSD in the Detroit area (Breslau et al. 1998); an additional question allowed the participant to briefly describe any other extraordinarily stressful situation or event that he or she had experienced. We then asked those participants who had experienced at least one traumatic event to choose which one they considered to be the worst. 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 this trauma, with responses ranging from 1 (not at all bothered) to

5 (extremely bothered). An additional PTSD section assessed symptoms based on a randomly chosen traumatic event (excluding the worst event) for those participants who had experienced more than one PTE. Respondents were considered affected by lifetime PTSD if all six DSM-IV criteria were met in reference to either the worst or the random event. The PTS symptom severity measure was then defined as the sum score of symptoms based on the worst event, which can range from 17 to 85.

To validate our identification of PTSD obtained from the telephone interview responses, we conducted clinical in-person interviews among a random subsample of 51 participants. A licensed clinician conducted one-hour clinical interviews after obtaining signed consent from participants, utilizing the Clinician-Administered PTSD Scale for DSM-IV (CAPS). The counselor was blinded to the information obtained from the participants during the telephone interview. Analysis of data from the in-person interviews showed that the PCL-C used during the telephone interviews had excellent internal consistency and high concordance. The PCL-C yielded a Cronbach coefficient alpha (α) of 0.93. Using clustering scoring based on DSM-IV criteria (i.e., to be a case, the participant's symptoms had to meet all six criteria), the instrument had a sensitivity (SE) of 0.24, specificity (SP) of 0.97, positive predictive value (PPV) of 0.80, negative predictive value (NPV) of 0.72, and an area under the ROC curve (AUC) of 0.76. Low sensitivity values imply that our survey-based PTSD prevalence estimates are conservative. Importantly, the high specificity ensures that our PTSD group is composed of true cases. All 100 individuals included in this pilot study had been exposed to at least one PTE; among these, 23 were affected by lifetime PTSD and 77 were trauma-exposed but unaffected.

Additional survey-based variables included in this study were demographic variables including race, sex, and age; number of traumatic events, which was a count of the different types of PTEs, ranging from 0 to 19 for each person; and whether a participant had ever smoked, a variable included because of the known influence of smoking on DNA methylation levels (Breitling et al. 2011). SEP was assessed on the basis of participants' reported highest level of educational attainment in order to capture cumulative social exposures, including both the educational opportunities of the individual participant and the opportunities and constraints encountered by the participant's parent(s) when making choices that influenced their children's socioeconomic circumstances (Galobardes, Lynch, and Smith 2007). Consistent with the evidence that attainment of more than a high school education is associated with improved health (Rogers et al. 2010), analyses were performed with SEP 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 next.

Analytic Methods

In this study we assess traumatic stress not only as a dichotomous outcome (i.e., PTSD), but also as a continuous (i.e., PTS symptom severity) outcome in order to (1) ensure that our results are robust to qualitative and quantitative assessments of PTSD; and (2) ascertain biological processes that may be operating at subthreshold levels, that is, processes that may not be apparent based on a strictly dichotomous PTSD diagnosis (Hawk et al. 2000). Presence or absence of lifetime PTSD was modeled using logistic regression. PTSD symptom severity was modeled using a general linear model. The severity measure was log-transformed for normality. We assessed the main effects of each outcome across all CpG sites on the array using the methylation beta value and SEP as predictors and controlling for demographic characteristics (age, race, sex), behavioral characteristics (smoking,

depression, GAD, medication use), and PBMC count. Methylation beta values were centered to the mean in both the PTSD and PTS symptom severity models. Following main effect analyses, we assessed the presence or absence of methylation-SEP interactions across all CpG sites on the array by including an interaction term in the main effects model and using high SEP as the reference group. In the main effects model, coefficients for gene methylation value and SEP were accepted as significant if $p < .01$ (uncorrected for multiple testing). In the interaction models, methylation-SEP interaction terms were accepted as significant if $p < .01$ (uncorrected for multiple testing).

Functional analyses of genes showing significant methylation-SEP interactions were performed using the functional annotation clustering (FAC) tool in the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang da, Sherman, and Lempicki 2009). DAVID is a publicly available resource that provides a comprehensive set of functional annotation tools to help investigators understand the biological meaning behind large sets of genes. In this study, results were obtained using the FAC tool, which clusters similar annotations based on the co-occurrence of particular gene sets. The tool also calculates an associated enrichment score for each cluster based on the geometric mean of the p values determined for each of its component annotations (which is then reported in log scale). For the FAC analyses reported here, options were set to their default values and annotations were accessed as indexed in July 2011. Clusters were identified by selecting overrepresented annotations that conveyed broad biological meaning within each FAC.

Results

Descriptive statistics and bivariate comparisons of participants with and without PTSD are presented in Table 1. The majority of the study sample was female and African American. The average PTS symptom severity was 38.7 ($SD = 16.01$). Participants with PTSD did not differ significantly from those without the disorder on age, gender, race/ethnicity, PBMC count, report of ever smoking, medication use, or SEP (education).

In main effect models assessing lifetime PTSD, significant coefficients (uncorrected $p < .01$) 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 (uncorrected $p < .01$) were obtained for methylation beta values in 80 CpG sites, corresponding to 79 unique genes. 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) comprised very similar annotations for both lifetime PTSD and PTS symptom severity, although their rank order differed slightly. Significant coefficient estimates (uncorrected $p < .01$) for SEP were obtained for only a few CpG sites in main effect models assessing lifetime PTSD ($n = 1$) and PTS symptom severity ($n = 6$), precluding their analysis with the FAC tool.

In interaction models assessing lifetime PTSD, significant methylation-SEP interaction coefficients (uncorrected $p < 0.01$) were obtained for 119 CpG sites, corresponding to 119 unique genes. In interaction models assessing PTS symptom severity, significant methylation-SEP interaction coefficients (uncorrected $p < 0.01$) were obtained for 55 CpG sites, corresponding to 55 unique genes. Nine CpG sites, corresponding to nine different genes, showed significant interaction effects for both outcomes; results of the main study predictors (CpG methylation, SEP, and their interaction, if applicable) for both main effect and interaction models are shown for these nine genes in Tables 3–6. Full results are presented for main effect and interaction models for these nine genes in Supplementary Tables 10–13, which are available alongside the online edition of this article.

Table 1
Descriptive statistics and bivariate comparisons of participants with and without PTSD

	Overall sample		PTSD ($n = 23$)		No PTSD ($n = 77$)		Test
	n/mean	%/SD	n/mean	%/SD	n/mean	%/SD	p
Age	45.32	16.78	46.70	15.19	44.91	17.29	.66
No. traumatic events	6.04	3.60	7.57	3.65	5.58	3.48	.02
No. PBMC	23.3376	8.05	23.29	7.25	23.35	8.31	.97
Female	60	60	15	65.22	45	58.44	.56
White	14	14	4	17.39	10	12.99	.61
African American	79	79	17	73.91	62	80.52	–
Others	7	7	2	8.7	5	6.49	–
Any medication	48	48	12	52.17	36	46.75	.65
Ever smoke	58	58	17	73.91	41	53.25	.08
PTSD	23	23	–	–	–	–	–
PTS symptom severity	38.7	16.01	56.70	11.86	33.32	12.88	<.0001
Generalized anxiety disorder diagnosis	17	17	10	43.48	7	9.09	<.0001
Depression diagnosis	33	33	12	52.17	21	27.27	.03
Low education	50	50	14	60.87	36	46.75	.23

Table 2
Functional annotation cluster analysis of genes showing significant methylation beta value coefficients in main effect analyses

Outcome	Cluster	Genes in cluster (n)	Enrichment score
PTSD	Cell adhesion	12	2.43
	Response to nutrient	8	1.88
	Secreted	37	1.34
PTS symptom severity	Secreted	22	1.26
	Response to nutrient	4	1.23
	Cell fraction	10	1.17

Notes: Functional annotation clusters (FACs) were bioinformatically inferred using DAVID (see Methods section for more details). Significance assessed at the $p < 0.01$ level. All genes are identified in terms of DAVID IDs; genes can appear in more than one FAC.

Results for the top three FACs for genes showing significant methylation-SEP interaction terms are summarized in Table 7 for both PTSD and PTS severity. 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 displayed in these FACs differed between PTSD and PTS symptom severity: the top three FACs for PTS symptom severity comprised annotations relating to synapse (e.g., postsynaptic cell membrane, synapse part), GTPase regulator activity, and oxidation reduction (oxioreductase,

Table 3
Main effect logistic regression model results of SEP and methylation predicting lifetime PTSD ($n = 100$)

CpG site	Gene symbol	RefSeq	b_{educ}	Se_{educ}	p_{educ}	b_{meth}	Se_{meth}	p_{meth}
cg04958389	*PRSS2*	NM_002770	1.07	0.65	0.10	0.75	2.51	0.77
cg06917325	*SLC22A8*	NM_004254	1.06	0.67	0.11	−0.49	2.47	0.84
cg08573687	*TH*	NM_199292	1.10	0.65	0.09	−2.21	2.82	0.43
cg14870461	*AER61*	NM_173654	1.13	0.65	0.08	−4.55	5.01	0.36
cg16426459	*MLPH*	NM_024101	1.07	0.65	0.10	0.87	3.25	0.79
cg16869108	*VHL*	NT_022517	1.12	0.65	0.08	−1.24	1.67	0.46
cg22753768	*BAP1*	NM_004656	1.11	0.64	0.09	4.44	5.20	0.39
cg26049501	*STARD13*	NM_052851	1.08	0.64	0.09	−2.70	4.41	0.54
cg26912636	*TMEPAI*	NM_020182	1.10	0.64	0.09	−0.13	2.91	0.96

Notes: Covariates are age, gender, race, sumpbmc (number of PBMCs), smoke, anymed (any medication), dep (depression), gad (generalized anxiety disorder), sumpte (number of potentially traumatic experiences), SEP (education), and gene methylation value. b = beta coefficient; se = standard error; p = *p* value.

Table 4
Main effect linear regression results of SEP and methylation predicting lifetime PTS symptom severity ($n = 100$)

CpG site	Gene symbol	RefSeq	b_{educ}	Se_{educ}	t_{educ}	p_{educ}	b_{meth}	Se_{meth}	t_{meth}	p_{meth}
cg04958389	*PRSS2*	NM_002770	0.15	0.07	2.02	0.05	0.13	0.27	0.50	0.62
cg06917325	*SLC22A8*	NM_004254	0.16	0.08	2.12	0.04	0.11	0.29	0.39	0.70
cg08573687	*TH*	NM_199292	0.15	0.07	2.07	0.04	−0.19	0.45	−0.42	0.67
cg14870461	*AER61*	NM_173654	0.15	0.07	2.05	0.04	0.42	0.48	0.89	0.38
cg16426459	*MLPH*	NM_024101	0.15	0.07	2.10	0.04	0.54	0.41	1.31	0.19
cg16869108	*VHL*	NT_022517	0.15	0.07	2.08	0.04	0.03	0.20	0.15	0.88
cg22753768	*BAP1*	NM_004656	0.16	0.07	2.13	0.04	0.72	0.56	1.29	0.20
cg26049501	*STARD13*	NM_052851	0.15	0.07	2.09	0.04	−0.34	0.47	−0.71	0.48
cg26912636	*TMEPAI*	NM_020182	0.16	0.07	2.11	0.04	0.31	0.34	0.92	0.36

Notes: Covariates are age, gender, race, sumpbmc (PBMC), smoke, anymed (any medications), dep (depression), gad (generalized anxiety disorder), sumpte (potentially traumatic experiences), SEP (education), and gene methylation value. b = beta coefficient; se = standard error; t = *t* value; p = *p* value.

mitochondrion). In contrast, the top three FACs for PTSD comprised annotations relating to hippocampus development, forebrain development, and RNA transport.

Further investigation of the CpG sites associated with significant interactions in the lifetime PTSD model revealed that 46 of the 119 sites showed positive interaction coefficients—that is, these sites were associated with increased risk of PTSD. FAC analyses of the 46 genes associated with these 46 sites determined that the top-ranked cluster was characterized by the nervous system–related annotation of synaptic transmission (Table 8); in contrast, FAC analyses of the 73 genes associated with the 73 CpG sites showing negative interaction coefficients did not reveal any annotations relating directly to nervous system function in the top three clusters.

Table 5
Interaction effect logistic regression model results of SEP-methylation predicting lifetime PTSD ($n = 100$)

CpG site	Gene symbol	RefSeq	b_{educ}	Se_{educ}	p_{educ}	b_{meth}	Se_{meth}	p_{meth}	b_{Int}	Se_{Int}	p_{Int}
cg04958389	*PRSS2*	NM_002770	0.85	0.73	0.24	−3.51	2.34	0.13	16.23	6.29	< 0.01
cg06917325	*SLC22A8*	NM_004254	1.18	0.74	0.11	−12.46	5.01	0.01	19.87	7.43	< 0.01
cg08573687	*TH*	NM_199292	0.17	0.80	0.83	−197.48	62.00	0.00	194.55	61.54	< 0.01
cg14870461	*AER61*	NM_173654	1.12	0.79	0.16	13.05	6.80	0.06	−44.06	15.36	< 0.01
cg16426459	*MLPH*	NM_024101	1.65	0.76	0.03	24.24	8.45	0.00	−30.48	10.55	< 0.01
cg16869108	*VHL*	NT_022517	1.52	0.80	0.06	−6.76	2.57	0.01	15.64	5.17	< 0.01
cg22753768	*BAP1*	NM_004656	1.27	0.77	0.10	−11.95	8.50	0.16	40.17	14.94	< 0.01
cg26049501	*STARD13*	NM_052851	1.79	0.82	0.03	−25.19	10.04	0.01	34.02	12.78	< 0.01
cg26912636	*TMEPAI*	NM_020182	1.62	0.80	0.04	−14.98	5.72	0.01	23.69	7.61	< 0.01

Notes: Covariates are age, gender, race, sumpbmc (PBMC), smoke, anymed (any medications), dep (depression), gad (generalized anxiety disorder), sumpte (potentially traumatic experiences), SEP (education), and gene methylation value. b = beta coefficient; se = standard error; p = *p* value.

Table 6
Interaction effect linear regression results of SEP-methylation predicting lifetime PTS symptom severity ($n = 100$)

CpG site	Symbol	RefSeq	b_{educ}	Se_{educ}	t_{educ}	p_{educ}	b_{meth}	Se_{meth}	t_{meth}	p_{meth}	b_{Int}	Se_{Int}	t_{Int}	p_{Int}
cg04958389	*PRSS2*	NM_002770	0.13	0.07	1.87	0.07	−0.37	0.30	−1.22	0.22	1.68	0.55	3.06	< 0.01
cg06917325	*SLC22A8*	NM_004254	0.14	0.07	1.95	0.05	−1.01	0.50	−2.00	0.05	1.58	0.59	2.68	< 0.01
cg08573687	*TH*	NM_199292	0.00	0.09	0.01	0.99	−16.07	5.01	−3.21	0.00	15.96	5.01	3.18	< 0.01
cg14870461	*AER61*	NM_173654	0.16	0.07	2.28	0.03	2.04	0.72	2.81	0.01	−2.76	0.96	−2.88	< 0.01
cg16426459	*MLPH*	NM_024101	0.16	0.07	2.25	0.03	2.60	0.73	3.57	0.00	−2.85	0.85	−3.34	< 0.01
cg16869108	*VHL*	NT_022517	0.15	0.07	2.17	0.03	−0.43	0.24	−1.76	0.08	1.25	0.40	3.11	< 0.01
cg22753768	*BAP1*	NM_004656	0.16	0.07	2.32	0.02	−0.48	0.66	−0.73	0.46	3.76	1.19	3.16	< 0.01
cg26049501	*STARD13*	NM_052851	0.16	0.07	2.17	0.03	−1.32	0.58	−2.26	0.03	2.60	0.97	2.69	< 0.01
cg26912636	*TMEPAI*	NM_020182	0.16	0.07	2.22	0.03	−0.60	0.45	−1.33	0.19	1.94	0.65	2.97	< 0.01

Notes: Covariates are age, gender, race, sumpbmc (PBMC), smoke, anymed (any medications), dep (depression), gad (generalized anxiety disorder), sumpte (potentially traumatic experiences), education, and gene methylation value. b = beta coefficient; se = standard error; t = *t* value; *p* = *p* value.

Table 7
Functional annotation cluster analysis of genes showing significant methylation-SEP interactions

Outcome	Cluster	Genes in cluster (*n*)	Enrichment score
PTSD	Hippocampus development	6	1.88
	Forebrain development	8	1.60
	RNA transport	4	1.50
PTS symptom severity	Synapse	6	1.18
	GTPase regulator activity	5	1.13
	Oxidation reduction	8	0.89

Notes: Functional annotation clusters (FACs) were bioinformatically inferred using DAVID (see Methods section for more details). Significance assessed at the $p < .01$ level. All genes are identified in terms of DAVID IDs; genes can appear in more than one cluster.

Table 8
Functional annotation cluster analysis of genes showing significant methylation-SEP interactions in PTSD

Interaction coefficient	Cluster	Genes in cluster (*n*)	Enrichment score
Positive	Synaptic transmission	6	1.41
	Regulation of GTPase activity	4	1.19
	GTPase regulator activity	4	1.09
Negative	Disulfide bond	26	0.85
	EGF-like	3	0.85
	Membrane	39	0.83

Notes: Functional annotation clusters (FACs) were bioinformatically inferred using DAVID (see Methods section for more details). Significance assessed at the $p < .01$ level. All genes are identified in terms of DAVID IDs; genes can appear in more than one cluster.

Similarly, in the PTS symptom severity model, 38 of the 55 CpG sites associated with significant interactions showed positive interaction coefficients, that is, they were associated with increased risk of PTS symptom severity. FAC analyses of the 38 genes associated with these 38 sites determined that among the top three highest-ranking clusters, the nervous system–related annotation of neuron projection characterized FAC 3 (Table 9); in contrast, FAC analyses of the 17 genes associated with the 17 CpG sites showing negative interaction coefficients did not reveal any annotations relating directly to nervous system function in the top three clusters. Results of all FAC analyses are listed in Supplementary Tables 14–21.

Discussion

Converging evidence from epidemiologic, molecular genetic, and animal model studies suggests that social exposures can influence DNA methylation in a manner salient to mental health. Using a subsample of participants from the DNHS, we conducted a pilot

Table 9
Functional annotation cluster analysis of genes showing significant methylation-SEP interactions in PTS symptom severity

Interaction coefficient	Cluster	Genes in cluster (*n*)	Enrichment score
Positive	Plasma membrane	20	1.31
	Ion transport	8	0.95
	Neuron projection	6	0.83
Negative	Small GTPase regulator activity	4	1.16
	DNA binding	9	0.94
	Nonmembrane-bound organelle	6	0.15

Notes: Functional annotation clusters (FACs) were bioinformatically inferred using DAVID (see Methods section for more details). Significance assessed at the $p < .01$ level. All genes are identified in terms of DAVID IDs; genes can appear in more than one cluster.

investigation of whether SEP modifies the association between genomic DNA methylation profiles and traumatic stress in a trauma-exposed cohort. We found that significant (uncorrected $p < .01$) methylation-SEP interactions occur preferentially in CpG sites associated with genes relating to nervous system function (which were notably absent from main effect analyses). Furthermore, when functional significance was assessed separately for CpG sites associated with significant positive and negative interaction coefficients, nervous system–related functions were especially apparent in the positive (i.e., risk-enhancing) gene set. Although the brain is the central organ mediating stress processes (McEwen 2007), many of the brain regions hypothesized to link SEP-related stress to health also regulate peripheral stress response axes that are important for health, including peripheral physiological reactivity (McEwen and Gianaros 2010). Taken together, these preliminary results suggest that SEP may preferentially modify DNA methylation profiles in biological pathways that enhance stress sensitivity and reactivity, which may, in turn, increase risk of PTSD.

Among the many annotations relating to nervous system function identified in this study, the identification of hippocampus-related annotations in the PTSD-based analyses is particularly noteworthy. The hippocampus is a brain region that originates from the telencephalon portion of the forebrain during development (Lagali, Corcoran, and Picketts 2010); is involved in memory, learning, and emotional regulation (McEwen 2001; McEwen 2007); and is known to be affected by stress (Sapolsky et al. 1990), including PTSD (Zhang, Tan, et al. 2011). Evidence from animal models has shown that chronic social stress can remodel this brain region (McEwen 2007), and human imaging studies indicate that chronic stressors are associated with decreased hippocampal volume, even in otherwise healthy individuals (Gianaros et al. 2007). Of importance to this study, recent work has found that children from lower-income households show lower hippocampal gray matter density, controlling for gender, age, parental education, and whole brain volume (Hanson et al. 2011). This finding is consistent with previous hypotheses regarding the likely neurobiological pathways that translate social stress, and particularly socioeconomic stress, into cognitive and health-related outcomes (Gianaros and Manuck 2010; Hackman and Farah 2009; McEwen and Gianaros 2010). Our own work, which is based on DNA methylation levels assessed in peripheral blood, cannot be tied directly to these brain-specific findings; nevertheless, a growing literature is exploring the concordance between molecular signatures obtained in central and peripheral tissues (Kato, Kakiuchi, and Iwamoto 2007; Kurian

et al. 2011; Le-Niculescu et al. 2009; Rollins et al. 2010), partly because of the existence of receptors for neurotransmitters that are expressed on both neurons and lymphocytes, including glucocorticoid receptors, dopamine receptors, GABA-A receptors, muscarinic and nicotinic receptors, serotonin receptors, and beta-adrenergic receptors (Gladkevich, Kauffman, and Korf 2004). Given the ready availability of peripheral samples and the scarcity of CNS tissues from living individuals, additional studies in this area are warranted.

Our study should be interpreted in light of a number of limitations. Chief among these is that our results were determined based on analyses that were not corrected for multiple testing; indeed, had we done so, none of our findings would have reached statistical significance. Instead, we adopted an uncorrected p value of $p < .01$ as a cutoff for subsequent functional analyses in order to aid our primary goal of identifying biological pathways that may be associated with DNA methylation modifications by SEP. In addition, while biologically meaningful interactions were detected between SEP and methylation in our study, the analyses presented in this work are cross-sectional, and we are thus unable to determine whether SEP exposures are truly causative of increased risk of PTSD. Our reliance on education as our SEP measure, however, is consistent with this hypothesis, as it is thought to represent a more cumulative index of SEP exposure (Galobardes, Lynch, and Smith 2007) that shapes health indicators throughout adult life. In addition, while we assessed DNA methylation variation at thousands of sites across the genome, providing a comprehensive picture of genome-scale variation, there are likely additional significant methylation-SEP interactions that we were unable to detect as a result of our relatively small sample size and incomplete coverage of the genome. Our findings would thus be strengthened by replication in larger, independent cohorts and by the usie of more recently developed microarrays that provide greater genomic coverage. Finally, our findings were based on a sample consisting predominantly of African American participants. Although the extent of racial/ethnic stratification in DNA methylation is currently unknown, this phenomenon is known to exist for DNA sequence variation (Li et al. 2008), which is known to affect DNA methylation (Hellman and Chess 2010); additionally, racial/ethnic variation in DNA methylation has been previously reported (Lee et al. 2007; Zhang, Cardarelli, et al. 2011). The extent to which our findings are generalizable to other populations thus remains to be determined.

In conclusion, the results presented here provide preliminary evidence that SEP modifies the relationship 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, confirming that the observed effect modification by SEP is consistent across qualitative and quantitative assessments of this disorder. Taken together, results from both PTSD and PTS symptom severity analyses help to shed light more broadly on how SEP interacts with epigenotype to predict risk of mental illness. These findings await confirmation from future studies conducted in independent cohorts.

References

Abdolmaleky, H. M., K. H. Cheng, S. V. Faraone, M. Wilcox, S. J. Glatt, F. Gao, C. L. Smith, et al. 2006. Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Hum Mol Genet* 15(21): 3132–3145.

Abdolmaleky, H. M., K. H. Cheng, A. Russo, C. L. Smith, S. V. Faraone, M. Wilcox, R. Shafa, et al. 2005. Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report. *Am J Med Genet B Neuropsychiatr Genet* 134B(1):60–66.

Ahern, J., and S. Galea. 2006. Social context and depression after a disaster: the role of income inequality. *J Epidemiol Community Health* 60(9): 766–770.

Bartholdi, D., M. Krajewska-Walasek, K. Ounap, H. Gaspar, K. H. Chrzanowska, H. Ilyana, H. Kayserili, I. W. Lurie, A. Schinzel, and A. Baumer. 2009. Epigenetic mutations of the imprinted IGF2-H19 domain in Silver-Russell syndrome (SRS): results from a large cohort of patients with SRS and SRS-like phenotypes. *J Med Genet* 46(3): 192–197.

Bjornsson, H. T., M. I. Sigurdsson, M. D. Fallin, R. A. Irizarry, T. Aspelund, H. Cui, W. Yu, et al. 2008. Intra-individual change over time in DNA methylation with familial clustering. *JAMA* 299(24): 2877–2883.

Borghol, N., M. Suderman, W. McArdle, A. Racine, M. Hallett, M. Pembrey, C. Hertzman, C. Power, and M. Szyf. 2012. Associations with early-life socio-economic position in adult DNA methylation. *Int J Epidemiol* 41(1): 62–74.

Breitling, L. P., R. Yang, B. Korn, B. Burwinkel, and H. Brenner. 2011. Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. *Am J Hum Genet* 88(4): 450–457.

Breslau, N., R. C. Kessler, H. D. Chilcoat, L. R. Schultz, G. C. Davis, and P. Andreski. 1998. Trauma and posttraumatic stress disorder in the community: the 1996 Detroit Area Survey of Trauma. *Arch Gen Psychiatry* 55(7): 626–632.

Brewin, C. R., B. Andrews, and J. D. Valentine. 2000. Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. *J Consult Clin Psychol* 68: 317–336.

Cairney, J., and N. Krause. 2005. The social distribution of psychological distress and depression in older adults. *J Aging Health* 17(6): 807–835.

Champagne, F. A., I. C. Weaver, J. Diorio, S. Dymov, M. Szyf, and M. J. Meaney. 2006. Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. *Endocrinology* 147(6): 2909–2915.

Delaval, K., A. Wagschal, and R. Feil. 2006. Epigenetic deregulation of imprinting in congenital diseases of aberrant growth. *Bioessays* 28(5): 453–459.

Dowd, J. B., A. E. Aiello, and D. E. Alley. 2009. Socioeconomic disparities in the seroprevalence of cytomegalovirus infection in the US population: NHANES III. *Epidemiol Infect* 137(1): 58–65.

Dowd, J. B., M. N. Haan, L. Blythe, K. Moore, and A. E. Aiello. 2008. Socioeconomic gradients in immune response to latent infection. *Am J Epidemiol* 167(1): 112–120.

Due, P., R. Krolner, M. Rasmussen, A. Andersen, M. Trab Damsgaard, H. Graham, and B. E. Holstein. 2011. Pathways and mechanisms in adolescence contribute to adult health inequalities. *Scand J Public Health* 39(6 suppl):62–78.

Eckhardt, F., J. Lewin, R. Cortese, V. K. Rakyan, J. Attwood, M. Burger, J. Burton, et al. 2006. DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat Genet* 38(12): 1378–1385.

Evans, G. W., and P. Kim. 2007. Childhood poverty and health: cumulative risk exposure and stress dysregulation. *Psychol Sci* 18(11): 953–957.

Galea, S., M. Tracy, K. J. Hoggatt, C. Dimaggio, and A. Karpati. 2011. Estimated deaths attributable to social factors in the United States. *Am J Public Health* 101(8): 1456–1465.

Galobardes, B., J. Lynch, and G. D. Smith. 2007. Measuring socioeconomic position in health research. *Br Med Bull* 81/82: 21–37.

Gianaros, P. J., J. R. Jennings, L. K. Sheu, P. J. Greer, L. H. Kuller, and K. A. Matthews. 2007. Prospective reports of chronic life stress predict decreased grey matter volume in the hippocampus. *Neuroimage* 35(2): 795–803.

Gianaros, P. J., and S. B. Manuck. 2010. Neurobiological pathways linking socioeconomic position and health. *Psychosom Med* 72(5): 450–461.

Gladkevich, A., H. F. Kauffman, and J. Korf. 2004. Lymphocytes as a neural probe: potential for studying psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 28(3): 559–576.

Hackman, D. A., and M. J. Farah. 2009. Socioeconomic status and the developing brain. *Trends Cogn Sci* 13(2): 65–73.

Hanson, J. L., A. Chandra, B. L. Wolfe, and S. D. Pollak. 2011. Association between income and the hippocampus. *PLoS ONE* 6(5): e18712.

Harrison, P. J., N. Freemantle, and J. R. Geddes. 2003. Meta-analysis of brain weight in schizophrenia. *Schizophr Res* 64(1): 25–34.

Havecker, E. R., L. M. Wallbridge, T. J. Hardcastle, M. S. Bush, K. A. Kelly, R. M. Dunn, F. Schwach, J. H. Doonan, and D. C. Baulcombe. 2010. The Arabidopsis RNA-directed DNA methylation argonautes functionally diverge based on their expression and interaction with target loci. *Plant Cell* 22(2): 321–334.

Hawk, L. W., A. L. Dougall, R. J. Ursano, and A. Baum. 2000. Urinary catecholamines and cortisol in recent-onset posttraumatic stress disorder after motor vehicle accidents. *Psychomatic Medicine* 62: 423–434.

Heijmans, B. T., E. W. Tobi, A. D. Stein, H. Putter, G. J. Blauw, E. S. Susser, P. E. Slagboom, and L. H. Lumey. 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A* 105(44): 17046–17049.

Hellman, A., and A. Chess. 2010. Extensive sequence-influenced DNA methylation polymorphism in the human genome. *Epigenetics Chromatin* 3 (1):11.

Hou, L., X. Zhang, D. Wang, and A. Baccarelli. 2012. Environmental chemical exposures and human epigenetics. *Int J Epidemiol* 41(1): 79–105.

Huang da, W., B. T. Sherman, and R. A. Lempicki. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4(1): 44–57.

Hulshoff Pol, H. E., H. W. Hoek, E. Susser, A. S. Brown, A. Dingemans, H. G. Schnack, N. E. van Haren, L. M. Pereira Ramos, C. C. Gispen-de Wied, and R. S. Kahn. 2000. Prenatal exposure to famine and brain morphology in schizophrenia. *Am J Psychiatry* 157(7): 1170–1172.

Kato, T., C. Kakiuchi, and K. Iwamoto. 2007. Comprehensive gene expression analysis in bipolar disorder. *Can J Psychiatry* 52(12): 763–771.

Kawachi, I., S. V. Subramanian, and N. Almeida-Filho. 2002. A glossary for health inequalities. *J Epidemiol Community Health* 56(9): 647–652.

Koenen, K. C., R. Harley, M. J. Lyons, J. Wolfe, J. C. Simpson, J. Goldberg, S. A. Eisen, and M. Tsuang. 2002. A twin registry study of familial and individual risk factors for trauma exposure and posttraumatic stress disorder. *J Nerv Ment Dis* 190(4): 209–218.

Koenen, K. C., T. E. Moffitt, R. Poulton, J. Martin, and A. Caspi. 2007. Early childhood factors associated with posttraumatic stress disorder: results from a longitudinal birth cohort. *Psychol Med* 37(2): 181–192.

Koenen, K. C., M. Uddin, S. C. Chang, A. E. Aiello, D. E. Wildman, R. de los Santos, E. Goldmann, and S. Galea. 2011. Methylation of SLC6A4 moderates effect of cumulative traumatic burden on risk for PTSD: a pilot study. *Depress Anxiety* 28(8): 639–647.

Kulka, R., W. Schlenger, J. Fairbank, R. Hough, B. K. Jordan, C. Marmar, D. Weiss, and D. Grady. 1990. *Trauma and the Vietnam War generation: report of the findings from the National Vietnam Veterans Readjustment Study*. New York: Brunner/ Mazel.

Kurian, S. M., H. Le-Niculescu, S. D. Patel, D. Bertram, J. Davis, C. Dike, N. Yehyawi, et al. 2011. Identification of blood biomarkers for psychosis using convergent functional genomics. *Mol Psychiatry* 16(1): 37–58.

Lagali, P. S., C. P. Corcoran, and D. J. Picketts. 2010. Hippocampus development and function: role of epigenetic factors and implications for cognitive disease. *Clin Genet* 78(4): 321–333.

Le-Niculescu, H., S. M. Kurian, N. Yehyawi, C. Dike, S. D. Patel, H. J. Edenberg, M. T. Tsuang, D. R. Salomon, J. I. Nurnberger, Jr., and A. B. Niculescu. 2009. Identifying blood biomarkers for mood disorders using convergent functional genomics. *Mol Psychiatry* 14(2): 156–174.

Lee, J. S., P. K. Lo, M. J. Fackler, P. Argani, Z. Zhang, E. Garrett-Meyer, and S. Sukumar. 2007. A comparative study of Korean with Caucasian breast cancer reveals frequency of methylation in multiple genes correlates with breast cancer in young, ER, PR-negative breast cancer in Korean women. *Cancer Biol Ther* 6(7): 1114–1120.

Li, J. Z., D. M. Absher, H. Tang, A. M. Southwick, A. M. Casto, S. Ramachandran, H. M. Cann, et al. 2008. Worldwide human relationships inferred from genome-wide patterns of variation. *Science* 319(5866): 1100–1104.

Link, B. G., and J. Phelan. 1995. Social conditions as fundamental causes of disease. *J Health Soc Behav* Spec. No.:80–94.

Lynch, J. W., G. D. Smith, G. A. Kaplan, and J. S. House. 2000. Income inequality and mortality: importance to health of individual income, psychosocial environment, or material conditions. *BMJ* 320(7243): 1200–1204.

Mattick, J. S., P. P. Amaral, M. E. Dinger, T. R. Mercer, and M. F. Mehler. 2009. RNA regulation of epigenetic processes. *Bioessays* 31(1): 51–59.

McEwen, B. S. 2001. Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. *Ann N Y Acad Sci* 933: 265–277.

———. 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev* 87(3): 873–904.

McEwen, B. S., and P. J. Gianaros. 2010. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Ann N Y Acad Sci* 1186: 190–222.

McEwen, B. S., and T. Seeman. 1999. Protective and damaging effects of mediators of stress: elaborating and testing the concepts of allostasis and allostatic load. *Ann NY Acad Sci* 896: 30–47.

McGowan, P. O., A. Sasaki, T. C. Huang, A. Unterberger, M. Suderman, C. Ernst, M. J. Meaney, G. Turecki, and M. Szyf. 2008. Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. *PLoS ONE* 3(5): e2085.

McGuinness, D., L. M. McGlynn, P. C. Johnson, A. MacIntyre, G. D. Batty, H. Burns, J. Cavanagh, et al. 2012. Socio-economic status is associated with epigenetic differences in the pSoBid cohort. *Int J Epidemiol* 41(1): 151–160.

Mill, J., T. Tang, Z. Kaminsky, T. Khare, S. Yazdanpanah, L. Bouchard, P. Jia, et al. 2008. Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *Am J Hum Genet* 82(3): 696–711.

Miller, G. E., E. Chen, A. K. Fok, H. Walker, A. Lim, E. F. Nicholls, S. Cole, and M. S. Kobor. 2009. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proc Natl Acad Sci U S A* 106(34):14,716–14,721.

Nicklett, E., and S. Burgard. 2009. Downward social mobility and major depressive episodes among Latino and Asian American immigrants to the United States. *Am J Epidemiol* 107(6): 793–801.

Pidsley, R., E. L. Dempster, and J. Mill. 2010. Brain weight in males is correlated with DNA methylation at IGF2. *Mol Psychiatry* 15(9): 880–881.

Riccio, A., A. Sparago, G. Verde, A. De Crescenzo, V. Citro, M. V. Cubellis, G. B. Ferrero, et al. 2009. Inherited and sporadic epimutations at the IGF2-H19 locus in Beckwith-Wiedemann syndrome and Wilms' tumor. *Endocr Dev* 14: 1–9.

Rogers, R. G., B. G. Everett, A. Zajacova, and R. A. Hummer. 2010. Educational degrees and adult mortality risk in the United States. *Biodemography Soc Biol* 56(1): 80–99.

Rollins, B., M. V. Martin, L. Morgan, and M. P. Vawter. 2010. Analysis of whole genome biomarker expression in blood and brain. *Am J Med Genet B Neuropsychiatr Genet* 153B(4):919–936.

Safaei, J. 2006. Is democracy good for health? *Int J Health Serv* 36(4): 767–786.

Sapolsky, R. M., J. Uno, C. S. Remert, and C. E. Finch. 1990. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *J Neurosci* 10: 2897–2902.

Shih, R., P. Belmonte, and P. Zandi. 2004. A review of the evidence from family, twin and adoption studies for a genetic contribution to adult psychiatric disorders. *Int Rev Psychiatry* 16: 260–283.

Smith, A. K., K. N. Conneely, V. Kilaru, K. B. Mercer, T. E. Weiss, B. Bradley, Y. Tang, C. F. Gillespie, J. F. Cubells, and K. J. Ressler. 2011. Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *Am J Med Genet B Neuropsychiatr Genet* 156(6): 700–708.

Steptoe, A., M. Hamer, K. O'Donnell, S. Venuraju, M. G. Marmot, and A. Lahiri. 2010. Socioeconomic status and subclinical coronary disease in the Whitehall II epidemiological study. *PLoS ONE* 5(1): e8874.

Talens, R. P., J. W. Jukema, S. Trompet, D. Kremer, R. G. Westendorp, L. H. Lumey, N. Sattar, H. Putter, P. E. Slagboom, and B. T. Heijmans. 2012. Hypermethylation at loci sensitive to the prenatal environment is associated with increased incidence of myocardial infarction. *Int J Epidemiol* 41(1): 106–115.

Tobi, E. W., P. E. Slagboom, J. van Dongen, D. Kremer, A. D. Stein, H. Putter, B. T. Heijmans, and L. H. Lumey. 2012. Prenatal famine and genetic variation are independently and additively associated with DNA methylation at regulatory loci within IGF2/H19. *PLoS ONE* 7(5): e37933.

Toumbourou, J. W., S. A. Hemphill, J. Tresidder, C. Humphreys, J. Edwards, and D. Murray. 2007. Mental health promotion and socio-economic disadvantage: lessons from substance abuse, violence and crime prevention and child health. *Health Promot J Austr* 18(3): 184–190.

Tsankova, N., W. Renthal, A. Kumar, and E. J. Nestler. 2007. Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci* 8(5): 355–367.

Uddin, M., A. E. Aiello, D. E. Wildman, K. C. Koenen, G. Pawelec, R. de Los Santos, E. Goldmann, and S. Galea. 2010. Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A* 107(20): 9470–9475.

Uddin, M., S. Galea, S. C. Chang, A. E. Aiello, D. E. Wildman, R. de los Santos, and K. C. Koenen. 2011. Gene expression and methylation signatures of MAN2C1 are associated with PTSD. *Dis Markers* 30(2–3):111–121.

Uddin, M., K. C. Koenen, A. E. Aiello, D. E. Wildman, R. de los Santos, and S. Galea. 2011. Epigenetic and inflammatory marker profiles associated with depression in a community-based epidemiologic sample. *Psychol Med* 41(5): 997–1007.

Weathers, F., and J. Ford. 1996. Psychometric review of PTSD checklist (PCL-C, PCL-S, PCL-M, PCL-R).” In *Measurement of stress, trauma, and adaptation*, ed. B. H. Stamm. Lutherville: Sidran Press.

Weaver, I. C., N. Cervoni, F. A. Champagne, A. C. D’Alessio, S. Sharma, J. R. Seckl, S. Dymov, M. Szyf, and M. J. Meaney. 2004. Epigenetic programming by maternal behavior. *Nat Neurosci* 7(8): 847–854.

Wong, C. C., A. Caspi, B. Williams, I. W. Craig, R. Houts, A. Ambler, T. E. Moffitt, and J. Mill. 2010. A longitudinal study of epigenetic variation in twins. *Epigenetics* 5(6): 516–526.

Zajacova, A., J. B. Dowd, and A. E. Aiello. 2009. Socioeconomic and race or ethnic patterns in persistent infection burden among U.S. adults. *J Gerontol* 642: 272–279.

Zhang, F. F., R. Cardarelli, J. Carroll, K. G. Fulda, M. Kaur, K. Gonzalez, J. K. Vishwanatha, R. M. Santella, and A. Morabia. 2011. Significant differences in global genomic DNA methylation by gender and race/ethnicity in peripheral blood. *Epigenetics* 6(5): 623–629.

Zhang, J., Q. Tan, H. Yin, X. Zhang, Y. Huan, L. Tang, H. Wang, J. Xu, and L. Li. 2011. Decreased gray matter volume in the left hippocampus and bilateral calcarine cortex in coal mine flood disaster survivors with recent onset PTSD. *Psychiatry Res* 192(2): 84–90.