Methylation Matters: Interaction Between Methylation Density and Serotonin Transporter Genotype Predicts Unresolved Loss or Trauma

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Background: Do genetic or epigenetic factors play a role in making some individuals more vulnerable than others to loss of attachment figures or other traumatic experiences?

Methods: DNA was obtained from growth phase entrained Epstein-Barr Virus (EBV) transformed lymphoblast cell lines from 143 adopted participants. Genotype of the serotonin transporter linked polymorphic region (5HTTLPR) was determined, and methylation ratios for each of the C-phosphate-G (CpG) residues were assessed using quantitative mass spectroscopy. Unresolved loss or trauma was established using the Berkeley Adult Attachment Interview.

Results: Higher levels of methylation of the 5HTT promoter associated CpG island were associated with increased risk of unresolved responses to loss or other trauma in carriers of the usually protective 5HTTLPR *ll* variant. The *ss* variant of 5HTTLPR predicted more unresolved loss or trauma, but only in case of lower levels of methylation. Higher levels of methylation of the *ss* variant were associated with less unresolved loss or other trauma.

Conclusions: Associations between 5HTTLPR polymorphisms and psychological problems are significantly altered by environmentally induced methylation patterns. Methylation may serve as the interface between adverse environment and the developing organism.

Key Words: 5HTTLPR, Adult Attachment Interview, attachment, methylation, unresolved loss or trauma

Loss of attachment figures or other traumatic events have a large impact on some individuals but not on others. Do genetic or epigenetic factors play a role in making some individuals more vulnerable than others? Experiences of maltreatment (1), genocidal violence (2), or loss of attachment figures (3) in childhood increase the rate of unresolved loss or trauma. Unresolved loss or trauma is apparent from an individual's narrative about attachment-related events of loss or trauma if it shows unpredictable lapses in the monitoring of speech during the well-validated Adult Attachment Interview (AAI) (3,4). These lapses may imply that the speaker continues to experience unusual absorption regarding the trauma. Unresolved loss or trauma is strongly associated with posttraumatic stress symptoms (5).

The serotonin transporter linked polymorphic region (5HTTLPR) at chromosome 17 is a variable nucleotide repeat in *SCL6A4*, the gene that codes for the serotonin transporter. Short or "*s*" alleles have been associated with decreased mRNA transcription (6–8), decreased protein production (9), and increased vulnerability to alcohol dependence, posttraumatic stress symptoms, and depression in the presence of stressors (10,11). Two meta-analyses did not support the predicted interaction effect of the less efficient serotonin polymorphisms and environmental adversity (12,13), whereas a third review showed that weaker assessment strategies used to measure adversity might be responsible for the failure to replicate the original gene by environment interaction (G × E) findings (14).

However, level of methylation may also be a source of diverging outcomes because it affects mRNA transcription. On the basis of extensive research in rodents, Meaney (15) argued that methylation may be mediating between environmental signals and the genome in the regulation of individual differences in behavior, cognition, and physiology and might be the way in which adverse environments become instantiated in the biological system. In a series of studies on humans, Philibert and colleagues showed that methylation levels of the CpG island upstream from *SCL6A4* were associated with reports of abuse during childhood (16) and that product levels of the serotonergic system differed according to degree of methylation (8). They argued that methylation may be a biological basis for the impact of adverse environments on human psychological development. Thus far, however, the consequences of methylation in the 5HTTLPR for psychological functioning have not been studied.

Here we hypothesize that the association of serotonin transporter gene polymorphisms with unresolved loss or trauma is moderated by the level of methylation. More methylation is expected to decrease gene expression, leading those individuals with long alleles to look more like those with short alleles.

Methods and Materials

Participants

The Iowa Adoption Studies are a long-standing series of studies on children (domestically) adopted in the first few months after birth into middle-class families. Biodata were collected in the last round of data collection since 2004 (17). Here we use data from predominantly Caucasian (91%) adoptees who completed the AAI and for whom genotyping of the 5HTTLPR locus and methylation measures were available (n = 143; 50% females; mean age 39 years, SD = 7.32). All procedures were approved by the University of Iowa Institutional Review Board.

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Genotyping and Methylation

Genotype of the 5HTTLPR locus was determined using polymerase chain reaction, electrophoresis, and detection conditions as previously described (6). DNA was obtained from growth phase entrained EBV transformed lymphoblast cell lines. The resulting DNA underwent bisulfite conversion of unmethylated cytosine residues to thymidine. Methylation ratios for each of the CpG residues were determined using quantitative mass spectroscopy by Sequenom (San Diego, California). Loci for CpG residues ranged from CpG1 (25,586,514 BP) to CpG71 (25,587,180 BP) (8). The weighted average of the residue values was used as index for methylation density (16,17). Genotypes were in Hardy–Weinberg equilibrium ($p = .93$).

Measures

Unresolved Loss or Trauma. Unresolved loss or trauma was assessed using an hour-long, semistructured interview, the AAI (4,3). In the context of this interview about childhood attachment experiences and the current relationship with the adoptive parents, questions were asked concerning experiences of loss and trauma (such as the death of family members or experiences of abuse). Scores for unresolved state of mind were assigned using a 9-point rating scale (3) when the subject reported at least one loss or other potentially traumatic experiences dating more than 1 year before the interview ($n = 133$). The transcribed interviews were coded "blindly" by reliable raters in accordance with the coding standards of the Berkeley laboratory of Mary Main and Erik Hesse. Intraclass correlation was $r = .76$.

Depressive Symptoms. The Brief Symptom Instrument (18) was administered after the AAI as a measure of concurrent mood disturbance. We derived *t* scores for symptoms of depression. Cronbach's alpha was good ($\alpha = .89$).

Results

The results of the analysis of variance of unresolved loss or trauma with gender and 5HTTLPR genotype (*ss, sl, ll*) as factors and depression and methylation density as covariates are presented in Table 1. No significant main effects were found. The interaction between 5HTTLPR and promoter methylation significantly predicted scores on unresolved loss or trauma, $F(2,121) = 4.87$, $p = .009$, partial $\eta^2 = .08$. Similar results were found when we controlled for age and when we included the nonsignificant interactions between gender and methylation, gender, and 5HTTLPR and the three-way interaction between gender, 5HTTLPR, and methylation. For the carriers of the *ss* variant, higher levels of methylation were associated with less unresolved loss or trauma, $r(18) = -.53$, $p = .02$; carriers of the *sl* variant showed no association with unresolved loss or trauma, $r(59) = .18$, $p = .18$; and for carriers of the *ll* variant, higher levels of methylation were marginally related to more unresolved loss or trauma, $r(46) = .28$, $p = .056$. Figure 1 shows the mean scores for unresolved loss as related to methylation density (lower, middle, and higher thirds) in each of the 5HTTLPR genotypes. Results did not alter when only Caucasian participants were included ($n = 118$).

Table 1. Analysis of Variance of Unresolved Loss or Trauma with Sex and 5-HTTLPR as Factors and Methylation and Depression as Covariates

Predictors	df	F	*p*	Partial η^2
Gender	1, 121	2.80	.10	.02
Depression	1, 121	1.44	.23	.01
5-HTTLPR	2, 121	.24	.79	.00
Methylation	1, 121	.12	.73	.00
5-HTTLPR × Methylation	2, 121	4.87	<.01	.08

Overall model $F(7,121) = 2.48$, $p = .02$, $\eta^2 = .12$.

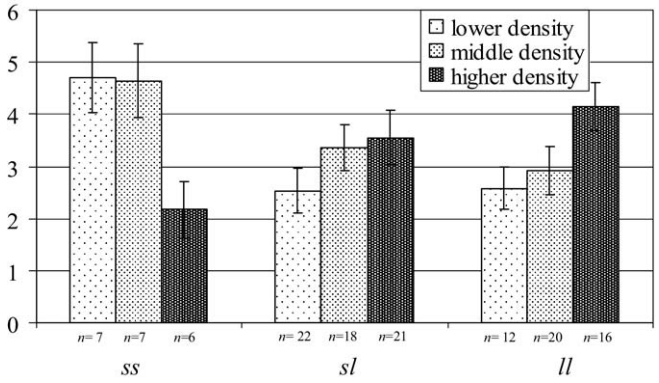

Figure 1. Scores for unresolved loss and trauma (M, SE) as related to 5HTTLPR genotype (*ss, sl, ll*) and methylation density (lower third, middle third, higher third). Lower density methylation: M = –0.41, SD = 0.12, range –0.69 to –0.26. Middle density methylation: M = –0.13, SD = 0.07, range –0.26 to 0.02. Higher density methylation: M = 0.38, SD = 0.31, range 0.02 to 1.48.

Discussion

In this study on participants with experiences of loss or other traumatic events, the 5HTTLPR genotype association with unresolved state of mind was dependent on methylation density. As expected, the long variant in combination with high methylation levels of the 5HTT promoter associated CpG islands predicted more unresolved loss or trauma. Methylation of alleles carrying the *ll* 5HTT variant seemed to hamper the expression of the otherwise protective *ll* variant (17) and to elevate the risk of unresolved responses to loss or other trauma. The short variant of 5HTT predicted more unresolved loss or trauma, but only when levels of methylation were low. Surprisingly, higher levels of methylation of the *ss* variant were associated with less unresolved loss or other trauma, indicating less negative affect about the traumatic experience. This suggests a discontinuous effect of greater methylation for those with the *ss* genotype, perhaps manifesting in decreased preoccupation and a different pattern of adjustment to trauma.

DNA methylation is an important determinant of gene expression, and it should therefore be taken into account when associations of DNA sequences with psychological problems are examined (15). Methylation of CpG islands may repress gene expression in some tissues, whereas the absence of methylation of such a site in other tissues corresponds with increased mRNA transcription. Methylation is found to be a common biological process influenced by environmental stressors such as abusive parenting (16, 19). Beach *et al.* (16) found that childhood experiences of sexual or physical abuse increased the level of methylation at 5HTTLPR. Child maltreatment has long-term developmental consequences (19), which suggests that methylation may serve as the interface between adverse environment and the developing organism. Future studies should focus on how the environment affects methylation patterns.

In an earlier study on methylation of the CpG islands in 5HTT, mRNA levels were significantly associated with level of methylation, but only if the influence of the 5HTTLPR genotype was controlled (8). The authors argued that 5HTT levels are tightly

regulated, and the counteracting effects of methylation on genetic variation may be an adaptive mechanism to maintain a desired level of gene transcription. The vulnerability of the *ss* variant of 5HTT for the development of psychological problems in response to adverse events may be lessened by higher levels of methylation. This may lower the risk for unresolved loss or trauma in carriers of the short variant of the serotonin transporter gene, entailing adaptive value.

A limitation of the study is that transformed cell lines were used and these sometimes can display markedly different methylation signatures than their cognate precursors. Fortunately, Grafodatskaya and colleagues (20) demonstrated a high correlation ($r = .95$) between lymphocyte DNA methylation and low passage lymphoblast DNA methylation. Another limitation is sample size, and replication in larger samples and in other ethnicities is needed to confirm the generalizability of our findings.

Our findings suggest that associations between 5HTTLPR polymorphisms and psychological problems are significantly altered by environmentally induced methylation patterns. By ignoring methylation, researchers may fail to find or replicate ubiquitous G×E interactions (15). Some G×E effects may in fact be environmental influences mediated by methylation patterns.

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On behalf of Dr. Philibert, the University of Iowa has filed intellectual property claims with respect to 5HTT. Dr. Philibert is a potential royalty holder on that application. All other authors reported no biomedical financial interests or potential conflicts of interest. All authors had complete access to raw data.

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