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DNA Methylation Across the Serotonin Transporter Gene Following Marital Separation: A Pilot Study

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Abstract

BACKGROUND: Marital separation and divorce are stressful life transitions associated with increased risk for a range of poor mental and physical health outcomes. A key task for research in this area is to identify individual differences that may index risk for these adverse outcomes. PURPOSE: To examine the association between DNA methylation across the serotonin transporter gene (SLC6A4) and self-reported emotional distress following marital separation. METHOD: Genomic DNA methylation (from buffy coat fractions of whole blood) was quantified in a sample of 47 adults following a recent marital separation; concurrent with the blood draw, participants completed questionnaires on their psychological adjustment to the separation experience. RESULTS: Relatively greater methylation of SLC6A4 was associated with less subjective separation-related psychological distress, and this association held after accounting for participants' age, length of the relationship, time since the separation, and SLC6A4 genotype, b = -211.99, SE = 94.94, p = .04, 95% CI: -402.22, -25.20. Significantly stronger negative associations were observed between methylation and psychological adjustment among participants who had more recently separated from their former partner. CONCLUSIONS: Although results derived from small samples must be considered preliminary and hypothesis generating, the current study raises new questions about the role of DNA methylation and psychosocial adaptation to stressful life events such as divorce, and the findings can inform future studies in this research area.

Key words: Marital separation, divorce, DNA methylation, genomics, coping, stress

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Marital separation and divorce are common and highly stressful negative life events. Although most adults are resilient in the face of these relationship transitions, the end of marriage confers risk for a range of poor health outcomes, including early death (1). One theme in the literature on coping with divorce—and other stressful events more broadly—is that background individual differences (e.g., genetic dispositions, attachment styles, personality, trait-like tendencies toward psychological rumination) are associated with and may shape the course of adjustment (2). Recent work suggests that polymorphisms in the serotonin transporter gene may condition adults' cardiovascular responses when reflecting over their recent marital separation experience. Separated adults who are carriers of the homozygous short allele for the serotonin transporter gene promoter region and who reported higher levels of psychological distress in response to the end of marriage evidenced greater decreases in heart rate variability when asked to mentally reflect on the history of their former relationship and their separation (3). A conclusion from this prior work is that adults who are homozygous carriers of the short allele for the serotonin transporter gene promoter region may struggle more with emotion regulatory challenges in the face of a stressful life transition like divorce. Despite this finding, most of the research on how adults cope with marital separation focuses on psychological variables with little attention to background genetic risk.

Although the study of DNA polymorphisms was initially viewed as a breakthrough for psychiatric genetics, the research in this area has not progressed as expected. In fact, a recent

meta-analysis found no evidence for the much-heralded life stress by serotonin transporter gene interaction in predicting the development of depression (4). One avenue of genetic research gaining increased traction in psychological science is the study of potential epigenetic regulation of static DNA. Epigenetics refers to chemical modifications of DNA that affect gene expression (5). One form of epigenetic regulation involves DNA methylation, in which a methyl group is attached to DNA base, either cysteine or adenine, altering activity for the specific gene (6). A provocative possibility emerging from this work is that "dynamic" epigenetic markers of risk, alone or in combination with "static" gene polymorphisms, may prove more highly predictive of bio-psycho-social outcomes than polymorphisms alone, or the study of polymorphisms in combination with life stressors (see 7). Whereas specific genetic polymorphisms may confer risk in a broad sense, variability in DNA methylation may provide a more precise or focused view on the differential accessibility of genes and thus the likelihood of specific patterns of gene expression (8). At the same time, it is important to recognize that this statement is largely a hypothesis that guides the research literature, and the exact connection between the genotype, DNA methylation and gene expression remains to be fully established (cf. 9).

A number of studies examine methylation in the serotonin transporter gene (SLC6A4), both in response to adverse life events and as a marker of risk for psychopathology (10,11). The general hypothesis guiding much of this work is that natural variability in the serotonergic system, regulated by the serotonin transporter, plays a key role in socio-emotional stress susceptibility and emotion regulation (11). Relative hypermethylation of the SLC6A4 is associated with lower levels of gene expression, resulting in lower production of the serotonin transporter and therefore a greater buildup of serotonin in the synaptic cleft (10). In general, hypermethylation of the SLC6A4 gene is positively correlated with a history of adverse

childhood experiences (12,13; though this is not always the case, see 14) as well as risk for mood disorders and other forms of psychopathology (10). Far fewer studies, however, have examined the role of SLC6A4 methylation as people navigate ongoing life stress (see 14). On one hand, hypermethylation, which serves to decrease the expression of SLC6A4— *presumably* shaped by early social experiences (14)—may canalize people toward risk for poor emotional outcomes and increased vulnerability to life stress. On the other hand, in situations that require ongoing adaptation to immediate environmental demands, the positive association between methylation of SLC6A4 and distress may not hold; in these situations, greater methylation levels may reflect efforts toward environmental adaptation rather than risk markers per se (11,15). This perspective provides a broader framework for understanding the adaptive value of methylation; coping with stressful events as they unfold may be a situation in which the putative risk associated with greater methylation instead provides adaptive value.

The current pilot study explores these ideas in the context of a recent marital separation, during a period in which adults are actively coping with the stress of their relationship transition. Sbarra, Hasselmo, and Nojopranoto (16) argued that the study of divorce provides an ideal "model system" for studying stress and health more generally, and other authors have called for a more complete investigation of background individual differences that may set the stage for better or worse adaptation when relationships end (2). Based on the literature linking SLC6A4 gene hypermethylation with risk for poor mental health outcomes (8,11), we expected to observe a positive correlation between methylation and subjective separation-related distress in the current study.

Method

Participants

This pilot study involved 47 adults (*n*= 16 men, mean age = 44.20, *SD*= 11.37 years) who reported a recent marital separation (mean time since separation = 3.61 months, *SD*= 2.45 months) and who took part in a larger NIH-funded study of psychosocial responses to marital separation (see 17,18). The average length of participants' prior relationship was 14.3 years (*SD*= 8.92 years). Separation in this case was defined as the date of permanent physical separation from a spouse. Sixty-four percent of the sub-sample reported their race as Caucasian, 21.3% reported they were Hispanic, 4.3% reported being African American, 2.1% reported they were Asian, while 8.5% indicated their race was "Other." Participants were phone screened prior to participation and required to have been married to their ex-partner for at least three years, and lived with them for at least two years. Participants were required to be 18 years of age or older and free of diagnoses for schizophrenia, bipolar disorder, suicidal ideation, or uncontrolled medical conditions (for further details on this pilot study sample see 19). At intake, participants reported an average score of 11.68 (*SD* = 7.25) on the Center for Epidemiologic Studies Depression Scale (CES-D; 20); 30% of the sub-sample reported depression symptoms above the clinical-cutoff on the CES-D. In general, participants in the sub-sample reported being generally healthly, with 71% of the participants endorsing a single item, "My health is excellent," as "Definitely True" or "Mostly True."

Procedure

Participants were recruited from a larger cohort study, the Divorce, Sleep, and Social Environment (DSE) study. Upon entry into the larger DSE study, participants were consented and completed questionnaires assessing their psychological response to the separation. To quantify DNA methylation, venous blood samples were collected at the Clinical and Translational Sciences Center at the University of Arizona Medical Center (details on blood

preparation procedures and DNA methylation quantification are available in the online Supplemental Materials, see osf.io/zstqc). All blood samples were collected within two weeks of collecting participants' self-reported adjustment to the separation.

Measures

Psychological adjustment composite. As reported elsewhere (see 3), we used a composite index to assess participants' self-reported psychological adjustment to the divorce; the composite included measures of the emotional impact of the divorce, depressed mood, grief, and self-concept disturbance. The internal consistency of this index was high (α = .93); as the primary outcome variable, we used a percent of maximum possible (POMP) scoring system (21) and higher scores on the composite index reflected greater self-reported separation-related psychological distress (details are available in the online Supplemental Materials, see osf.io/zstqc).

DNA methylation. A methylation composite score was computed as the average methylation across 16 probe sites in the SLC6A4 gene promoter region. (The specific sites are listed in the Supplemental Materials.) Methylation status was determined using the Illumina HumanMethylation450K BeadChip; quantification was conducted at the University of Utah's Genomics Core Facility using the protocol specified by the manufacturer. We used the Bioconductor package, minfi (see 22), to preprocess the data and quality control (QC) assessments; custom R scripts were utilized to apply internal minfi normalization method to preprocess and normalize the 450kMethylation arrays data for 47 subjects and compare differences in DNA methylation levels (β-values) across the whole genome.

Control variables. In our regression analyses predicting the psychological distress composite, we statistically accounted for participants' age, gender, relationship length (in months), and time since the separation (in months). Based on evidence suggesting DNA polymorphisms in the promoter region can interact with methylation to alter gene expression (23), we also accounted for alleles in the promoter region (see the online Supplemental Material). Following Hasselmo et al. (3), we computed two orthogonal linear contrasts that (1) compared carriers who were homozygous for the short allele to all other genotypes, and (2) compared carriers who were heterozygous to those who were homozygous for the long allele.

Data Analysis

To test the main hypothesis of interest— that relative hypermethylation across the SLC64A transporter region would be positively associated with self-reported separation-related distress— we first explored the zero-order correlation between these variables, then examined the robustness of the association in a multiple regression analysis. Within the regression framework, we sought to determine if methylation status was associated with separation-related distress after accounting for the competing predictors of age, length of the relationship, time since the separation, and, importantly, SLC6A4 genotype (using a series of orthogonal linear contrasts—see Table 2). The key question for the regression analysis is whether the association between separation-related distress and methylation persists after accounting for the relevant covariates, all of which may explain variability in the outcome. All analyses were conducted with SPSS 24.0, and the exploratory analyses were conducted using Hayes's PROCESS macro for conditional process analysis (24).

Results

Table 1 presents the descriptive statistics for and zero-order correlations among the predictor, covariate, and outcome variables. As shown and in contrast with our main study prediction, the correlation between the psychological adjustment composite and methylation of the SLC6A4 gene was negative and reliably different from zero, r = -.31, 95% CI [-0.54, -0.04]. As illustrated in Figure 1, relatively greater methylation in the SLC6A4 gene was associated with less subjective separation-related psychological distress. We examined the robustness of the methylation-distress analysis using multiple regression. As shown in Table 2 (Model 1), after accounting for participants' age, sex, relationship length, and time since the separation event, methylation of the SLC6A4 gene remained a significant predictor of subjective separation-related psychological distress. In the final analysis (Table 2, Model 2), we accounted for SLC6A4 genotype data (i.e., the orthogonal linear contrasts) in the regression model and the results remained unchanged.

Given that the observed association between SLC6A4 gene methylation and separation-related psychological distress was negative and in direct contrast with our main study prediction, we explored a series of supplemental analyses to investigate whether the inverse association might reflect ongoing adaptation to the separation event. If this were the case, it is reasonable to expect a *stronger* negative association between methylation and self-reported distress immediately following the separation (possibly through conferring an "adjustment advantage" in unstable environments). We tested this possibility formally by adding the Methylation X Separation Length interaction to Model 2 (Table 2) and the interaction effect was reliably different from zero, b = 77.85, SE = 35.36, t = 2.12, p = .04. A deconstruction of the simple slopes revealed significantly stronger negative associations between methylation and self-reported distress among participants who had more recently separated from their former partner.

Specifically, a Johnson-Neyman step-down procedure, which identifies points along a continuous moderator where the association between a predictor and outcome becomes reliably different from zero (see 24), revealed the association between methylation and adjustment was significant for participants who entered the study *within* 4 months of their separation; the conditional effects of the negative association between methylation and separation-related psychological distress were significant at 4 months and all time points below 4 months since participants physically separated from their partner. When including the interaction effect in the model, the main effect of SLC6A4 remained reliably different from zero and the standardized effect was unchanged relative to what we observed in the main-effect-only model.

Discussion

Marital separation and divorce are relatively common stressful events that are associated with an increased risk for a range of health morbidities as well as early mortality. Results from this pilot study revealed that relative hypermethylation across the SLC6A4 gene is associated with less separation-related emotional distress. The chief limitation of this work is the small sample size, as it is well known that the precision and stability of statistical effects are limited in sample sizes lower than 200 (25); consequently, small sample sizes are prone to yield spurious results. These limitations notwithstanding, the characterization of DNA methylation in the SLC6A4 gene in a sample of recently separated adults who are actively coping with the end of marriage is unique and may provide a rare opportunity to consider the role of SLC6A4 methylation in the context of ongoing adaptation to an acutely stressful event. In this way, we view these findings as generative, exploratory, or hypothesis-guiding rather than hypothesis testing and confirmatory, which will require larger, more adequately powered samples (as well as a preregistration of a specific hypothesis and analysis plan).

Although we expected relative *hyper*methylation to be associated with greater separation distress, we observed an inverse association between methylation status and distress. In seeking to understand this pattern, it may be most informative to consider the positive association between relative *hypo*methylation of SLC6A4 and greater distress. The observation that SLC6A4 relative hypomethylation is associated with poorer psychological adaptation to marital separation is somewhat at odds with the existing literature and in contrast to our main study prediction. Prior work largely suggests that hypermethylation is associated with both the experience of early adversity and greater risk for pathological outcomes in adulthood (10,12). In the current study, however, it would be inaccurate to equate subjective distress with pathology per se; most people are resilient in the face of divorce and greater distress in the months immediately after a separation may constitute a normative (adaptive) grief response. Other investigators have speculated that increased SLC6A4 methylation may reflect efforts to adapt to environmental adversity (11,15) and nurses working in a high stress environment, relative to a low stress environment, evidenced lower methylation in the SLC6A4 promoter region (14). Our supplemental/exploratory analyses suggested that the negative association between methylation and adjustment operates only among people within 4 months of their separation. These findings raise the intriguing possibility that relative hypomethylation reflects ongoing efforts to adapt to acutely stressful events. This possibility also runs counter to the idea that methylation patterns are static risk indicators, but far more research is needed to understand under what contexts methylation patterns may vary as people adapt to stressful events.

The findings from this study should be considered in light of its limitations. First, the sample size in this report is far too small to draw high-confidence conclusions, and this is especially relevant for considering the Methylation X Separation Length interaction effect;

although 66% of the sample had separated within the prior 4 months, the small overall sample size limits the precision of the simple slope estimates. Accordingly, we have made efforts to be tentative in our observations and cautious in our conclusions. Despite this limitation, we believe the current findings have generative potential regarding the role that DNA methylation can play in adaptation to divorce. The cross-sectional design of our study precludes any directional conclusions about methylation/adjustment association. A primary goal for future research will be to explore ways in which methylation changes may be associated with subjective experience, and how these processes may unfold together over time as adults adapt to stressful events. For adults experiencing acutely stressful events, it would also be informative if future studies collected reports on early adversity; this information would be useful in exploring the ways in which methylation, early adversity, and stressful experiences in adulthood operate together in their association with subjective distress.

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Table 1

Correlation Matrix and Descriptive Statistics for Study Variables

	1	2	3	4	5	6	7	8
Age (1)	1							

Gender (2)	.16	1						
Length (3)	.60**	.11	1					
Time Since (4)	.04	.00	.07	1				
Distress (5)	.03	.23**	-.17*	.02	1			
SERTMeth (6)	.20	-.25	-.05	.01	-.31*	1		
Contrast 1 (7)	.04	-.11	.05	.02	-.07	.02	1	
Contrast 2 (8)	-.11	-.09	-.07	-.06	-.04	.12	.21*	1
Mean	44.12	64.5[a]	11.91	3.64	28.65	.38	28.9[b]	51.1[c]
SD	11.26		8.25	2.44	17.99	.03		
Range	25-64		37-386	.25-12.75	2.03-73.43	.33-.44		

Note. Gender is coded 0 = female, 1 = male; [a] = % female; Length = length of the relationship in years; Time Since = time since the separation in months; Distress = separation-related psychological distress; SERTMeth = methylation of the serotonin transporter gene (SLC6A4; values range from 0 – 1) Contrast 1 = contrast code comparing SLC6A4 short/short allele carriers to all other participants ([b] = % homozygous for short allele); Contrast 2 = contrast code comparing SLC6A4 short/long allele carriers to long/long allele carriers ([c] = % with one short allele). *p <.05; **p<.01.

Table 2

Regression Models Predicting Psychological Distress Following Marital Separation

Model 1	*B*	*SE*	*p*	95% CI	*B*
Constant	98.46	34.80		[29.64, 168.71]	
Age	.32	.29	.24	[-.25, .91]	.21
Gender	9.43	6.01	.09	[-2.70, 21.18]	.14
Length	-.07	.02	.02	[-.11, -.020]	-.41
Time Since	.36	1.05	.71	[-2.07, 2.29]	.36
SERTMeth	-199.61	89.33	.04	[-371.55, -26.23]	-.32
Model 2	***B***	***SE***	***p***	**95% CI**	***B***
Constant	101.93	36.90		[32.78, 178.38]	
Age	.52	.28	.06	[-.04, 1.09]	.32
Gender	4.91	6.05	.38	[-7.12, 16.67]	.13
Length	-.09	.03	.003	[-.13, -.04]	-.50
Time Since	.23	1.22	.81	[-3.01, 1.94]	.03
SERTMeth	-211.99	94.91	.03	[-402.22, -25.21]	-.32
Contrast 1	-1.97	2.08	.28	[-6.15, 2.01]	-.15
Contrast 2	-7.77	3.28	.02	[-15.05, -1.74]	-.34

Note. Gender is coded 0 =female, 1 = male; Length = length of the relationship; Time Since = time since the separation; SERTMeth = methylation of the serotonin transporter gene (SLC6A4). Contrast 1 = contrast code comparing SLC6A4 short/short allele carriers to all other participants; Contrast 2 = contrast code comparing SLC6A4 short/long allele carriers to long/long allele carriers. *B* = unstandardized regression estimate; *SE* = standard error; *B* = standardized regression estimate. *p <.05; **p<.01.

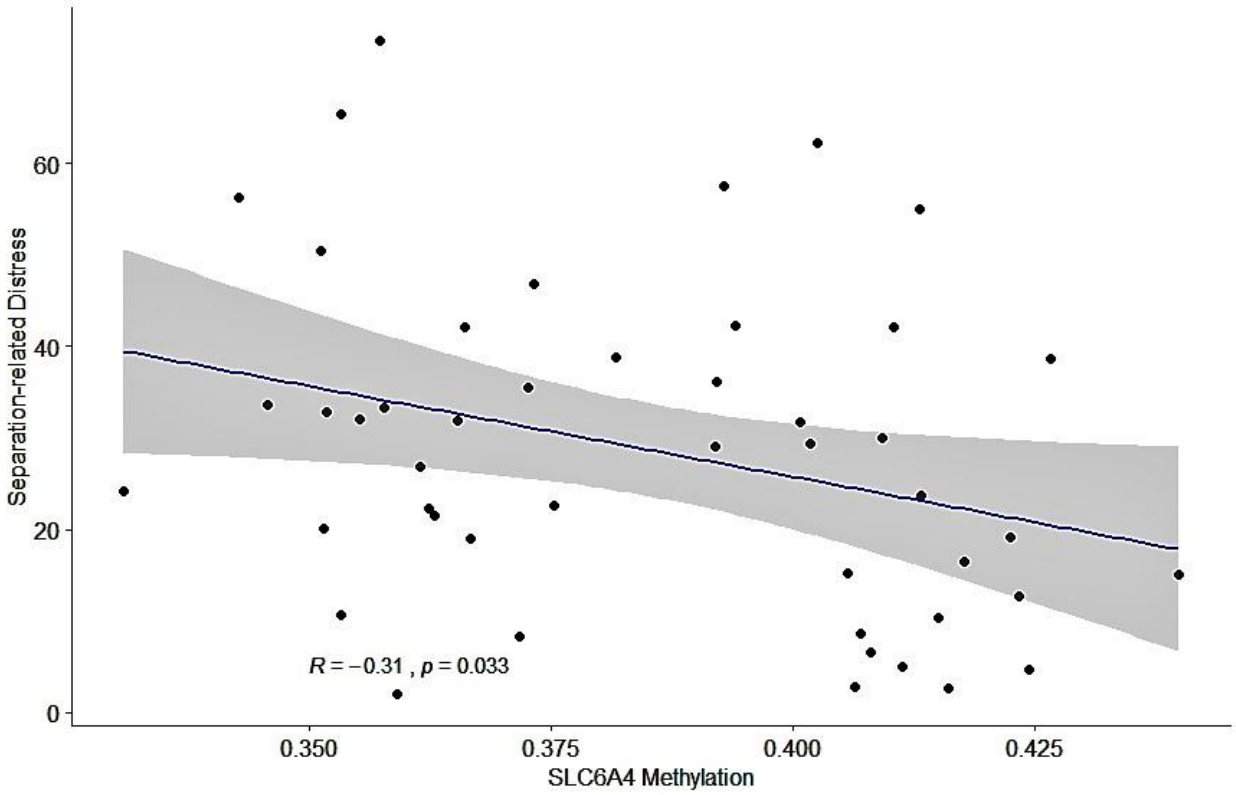

Figure 1. Scatterplot of the association between separation-related emotional distress and DNA methylation across the serotonin transporter gene promoter region.