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Association Between Promoter Methylation of Serotonin Transporter Gene and Depressive Symptoms: A Monozygotic Twin Study

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Abstract

Objective—Epigenetic mechanisms have been implicated in the pathogenesis of psychiatric disorders. The serotonin transporter gene (*SLC6A4*) is a key candidate gene for depression. We examined the association between *SLC6A4* promoter methylation variation and depressive symptoms using 84 monozygotic twin pairs.

Methods—DNA methylation level in the *SLC6A4* promoter region was quantified by bisulfite pyrosequencing using genomic DNA isolated from peripheral blood leukocytes. The number of current depressive symptoms was assessed using the Beck Depressive Inventory II (BDI-II). The association between methylation variation and depressive symptoms was examined using matched twin-pair analyses, adjusting for body mass index, smoking, physical activity, and alcohol consumption. Multiple testing was controlled by adjusted false discovery rate (*q* value).

Results—Intrapair difference in DNA methylation variation at 10 of the 20 studied CpG sites is significantly correlated with intrapair difference in BDI scores. Linear regression using intrapair differences demonstrates that intrapair difference in BDI score was significantly associated with intrapair differences in DNA methylation variation after adjusting for potential confounders and correction for multiple testing. On average, a 10% increase in the difference in mean DNA methylation level was associated with 4.4 increase in the difference in BDI score (95% confidence interval = 0.9–7.9, *p* = .01).

Conclusions—This study provides evidence that variation in methylation level within the promoter region of the serotonin transporter gene is associated with variation in depressive symptoms in a large sample of monozygotic twin pairs. This relationship is not confounded by genetic and shared environment. The *5-HTTLPR* genotype also does not modulate this association.

Keywords

DNA methylation; *SLC6A4*; depressive symptoms; monozygotic twins

INTRODUCTION

Depression is the most common mental health problem in the United States and is one of the leading causes of total disability and financial burden (1). Interindividual variability in the vulnerability of depression results from the complex interplay between genetic and environmental factors (2,3). Despite substantial research, understanding of the molecular processes underlying depression remains a major challenge. This uncertainty greatly hampers our ability to implement early diagnosis, prevention, and treatment of this debilitating disorder.

A growing body of evidence suggests that epigenetic mechanisms, especially DNA methylation, play an important role in the susceptibility to depression (4–7). Epigenetic mechanisms act at the interface between external environmental factors and internal genetic landscape on the development of psychiatric disorders through dynamic regulation of gene expression (8–10). Epigenetic factors provide an appealing explanation for many clinical features of depression such as discordance in identical twin pairs, close correlation with adverse life events, sex differences, and remission/relapse episodes (4–6). However, despite the current promise of epigenetic research, the specific genes or biological pathways involved in depression remain poorly understood.

Serotonin (5-hydroxytryptamine [5-HT]) plays a critical role in the pathogenesis of depression. Serotonin transporter (5-HTT; encoded by the *SLC6A4* gene) is responsible for the reuptake of serotonin into the presynaptic neuron and regulates the extracellular serotonin concentration, the strength of serotonin signals, and the duration of postsynaptic responses to serotonin. Thus, it plays a pivotal role in serotonin turnover and serotonin level in the synaptic cleft (11). Consequently, any defects of 5-HTT may lead to dysregulation of the serotonergic system and could potentially contribute to the pathogenesis of depression. Indeed, a polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) has been associated with depression in multiple association studies (12,13), although results are mixed (14,15). This polymorphism involves a 44-base pair insertion/deletion region, rendering either a short (S allele) or long allele (L allele). The S allele of this polymorphism causes reduced uptake of the neurotransmitter serotonin into the presynaptic cells in the brain (11), resulting in a lower functional activity of the 5-HTT compared with the L allele (11,16). Recent evidence also demonstrates that increased *SLC6A4* promoter methylation level was associated with decreased messenger RNA (mRNA) level in lymphoblast cell lines (17,18), although mRNA expression was not associated with lifetime history of major depression in this study (17).

The goal of this study is to investigate the association between methylation variation in a serotonin transporter gene promoter region and current depressive symptoms assessed by Beck Depressive Inventory II (BDI-II). Because both epigenetic variation (19) and depressive symptoms (20) are under genetic control, it is critical to take into account the shared genetic influences between DNA methylation and depressive symptoms. In addition, it is important to control for environment because epigenetic variation is influenced by early life experience, which may have a long-lasting impact on the epigenome that will likely affect future risk for depression (21). A monozygotic (MZ) cotwin control design controls for shared genes and shared environment, thus representing a useful model for epigenetic research of complex traits such as depressive symptoms. As far as we are aware, this is the first study to examine the association between *SLC6A4* promoter methylation and current depressive symptoms, while controlling for the confounding by genetic and/or shared environment in a large sample of MZ twin pairs.

METHODS

Study Population

Twins included in this study were drawn from the Vietnam Era Twin Registry, one of the largest twin registries in the United States (22). All twins were male veterans who were born between 1946 and 1956. A total of 307 twin pairs (who were raised in the same household) were recruited by the Emory Twin Studies (ETS), which included two companion studies to investigate the role of psychological, behavioral, and biological risk factors for subclinical cardiovascular disease in twins. The ETS include male-male twin pairs, including 187 MZ pairs and 120 dizygotic pairs, with an inclusion of two samples of twin pairs discordant for major depression or posttraumatic stress disorder. The ETS protocol has been described elsewhere (23). This research was approved by the Emory Institutional Review Board, and all participants signed an informed consent form.

The current analysis included 84 MZ twin pairs, among which 43 pairs were discordant for major depression and 16 pairs were discordant for posttraumatic stress disorder. These twin pairs were selected from the ETS based on the availability of DNA samples and phenotype data for both members of a twin pair. All twins were examined in pairs at the Emory University General Clinical Research Center between 2002 and 2010, where their medical history was updated. All participants were white. Zygosity information was determined by DNA analysis.

Assessment of Current Depressive Symptoms

We measured current depressive symptoms using the BDI-II (24), a standardized scale providing a continuous measure of depressive symptoms. The BDI is one of the most widely used self-rating scales for measuring depression (25). Its psychometric properties have been extensively validated in community and clinical samples (26). The questionnaire includes 21 items, with each item rating severity of depression on a 4-point scale ranging from 0 to 3. The measures ask respondents to provide evaluative statements on how they have been feeling over the past two weeks. The total score for all 21 items ranges from 0 to 63, with higher scores indicating higher levels of depression.

We also measured lifetime and current major depression using the structured clinical interview for DSM-III-R, which yields a clinical diagnosis of major depressive disorder based on lifetime history of major depressive episodes (27). However, only few twins in ETS met the criteria for current depressive episode assessed by structured clinical interview for DSM-III-R. In addition, most twins experienced only a single lifetime episode, and only few participants had a major episode of depression within the past year, whereas most had their last episode 3 or more years before. Therefore, our analyses focus on the continuous measure of BDI scores.

Other Measurements

All measurements were performed in the morning after an overnight fast, and both members of a pair were tested at the same time. A medical history and a physical examination were obtained from all twins. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Cigarette smoking was classified into current smoker (any number of cigarettes) versus never or past smoker. A pack-year was calculated by multiplying the number of packs of cigarettes a person has smoked per day by the number of years the person has smoked. Physical activity was assessed by means of a modified version of the Baecke Questionnaire of Habitual Physical Activity used in the Atherosclerosis Risk in Communities study (28), a 16-question instrument documenting the level of physical activity at work, during sports, and nonsports activities. The total physical

activity score was used in the analysis. Information on alcohol consumption was collected by asking about the number of alcoholic drinks (beer, wine, or liquor) consumed in a typical week. The total amount of alcohol consumption (in grams) per week was estimated based on the following algorithms: 4 oz of wine contains 10.8 grams, 12 oz of beer contains 13.2 grams, and 1.5 oz of liquor contains 15.1 grams of ethanol.

DNA Methylation Analyses by Quantitative Bisulfite Pyrosequencing

Genomic DNA was isolated from peripheral blood leukocytes by standard methods. Promoter methylation of the serotonin transporter gene (*SLC6A4*) was determined using quantitative bisulfite pyrosequencing by the EpigenDx Inc. (Worcester, MA). Briefly, we assayed 20 CpG dinucleotides in the promoter region of the *SLC6A4* from -213 to -69 base pairs from the transcriptional start site, based on Ensembl Gene ID ENSG00000108576 and the Transcript ID ENST00000394821. The *SLC6A4* assay was designed to capture CpG sites in the promoter region immediately upstream of the transcriptional start site because this region was reported to be sensitive to methylation alterations and influence downstream gene expression in other genes (29). To sequence the selected CpG sites, we designed pyrosequencing assays and tested for polymerase chain reaction (PCR) preferential amplification and quantitative pyro-sequencing analysis. The assay is targeted to the antisense sequence of the *SLC6A4* gene.

The bisulfite conversion was performed with 500-ng genomic DNA using the EZ DNA methylation kit (ZymoResearch, Inc., Irvine, CA). The PCR reaction was performed with 0.2 μ M of each primer with one of the PCR primers being biotinylated to purify the final PCR product using Sepharose beads. The PCR product was bound to Streptavidin Sepharose HP (Amersham Biosciences, Uppsala, Sweden), and the Sepharose beads containing the immobilized PCR product were purified, washed, and denatured using 0.2 M NaOH solution and rewashed using the pyrosequencing Vacuum Prep Tool (Pyrosequencing; Qiagen), as recommended by the manufacturer. Then 0.5 μ M pyrosequencing primer was annealed to the purified single-stranded PCR product. Ten microliters of the PCR products was sequenced by pyro-sequencing PSQ96 HS System (Pyrosequencing; Qiagen), following the manufacturer's instructions (Pyrosequencing; Qiagen). The methylation status of each CpG site was analyzed individually as an artificial T/C SNP using QCpG software (Pyrosequencing; Qiagen). The methylation level at each CpG site for each sample was calculated as the percentage of the methylated alleles over the sum of methylated and unmethylated alleles. The mean methylation level was calculated using methylation levels of all measured CpG sites within the targeted region. Pyrosequencing assay was done on duplicate samples, with a correlation of more than 99.8% between the two runs for a same sample. The average methylation level was used in the statistical analysis. For quality control, each experiment included non-CpG cytosines as internal controls to verify efficient sodium bisulfite DNA conversion. We also included unmethylated and methylated DNAs as controls in each run. In addition, we performed PCR bias testing using pyrosequencing by mixing the unmethylated DNA control and in vitro methylated DNA at different ratios (0, 20%, 40%, up to 100%), followed by bisulfite modification, PCR, and pyrosequencing analysis. The percent methylation obtained from the mixing study showed high correlation with expected methylation percentages ($r^2 = 0.97$), indicating high-quality methylation data.

Genotyping of the 5-HTTLPR Polymorphism

To examine whether the association between DNA methylation and depressive symptoms is confounded by the 5-HTTLPR variant, we genotyped this promoter polymorphism in all twins. Methods for genotyping have been described previously (30). Figure 1 presents a schematic diagram for the 20 CpG sites assayed in our study with respect to the location of 5-HTTLPR variant in the promoter region of *SLC6A4*.

Statistical Analyses

We conducted matched pair analyses to examine the association between *SLC6A4* promoter methylation variation and current depressive symptoms. Because MZ twins share genetic material and raising environment, a matched pair analysis using within twin-pair difference should rule out the potential confounding by gene and/or shared environment. To do so, we first calculated intrapair difference in DNA methylation level within a twin pair, defined as the difference in methylation level of each CpG site between two members of a twin pair. The intrapair differences in other continuous variables, including BDI scores, BMI, smoking (pack-year), physical activity level, and alcohol consumption (grams/wk), were similarly calculated. We then estimated the correlations (Spearman rank) between intrapair differences in BDI score and intrapair differences in DNA methylation level at each CpG residue. We also conducted linear regression analysis (31), in which intrapair difference in BDI score was the dependent variable and intrapair difference in DNA methylation level at each CpG site was the independent variable. Covariates including intrapair differences in BMI, pack-year, alcohol consumption, and level of physical activity were adjusted in the regression model. These analyses were done using robust regression (PROC ROBUSTREG in SAS), which is robust to outliers and/or nonnormality for depressive symptoms and methylation data.

To examine whether *5-HTTLPR* genotype modulates the association between methylation variation and depressive symptoms, we stratified the analyses by *5-HTTLPR* genotypes. We also performed sensitivity analysis to examine whether treatment with antidepressants influences our results by removing twins on antidepressant medications (7 pairs in which both members were on medication plus 13 singletons and their cotwins) from the statistical analysis.

RESULTS

Table 1 presents the demographic characteristics of the twins included in this analysis. The age of the twins ranged from 48 to 61 years, with a mean of 55 years. The distribution of current depressive symptoms, measured with BDI-II, is as follows: BDI of 6 or less, 64.1%; BDI of 7 to 9, 9.6%; BDI of 10 to 13, 7.8%; and BDI of 14 or greater, 18.6%. The mean (standard deviation) BDI score of the twin participants was 6.68 (7.98; ranging from 0 to 43).

Among the 20 examined CpG residues, methylation levels at 19 sites were highly correlated (all p values $<.0001$). Methylation level of one CpG site (Position 3) was significantly correlated ($r = 0.72$; $p < .0001$) with that of another site (Position 4), but not others. The mean (standard deviation) methylation level of the 20 CpG sites across the promoter region was 11.0% (4.2%; ranging from 6.3% to 32.4%). DNA methylation levels of the two members within a pair were significantly correlated at 17 of the 20 examined CpG residues (Table 2). The mean DNA methylation level of the two twins within a pair was also significantly correlated ($r = 0.41$, $p = .0001$). Intrapair differences in DNA methylation variation were significantly correlated with intrapair difference in BDI scores at 10 of the 20 examined CpG sites (Table 3). Intrapair difference in mean DNA methylation across all studied CpG sites was also correlated with intrapair difference in BDI score of the two members within a pair ($r = 0.19$, $p = .05$).

Linear regression based on intrapair differences demonstrated that intrapair difference in BDI score was significantly associated with intrapair differences in DNA methylation level at 10 CpG sites, after adjusting for covariates including BMI, smoking (pack-year), physical activity and alcohol consumption, and multiple testing by adjusted false discovery rate (q value) at the .05 significance level. On average, a 10% increase in the difference in mean

DNA methylation level was associated with 4.4 increase in the difference in BDI scores (95% confidence interval = 0.9–7.9, $p = .01$). In the multivariate regression model, physical activity level was also significantly associated with depressive symptoms, but other covariates (e.g., BMI, alcohol drinking, and smoking) were statistically insignificant. Table 4 shows the association between *SLC6A4* promoter methylation and depressive symptoms by matched pair analysis based on within twin-pair differences in DNA methylation levels and BDI scores.

Statistical analyses stratified by *5-HTTLPR* genotypes demonstrated that, compared with twins without the S allele, those carrying the S allele did not exhibit a significant difference in either depressive symptoms or DNA methylation level. The average within-pair differences in methylation level or depression scores did not differ by *5-HTTLPR* genotypes. These results suggest that the observed association between methylation variation and depressive symptoms may not be confounded by the *5-HTTLPR* genotype. Results for genotype-stratified analyses are shown in Table 5. Sensitivity analyses also showed that the use of antidepressants did not affect the relationship between *SLC6A4* methylation and depressive symptoms. After excluding twins on antidepressants, the observed associations remained statistically significant.

DISCUSSION

Using a well-matched MZ twin sample, we found that higher methylation variation in the promoter region of the serotonin transporter receptor gene in peripheral blood leukocytes was significantly associated with a higher level of depressive symptoms. This association was not confounded by genetic, including the *5-HTTLPR* genotype, and other shared environmental factors among the twins.

The serotonergic system is involved in the regulation of behavioral, psychological, and physiological processes. A sufficient amount of serotonin in the central nervous system is necessary for the normal functioning of these biological activities. Deficiency in the serotonin system is associated with depression and other psychiatric conditions (32). The serotonin transporter is the principal regulator of serotonergic activity, and epigenetic alteration at this locus may thus be an important contributor to the vulnerability of depression. In this context, the observed association between a higher methylation variation of the serotonin transporter gene and a higher level of depressive symptoms found in our study is in line with previous findings. Because DNA methylation usually silences gene expression (33), increased methylation of the serotonin transporter gene could repress the expression of *SLC6A4*, which, in turn, could lead to decreased serotonin uptake and a deficiency of serotonin activity, thereby increasing the risk for depression.

Few previous studies have investigated the association between serotonin transporter gene methylation and depression. Philibert et al. (17) observed a trend for an increased overall *SLC6A4* methylation with lifetime history of major depression in unrelated individuals recruited by the Iowa Adoption Studies, but the association was statistically insignificant (17.3% in participants with major depression versus 15.6% in those without, $p < .07$). In addition, the trend disappeared when male and female samples were analyzed separately, indicating that sex may be a potential confounder for the observed trend. The authors also found an association of increased methylation with reduced levels of mRNA expression in lymphoblast cell lines. This association was evident only when the *5-HTTLPR* genotype was taken into account, implying that genetic factors may potentially confound the observed association of DNA methylation and mRNA expression. Moreover, mRNA expression was not associated with lifetime history of major depression in that study (17), leading to intriguing results with respect to the relationships among *5-HTT* gene methylation, mRNA

production, and depression. Our study, however, did not detect an association of the 5-*HTTLPR* genotype with either methylation variation or depressive symptoms, suggesting that this genotype is unlikely to mediate the relationship between *SLC6A4* methylation and depressive symptoms in our sample. The use of MZ twin pairs in our study rules out the confounding influences of genetic and/or shared environmental heterogeneity, thus allowing elucidation of the etiological role of epigenetic variation in disease development. The same-sex cotwin control design provides additional control for sex, a potential confounder that is known to be implicated in both depression (34) and epigenetic variation (35).

In this study, we observed that DNA methylation level at individual CpG sites showed considerable variability within MZ twin pairs (ranging from 3.0 to 16.7). This agrees with findings by us (36) and other investigators (37,38) in the investigation of complex disease-associated epigenetic variation. In addition, the magnitude of DNA methylation variation is relatively small compared with DNA methylation alterations generally observed in cancer. However, the magnitude of methylation variation in our study is comparable with many of the previous studies on nonmalignant complex disorders (36,38–44). It is most likely that epigenetic variation at multiple CpG sites is involved in the pathogenesis of a complex disease, but each site individually confers only a small risk effect to disease. This phenomenon seems to be the norm for human complex disorders and parallels with findings from genome-wide association studies in which many genetic variants contribute to disease risk, but the predicted risk associated with each variant is generally small (45).

Our study has several limitations. First, because of practical difficulties in obtaining tissues from living individuals, methylation levels were tested in peripheral blood leukocytes, but not directly from brain, the primary affected organ in depression. Therefore, our results may not provide a direct index of DNA methylation in the central nervous system. However, there is increasing evidence that leukocytes may be a useful cellular model to evaluate epigenetic changes because epimutations may not be limited to the affected tissue but can also be detected in peripheral blood leukocytes (46). Importantly, blood samples are much easier to obtain and could be used for large-scale epidemiologic studies, tracking changes associated with depression as the disease develops. Second, our epigenetic data were collected from DNA derived from whole blood leukocytes, which is a mixture of many cell types. As such, we were unable to assess cell-specific differences in methylation changes, which could have to be accounted for when replicating the present findings in future studies. In addition, we were unable to evaluate the functional significance of the observed methylation changes because of the lack of brain tissue or fresh leukocytes. Third, the magnitude of the observed within twin-pair difference in DNA methylation was relatively small, and the clinical significance of these small epigenetic alterations with respect to the change in depressive symptoms remains to be determined. Fourth, our analyses only focused on a small region in the promoter of the serotonin transporter gene and thus may not reflect the methylation status of other genomic regions, which should be examined in future research. Finally, our twin sample was derived from a middle-aged sample of male military veterans; therefore, the generalizability to women and other younger or older populations is not known. In addition, because our study was cross sectional, the causal relationship between methylation variation and depressive symptoms cannot be determined in the current analysis.

Nonetheless, to our best knowledge, this is the first study to demonstrate the association of DNA methylation variation in the promoter region of the serotonin transporter gene with current depressive symptoms in a large sample of MZ twin pairs. Previous studies have shown that interindividual epigenetic processes have genetic predispositions (47,48). Therefore, the study of the causal impact of epigenotype on disease etiology requires careful matching on or control for genetic background and other potential confounding variables.

Monozygotic twins match exactly on genetic background, age, and sex, thus eliminating the effects of these important confounders (49,50). In addition, identical twins in general share raising environment, providing further control for confounding by early life experience, which has a long-lasting impact on the epigenetic plasticity of human genome (51).

In summary, this study provides initial evidence that variation in DNA methylation level of the serotonin transporter gene promoter region is associated with variation in depressive symptoms. This association is not confounded by gene and/ or shared environmental factors. Because epigenetic processes are potentially reversible, our finding may provide an opportunity for the development of novel therapeutic approaches for the prevention and treatment of depression and its associated disorders.

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Glossary

SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
5-HTTLPR	serotonin-transporter–linked polymorphic region
5-HTT	serotonin transporter
BDI-II	Beck Depressive Inventory II

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Figure 1.

A schematic diagram for the studied CpG sites in *SLC6A4* promoter region in relation to 5-*HTTLPR* variant. The sequence shown represents a 145-bp fragment (–213 to –69 with respect to TSS) in the 5'-untranslated region of *SLC6A4*. Numbers 1 to 20 refer to locations of the CpG sites assayed in this study. TSS = transcriptional start site; ATG: translational start codon. *SLC6A4* = solute carrier family 6 (neurotransmitter transporter, serotonin), member 4; 5-*HTTLPR* = serotonin-transporter–linked polymorphic region.

TABLE 1Characteristics of the Twin Participants ($n = 168$)

Variable	Mean (SD) or %
Age, y	55.1 (2.8)
Type 2 diabetes, %	11.4
Hypertension, %	36.5
Body mass index, kg/m ²	29.4 (4.8)
Cigarette smoking, pack-year	22.9 (24.1)
Alcohol consumption, grams/wk	12.5 (36.3)
Physical activity score	7.4 (1.9)
Systolic blood pressure, mm Hg	129.3 (17.5)
Diastolic blood pressure, mm Hg	81.4 (11.6)
High density lipoprotein, mg/dl	37.6 (10.7)
Low density lipoprotein, mg/dl	123.3 (36.8)
Total triglyceride, mg/dl	177.5 (102.5)
Mean BDI score	6.68 (7.98)
Mean DNA methylation level, %	11.02 (4.20)

BDI = Beck Depressive Inventory; SD = standard deviation.

TABLE 2

Intrapair Correlation (One Twin and His Cotwin Brother in a Pair) Between BDI Score and DNA Methylation at *SLC6A4* Locus

CpG site	Correlation	<i>p</i>
1	0.001	.95
2	0.51	<.001
3	0.16	.15
4	0.13	.26
5	0.52	<.001
6	0.43	<.001
7	0.24	.02
8	0.44	<.001
9	0.30	.006
10	0.49	<.001
11	0.36	.002
12	0.35	.001
13	0.39	<.001
14	0.40	<.001
15	0.48	<.001
16	0.41	<.001
17	0.42	<.001
18	0.39	<.001
19	0.40	<.001
20	0.39	<.001
BDI score	0.43	<.001

BDI = Beck Depressive Inventory; *SLC6A4* = solute carrier family 6 (neuro-transmitter transporter, serotonin), member 4.

TABLE 3

Correlation Between Intrapair Difference in Depressive Symptoms and Intrapair Difference in *5-HTTLPR* Promoter Methylation Variation in a Monozygotic Twin Sample ($n = 84$ pairs)

Position	Distance Relative to TSS (bp)	Genomic Position (Chr17, GCRh37/hg19)	Mean Methylation, mean (SD), %	Intrapair Correlation (p Value)
1	-69	28562784	3.02 (1.75)	0.05 (.60)
2	-72	28562787	3.39 (1.91)	0.19 (.05)
3	-99	28562814	4.30 (3.67)	0.08 (.49)
4	-112	28562827	9.63 (6.82)	0.15 (.16)
5	-133	28562848	13.27 (5.57)	0.26 (.01)
6	-135	28562850	11.28 (4.06)	0.27 (.01)
7	-139	28562854	11.65 (4.86)	0.17 (.05)
8	-141	28562856	9.23 (3.48)	0.12 (.07)
9	-147	28562862	16.72 (7.18)	0.07 (.52)
10	-149	28562864	11.03 (3.92)	0.18 (.05)
11	-155	28562870	13.54 (5.78)	0.21 (.04)
12	-170	28562885	15.67 (4.61)	0.11 (.12)
13	-174	28562889	14.76 (6.83)	0.22 (.03)
14	-188	28562903	15.98 (7.77)	0.19 (.05)
15	-190	28562905	10.03 (4.27)	0.24 (.03)
16	-195	28562910	13.58 (5.75)	0.11 (.27)
17	-200	28562915	10.12 (4.74)	0.16 (.08)
18	-207	28562922	11.59 (4.72)	0.18 (.06)
19	-209	28562924	8.76 (3.68)	0.14 (.17)
20	-213	28562928	12.79 (6.61)	0.21 (.03)
Mean			11.02 (4.20)	0.19 (.05)

5-HTTLPR = serotonin-transporter-linked polymorphic region; TSS = transcription start site; bp = base pairs; SD = standard deviation.

TABLE 4

Association Between Intrapair Difference in Methylation and Intrapair Difference in BDI by Matched Pair Analysis ($n = 84$ pairs)^a

CpG Site	Regression Coefficient β (95% CI) ^b	p
1	1.9 (−8.4 to 4.6; 0.56)	.56
2	3.6 (−1.2 to 5.0)	.41
3	0.5 (−0.03 to 10.8)	.051
4	1.7 (−0.1 to 3.4)	.058
5	3.9 (1.0 to 6.8)	.009 ^c
6	4.6 (1.0 to 8.2)	.012 ^c
7	4.0 (1.7 to 6.4)	<.001 ^c
8	5.4 (1.0 to 9.8)	.015 ^c
9	2.4 (0.6 to 4.2)	.007 ^c
10	2.9 (1.1 to 7.0)	.028 ^c
11	2.4 (−0.1 to 4.9)	.064
12	2.5 (−0.6 to 5.6)	.12
13	2.3 (0.1 to 4.5)	.038 ^c
14	2.1 (0.1 to 4.0)	.044 ^c
15	3.3 (0.6 to 7.5)	.034 ^c
16	1.9 (−0.1 to 4.5)	.13
17	2.3 (−0.1 to 5.5)	.16
18	2.7 (−0.07 to 6.1)	.12
19	3.9 (−0.05 to 8.3)	.083
20	2.4 (0.2 to 4.7)	.032 ^c
Mean	4.4 (0.9 to 7.9)	.014 ^c

BDI = Beck Depressive Inventory; CI = confidence interval.

^a Increase in BDI score difference per 10% increase in methylation difference.

^b Adjusted for intrapair differences in body mass index, pack-year, alcohol consumption, and physical activity level.

^c Remained significant after correction for multiple testing by adjusted false discovery rate (q value) at the .05 level (two sided).

TABLE 5Sensitivity Analysis According to *5-HTTLPR* Genotypes (*n* = 84 Pairs)

	LL (<i>n</i> = 30)	SL (<i>n</i> = 39)	SS (<i>n</i> = 15)	<i>p</i>^a
DNA methylation level, %	11.4 (4.4)	10.8 (3.7)	10.8 (5.0)	.74
Mean intrapair difference in methylation	0.9 (3.6)	0.7 (3.8)	0.9 (7.4)	.66
BDI scores	6.6 (7.0)	7.4 (9.1)	4.5 (6.2)	.10
Mean intrapair difference in BDI scores	1.2 (8.5)	0.3 (8.1)	4.1 (9.1)	.10

Data are shown as mean (SD). *5-HTTLPR* = serotonin-transporter-linked polymorphic region; LL=long allele homozygotes; SL=heterozygotes; SS=short allele homozygotes; SD = standard deviation; BDI = Beck Depressive Inventory.

^a S allele carriers versus noncarriers.