Depression in systemic lupus erythematosus patients is associated with link-polymorphism but not methylation status of the 5HTT promoter region

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What is This?
Depression in systemic lupus erythematosus patients is associated with link-polymorphism but not methylation status of the 5HTT promoter region

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A higher prevalence of depression in systemic lupus erythematosus (SLE) patients has been reported, though the mechanism underlying this phenomenon remains unclear. The present study was conducted to explore whether the polymorphism and methylation status of the serotonin transporter gene (5HTT) promoter region (PR-5HTT) contribute to depression in SLE patients from both genetic and epigenetic perspectives. In this study, 96 SLE patients and 96 healthy controls (HCs) were recruited. Depression levels of all subjects were evaluated using the Hamilton Depression Rating Scale (HDRS). The serotonin transporter-linked polymorphism (5HTTLPR) and the DNA methylation status of PR-5HTT were detected in peripheral lymphocytes of SLE patients and HCs. The differences in 5HTTLPR and DNA methylation of PR-5HTT between SLEs and HCs were compared. In SLE patients, the frequencies of short allele (S) and SS genotype of 5HTTLPR were higher in depressive SLE (SLE-D) patients than in non-depressive SLE (SLE-ND) patients. The mean HDRS score of SS homozygote patients was higher than that of patients with SL/LL genotypes. Conversely, PR-5HTT was hypomethylated in HCs as well as SLE patients. There was no difference in the methylation status between HCs and SLEs. Thus, the functional expression of PR-5HTT may be primarily regulated by gene polymorphism and not by DNA methylation. The risk allele of 5HTTLPR appears to be a major contributor to depression in SLE patients. Lupus (2013) 22, 1001–1010.

Key words: Systemic lupus erythematosus; depression; DNA methylation; serotonin transporter-linked polymorphism

Introduction

Systemic lupus erythematosus (SLE) is a common autoimmune disease affecting multiple organs and bodily systems, particularly the central nervous system (CNS). SLE is closely related to highly prevalent mental disorders and distinct psychological disorders.1–4 Comorbidity of depression and SLE is quite common, with morbidity rates ranging from 10.8% to 39.6%,5,6 significantly higher than in the general population.7 Gao et al.8 identify the brain as the primary organ affected in lupus patients and states that depression-like behaviors may occur at a very early stage of the disease, despite only slight immediate changes in autoimmunity. However, the exact mechanisms of this connection remain unclear.

Researchers have long suspected that genetics play a role in both SLE and depression. Recent genome-wide9 and association studies10 found a relationship between signal transducers and activators of the transcription (STAT) 4 gene and SLE, while the STAT pathway is also involved with synaptic plasticity in the brain.11 Brain-derived
neurotrophic factor (BDNF) gene is an important candidate gene for depression. The BDNF Met66 allele is also associated with cognitive functioning in the psychomotor and motor domains of SLE patients. These results suggest some degree of shared genetic background in both SLE and depression. On the other hand, as they can regulate gene expression without altering DNA sequences, epigenetic mechanisms, including DNA methylation, have been implicated in the regulation of complex behavior, including abnormalities in psychiatric disorders such as depression. Some specific epigenetic modifications have also been identified in mood disorders and depression. Postmortem studies revealed epigenetic changes in the frontal cortex of depressed suicide victims. Adverse alterations of gene expression profiles, including the glucocorticoid receptor or BDNF, were shown to be inducible by early life stress and reversible by epigenetic drugs. Furthermore, there are a number of studies on the effects of methylation on specific immunoregulation genes in SLE. Abnormal DNA methylation has been linked to the pathogenesis of lupus, particularly with respect to lymphocytes.

The serotonin (5HT) dysfunction hypothesis is one of the most popular proposed mechanisms of depression. Importantly, 5HT has also been recognized as a peripheral immune regulation factor and can regulate both innate and adaptive immunity when stimulated by antigens. This regulation includes activating and inhibiting effects on T cells, B cells, natural killer (NK) cells, and macrophages. 5HT binding with the 5HT receptor on the immune cell membrane can induce the production of a second messenger, which then regulates immune system functions.

5HT transporters (5HTT) at the presynaptic membrane reuptake 5HT from the synaptic cleft and accurately regulate the quantity and duration of 5HT neurotransmission. Indeed, mutations in the 5HTT gene have often been linked to depression. Lesch et al. reported a variable nucleotide repeat, termed a serotonin transporter linked polymorphic region (5HTLPR), with a 22 base pair (bp) repeat unit 1400 bp upstream of the 5HTT transcription start site. The short (S) variant of 5HTLPR containing 10 repeats reduced transcriptional efficiency by approximately 50% compared to the long (L) 12 repeat allele, with the S variant having a dominant effect on both mRNA transcription and clinical phenotype. The 5HTTLPR can affect psychiatric disorders such as depression. Peripheral 5HTT shares the same genetic coding with central 5HTT. There are reported reductions of 5HTT in the brain, blood platelets, and lymphocytes of patients with major depression. Previous research using lymphoblast lines reported that 5HTT mRNA levels were associated with the methylation of an upstream CpG island via the 5HTTLPR. An interaction of 5HTTLPR and methylation was also found. Individuals may experience a greater sense of loss and trauma if their less functional 5-HTTLPR “SS” allele was lightly methylated, or conversely, if their high-functioning “LL” allele was “silenced” by heavy methylation. Meyerhoff et al. have reported decreased levels of platelet 5HT in SLE patients, with the lowest levels being found among those with the most serious SLE indicators. However, studies concerning the role of 5HTTLPR in SLE pathology or its concurrence with depression have been inadequate. As 5HTT deficits can be detected in both SLE and depression, it is possible that the epigenetic regulation and genetic variation of the 5HTT may contribute to the pathophysiology of SLE or to comorbid depression.

In our previous results, we found that hypomethylation of the promoter area of the serotonin 1A receptor (HTR1A) and overexpression of HTR1A might contribute to SLE. However, the genetic or epigenetic regulation of the 5HTT, as well as its contribution to depression in SLE, remains unclear. In this study, we attempted to detect 5HTTLPR status and DNA methylation of the serotonin transporter gene promoter region (PR-5HTT) in SLE patients with (SLE-D) or without (SLE-ND) depression and in corresponding controls to understand the role of gene polymorphism and DNA methylation in the pathology of SLE and the comorbidity of depression.

Material and methods

Subjects

A group of SLE inpatients or outpatients from the Department of Rheumatology and Immunology of the First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China (Chinese SLE Treatment and Research Group (CSTAR) member units), were recruited for this study. All were investigated via a standardized protocol and were followed by the same investigator. The inclusion criteria included the following factors: (1) diagnosis of SLE confirmed when patients presented with four or more items, according to the 1997 revised American College of Rheumatology (ACR) criteria for the classification of lupus;
(2) aged from 18 to 50; and (3) willingly gave written consent to participate in this study.

The exclusion criteria included the following factors: (1) patients presenting with ACR standards of rheumatoid arthritis, systemic sclerosis, Sjögren’s syndrome, idiopathic osteoporosis, or other connective tissue diseases, and the standard drug-induced SLE; (2) patients with present or previous diagnosed neurological or psychiatric disease (e.g., head trauma, stroke, Parkinson’s disease, epilepsy, schizophrenia, bipolar disorder); (3) patients with psychiatric symptoms (e.g., illusions, delusions, disorganized behavior); (4) patients with a history of substance abuse; (5) patients who were pregnant; or (6) patients with other physical diseases such as high arterial blood pressure, diabetes or insufficient renal function.

Adhering to the above inclusion and exclusion standards, a group of 96 consecutive SLE patients were recruited in the present study. Ninety-six healthy controls (HCs), matched by sex and age, were also recruited. All HC group members received thorough physical examinations to exclude major physical disorders, particularly neurological problems. An experienced rheumatologist and a neurologist performed these examinations. Participants were also examined and selected by an experienced psychiatrist via the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), which is based on the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV). All participants gave written consent after being informed of the research process. The Ethics Committee of the First Affiliated Hospital of Kunming Medical University approved this research (ClinicalTrial.gov: NCT00703742).

Collection of population statistics, clinic data and depression evaluation

All participants were Chinese Han people. Demographic and clinical data for each patient were collected, including sex, age of disease onset, and course of disease.

Course of disease was defined as the duration from the primary clinical manifestation of confirmed SLE to the instance of peripheral blood collection. All clinical manifestations and lab examinations were based on the ACR Standard Record. Depression was evaluated via the 17 items of the Hamilton Depression Rating Scale (HDRS) by an experienced psychiatrist, and subjects were confirmed as presenting with depression when they scored over 14. Other mental health symptoms were examined and confirmed via the structured clinical interview provided by the SCID for Patients Edition. The disease activity of SLE patients was measured by the systemic lupus erythematosus disease activity index (SLEDAI).

Test of the 5HTTLPR

The 5HTTLPR of all subjects was analyzed using the method described by Kluck and Cook. In a 200 μl nuclelease-free microcentrifuge tube, the following were combined according to the manufacturer’s instructions (TaKaRa Biotechnology Co., Ltd.): TaKaRa LA Taq (5 U/μl) 0.25 μl, 10 × LA polymerase chain reaction (PCR) Buffer (Mg2+ Plus) 2.5 μl, dNTP mixture (each 2.5 mM) 4 μl, sense primer (5’- GGCGTTGCGCTCTGAATGC-3’) 0.5 μl, antisense primer (5’-GAGGACTGAGCTGGA CAACCAC-3’) 0.5 μl, template DNA (3 μl of patient, 1 μl of normal control), and nuclelease-free water to 25 μl reaction volume. The lengths of PCR products of these primers were 484 bp (S) and 528 bp (L). Touchdown PCR procedures were carried out in the following manner: initial denaturation at 95°C for five minutes and 95°C for 30 seconds and annealing temperature from 65°C to 55°C (decreasing by 1°C per cycle) for 30 seconds and then 72°C for 30 seconds. At the extension temperature of 55°C, 25 cycles were performed; the temperature was then held at 4°C. The PCR products were separated by 3% agarose gel electrophoresis and visualized on ethidium bromide-stained agarose gel. Bands were visualized by ethidium bromide staining under ultraviolet (UV) illumination. The 44-bp deletion was designated as the “S,” or “short” variant, while the 44 bp insertion was designated as the “L,” or “long” variant. Thus, the three possible genotypes were “SS” homozygote, “SL” heterozygote, and “LL” homozygote (Supplementary Figure 1).

Detecting methylation of the PR-5HTT: DNA sequence analysis and primer design

The PR-5HTT was derived from the sequence annotated in the database of the National Center for Biotechnology Information (NCBI) (GenBank accession number: EF179203.1). According to the definition of a CpG, the PR-5HTT ranges from −16169 bp to −14,992 bp. The CpG islands were predicted via CpG Island Searcher (http://www.urogene.org/methprimer/index.html) (Figure 1). When designing the primers, cytosine (C) in the original sequence was substituted with thymine (T). A new sequence was produced, for which nested

Lupus
PCR primers were designed as an outer-side primer pair (5HTT-Osense: 5'-TTTAGGATTTTTTGTGGAATTTTTATTATT-3'/5HTT-Oantisense: 5'-AAAAACTCTACCTCTCCCCAAAA-3') and an inner-side primer pair (5HTT-Isense: 5'-GGTTAGGATTTTTTGGTTAGGATTTTTT-3'/5HTT-Iantisense: 5'-AAAAAACTCTACCTCTCTCCCCAAAA-3'). The primer sequence is marked in Figure 1. The length for amplification was 236 bp.

Peripheral blood lymphocyte cell (PBLC) isolation

Peripheral blood samples of all subjects were taken on the initial day of investigation. Peripheral blood lymphocytes were then isolated via the standard Ficoll-Hypaque density gradient centrifugation procedure. The isolated lymphocytes were then used for DNA extraction.

Genomic DNA extraction and bisulfite modification

DNA was extracted from peripheral lymphocytes via a standard proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. The DNA samples were tested using a spectrophotometer with the ratio of A260:A280 between 1.6 and 1.8. Bisulfite modification of genomic DNA was conducted with the EZ DNA Methylation-Gold Kit (Zymo Research Corporation, California, USA) according to the manufacturer’s instructions. This kit has been validated as highly effective in converting unmethylated cytosine into uracil, thereby enabling subsequent PCR extension products to contain T in place of unmethylated cytosine. Here, the methylated cytosines (mC) were not converted. During the final step, converted DNA was eluted with 35 μl of elution buffer.
rather than with 10 μl as directed by the instructions. Samples were then stored at −20°C for future use.

**PCR and sequencing**
We used bisulfite modification sequencing methods to detect the methylation of PR-5HTT. First, PCR amplification of PR-5HTT was conducted by combining nested and touchdown PCR. Methylation status was then analyzed by bisulfite modification sequencing. This method accurately measures methylation due to the conversion of unmethylated cytosine into uracil, leaving mC unchanged. We were then able to easily identify mC through sequence alignment.

The first PCR reaction was performed in a volume of 12.5 μl containing no less than 3 μl of DNA template that was converted using LA Taq polymerase (TaKaRa Biotechnology (Dalian) Co., Ltd.). Touchdown PCR was carried out under the following conditions: initial denaturation occurred at 95°C for five minutes and at 95°C for 30 seconds, and the annealing temperature ranged from 62°C to 52°C (decreasing by 2°C every two cycles) for 30 seconds and then 72°C for one minute. An additional 18–30 cycles were performed at 95°C for 30 seconds, at 50°C for 30 seconds, and at 72°C for one minute, with a final extension at 72°C for 10 minutes. In the second PCR, 2.5 μl of the first PCR product was added to 47.5 μl (total 50 μl) of the reaction mix using Ex Taq (TaKaRa Biotechnology (Dalian) Co., Ltd.). Touchdown PCR was also performed as described above, changing only the annealing temperature (from 62°C to 51°C, decreasing by 1°C per cycle). The PCR products were separated by 1.2% agarose gel electrophoresis, stained with ethidium bromide and visualized by UV transillumination (Beckman, USA). PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA). These purified products were cloned into pMD 19-T Vectors (TaKaRa Biotechnology (Dalian) Co., Ltd.). The products were sent to Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. for two-direction sequencing. We sequenced seven to 10 clones of each cloned product.

**Statistical analysis**
Statistical analysis was conducted with SPSS 17.0 (SPSS Inc, 1989–2004). The two group t tests were used to compare the difference of HDRS score for SLE and HCs, as well as the difference of SLEDAI for SLE-ND and SLE-D. The allelic frequencies and genotypes for SLEs and HCs, as well as the quantity of mCs and the frequencies of mCs between the two groups, were analyzed by the chi square test and Fisher’s exact tests. Student’s t tests were used to compare the difference of age, HDRS, and SLEDAI between the SS group and L carrier group. One-way factorial analyses of variance (ANOVA) were used to compare the difference of age, HDRS, and SLEDAI between different genotype groups. The least-significant difference (LSD) tests were used for post-hoc multiple comparisons. The results were statistically significant when p < 0.05. All statistical tests were two sided.

**Results**

**Demographical and clinical data**
This research involved 96 SLE patients and 96 HCs matched by age and sex. In each of the two groups, there were 19 males and 77 females. There were 36 patients (37.5%) identified as concomitant with depressive mood, with an HDRS score above or equal to 14. The mean HDRS score for SLE-D was 18.17 (SD = 3.63), while the HDRS score for SLE-ND was 6.78 (SD = 2.89). There were no significant differences in the severity and activity of SLE between depressed and non-depressed patients (Table 1).

**5HTTLPR’s relationship with depression in SLE**
The 5HTTLPR in HCs and SLE patients was detected. Observed genotype frequencies for all participants were consistent with the Hardy-Weinberg Equilibrium (p = 0.907 for HCs and p = 0.13 for SLE, all p > 0.05). There were no significant variances of allelic frequencies and genotypes between HCs and SLEs (χ² = 0.202, p = 0.653 for allelic frequencies, and χ² = 0.333, p = 0.564 for genotypes) (Table 1). However, there was a higher frequency of the long allele (L) in SLE-ND than in SLE-D (χ² = 7.482, p = 0.006, odds ratio (OR) = 0.371, 95% confidence interval (CI) = 0.180–0.766). The frequency of SS homozygotes was also higher in SLE-D than in SLE-ND (χ² = 6.160, p = 0.013, OR = 2.972, 95 CI% = 1.240–7.121) (Table 2). The HDRS score of SS genotype patients was higher than that of LL genotype patients (p = 0.042, Supplementary Table 1), while the L carrier group had lower HDRS scores than that of the SS homozygote group (t = −2.133, p = 0.036). There were no significant differences of SLEDAI scores between different genotypes (Supplementary Table 1).
Methylation status of PR-5HTT in SLEs and HCs

Using the bisulfate sequencing method, we could detect the methylation state of a segment of PR-5HTT with a length of 178 bp. There were 15 potential CpG sites we tested in the PR-5HTT of HCs (Figure 1). However, we found only 11 methylated CpG sites. This region showed less methylation in most HC subjects. In 96 HCs, only 25 individuals had mC (26.04%). Another 71 people had no mC at all. In total, there were only 33 mC out of all 1440 potential CpG sites for all HCs (2.29%).

This research also analyzed methylation of the PR-5HTT in 96 SLE patients. In all 15 potential CpG sites, only eight CpG sites were methylated in SLE patients, particularly at the $/C0_{15119}$, $/C0_{15108}$, and $/C0_{15101}$ sites. Of the 96 patients, there were only 17 cases with mC. There were only 23 mCs out of the 1440 potential CpG sites for all SLE patients (1.60%). Among the SLE patients and HCs, there was no significant difference in the number of subjects with mC ($\chi^2 = 1.950$, $p = 0.163$) or the total number of mC ($\chi^2 = 1.821$, $p = 0.177$; Table 1). Likewise, there was no significant difference in the number of mC occurring in each CpG site between the SLE and HCs groups. The percentage of mC at site $-15108$ appeared to be higher, but the differences were not statistically significant compared with HCs (Supplementary Table 2).

Methylation status of PR-5HTT between SLE-D and SLE-ND

We compared the degree of methylation of the PR-5HTT in SLE-D and SLE-ND to explore the relationship between methylation and depression. Only five of 36 SLE-D subjects had mC (13.89%) for a total of 8 mC (1.48%). In contrast, 12 of 60 SLE-ND subjects had mC (20.00%) for a total of 15 mC (1.67%). There was no significant difference in the overall number of mC between depressive and non-depressive SLE patients ($\chi^2 = 0.074$, $p = 0.786$), neither was there a difference in the number of individuals with mC in these two groups ($\chi^2 = 0.577$, $p = 0.448$; Table 1). The numbers of mC occurring in each CpG site between the SLEs and HCs or between SLE-D and SLE-ND showed no significant difference (Supplementary Table 2). There was no difference of mC in different genotypes between SLEs and HCs or between SLE-D and SLE-ND (Table 2).

Discussion

In this study we report a correlation between the short allele of SHTTLPR and depression in SLE patients. SLE-D patients exhibited higher frequencies of the S allele than did SLE-ND patients. The SS homozygocity occurred more often in SLE-D patients. Additionally, depressive severity of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographical data and the distribution of 5HTT methylation and SHTTLPR for SLE and HC groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC (male, female)</td>
</tr>
<tr>
<td>N</td>
<td>96 (19, 77)</td>
</tr>
<tr>
<td>HDRS score (Mean±SD)</td>
<td>1.26±1.56</td>
</tr>
<tr>
<td>Number of subjects with mC (%)</td>
<td>33 (2.29)</td>
</tr>
<tr>
<td>L (%)/S (%)</td>
<td>58 (30.21)/134 (69.79)</td>
</tr>
<tr>
<td>L carrier (%)/SS (%)</td>
<td>49 (35.71)/47 (48.96)</td>
</tr>
</tbody>
</table>

The two group t tests were used to compare the difference of HDRS score for SLE and HCs, as well as the difference of SLEDAI for SLE-ND and SLE-D. The allelic frequencies and genotypes for SLEs and HCs, as well as the quantity of mCs and the frequency of mCs between the two groups, were analyzed by the chi-square test and Fisher’s exact tests. SLE: systemic lupus erythematosus; HC: healthy controls; HDRS: Hamilton Depression Rating Scale; SLEDAI: systemic lupus erythematosus disease activity index; SLE-D: depressive SLE patient; SLE-ND: non-depressive patient.
patients with the SS genotype was higher than that of L carrier patients. These results indicate that the S allele is a risk factor for depression in SLE, while the L allele showed some protective effect of depression in SLE (OR = 0.371, 95% CI = 0.180–0.766; Table 2).

The relationship between 5HTTLPR and depression has been widely studied, and the 5-HTTLPR S allele has been associated with anxiety-related personality traits and with depression. A recent meta-analysis supports the hypothesis that 5-HTTLPR moderates the relationship between stress and depression. The meta-analysis found a statistically significant relationship between the presence of the S allele of 5-HTTLPR and increased stress sensitivity in two of the subgroups: 1) individuals who had suffered abuse in childhood and 2) individuals who developed a specific stress-related medical condition. Another study found that carriers of the S allele of 5-HTTLPR are more vulnerable to depression, to perceived stress, and to high norepinephrine secretion among patients with chronic illness. These factors may contribute to more severe cardiovascular outcomes in these patients. Although the specific mechanism(s) of how 5HTTLPR affects the function of 5HTT remains unclear, 5HTTLPR has been identified as an important regulator of 5HTT activity. Findings by Lesch et al. show that the S and L alleles are associated with low and high 5HTT activity, respectively. Further research has also shown that the transcriptional activity of the long L segment is 2.5 times higher than that of the short S segment. The L allele can strengthen 5HT transcription and the expression of the 5HT protein, promoting the rate of reuptake of 5HT. Thus, the L and S alleles are thought to function differently in 5HT gene transcription and transporter protein expression.

Studies focused on the effects of the 5HTT polymorphism on 5HT function in blood platelets have also revealed that the average binding fraction of individuals’ blood platelets with the S allele is fairly low. Consistent with these results, it is possible that the S allele may also affect 5HTT activity or stress sensitivity in SLE patients.

To our surprise, we found that the PR-5HTT presented with very low rates of methylation (only approximately 2% of CpGs were methylated) in SLE patients and HCs. Our study did not find a difference in the methylation state of the 5HTT promoter between HCs and SLE patients. 5HTT promoter methylation in depressed and non-depressed patients was approximately equal. There are about 29,000 CpG islands in the human genome, and 50%–60% of all genes contain a CpG island. In vertebrates, 70% of CpGs are methylated. However, the promoter regions of active genes are less than 30% methylated. Usually, CpG islands of expressed genes have low levels of methylation. Methylated genes are relatively transcriptionally inactivate while unmethylated genes are more active. The hypomethylation of the PR-5HTT might suggest a higher level of transcription or an activated

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### Table 2  Distribution of 5HTTLPR and the methylation status of different 5HTTLPR genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SLE (n=96)</th>
<th>HC (n=96)</th>
<th>X²</th>
<th>p</th>
<th>OR</th>
<th>95% CI</th>
<th>SLE-D (n=36)</th>
<th>SLE-ND (n=60)</th>
<th>X²</th>
<th>p</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL (%)</td>
<td>9 (9.38)</td>
<td>9 (9.38)</td>
<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.379–2.635</td>
<td>1 (2.78)</td>
<td>8 (13.33)</td>
<td>/</td>
<td>0.147*</td>
<td>0.186</td>
<td>0.022–1.551</td>
</tr>
<tr>
<td>SL (%)</td>
<td>36 (37.50)</td>
<td>40 (41.67)</td>
<td>0.125</td>
<td>0.723</td>
<td>0.900</td>
<td>0.502–1.612</td>
<td>10 (27.78)</td>
<td>26 (43.33)</td>
<td>2.323</td>
<td>0.127</td>
<td>0.503</td>
<td>0.206–1.225</td>
</tr>
<tr>
<td>SS (%)</td>
<td>51 (53.13)</td>
<td>47 (48.96)</td>
<td>0.333</td>
<td>0.564</td>
<td>1.182</td>
<td>0.671–2.082</td>
<td>25 (69.44)</td>
<td>26 (43.33)</td>
<td>6.160</td>
<td>0.013</td>
<td>2.972</td>
<td>1.240–7.121</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36</td>
<td>60</td>
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<tr>
<td>Allelic frequency</td>
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<tr>
<td>L (%)</td>
<td>54 (28.13)</td>
<td>58 (30.21)</td>
<td>0.202</td>
<td>0.653</td>
<td>0.904</td>
<td>0.582–1.404</td>
<td>12 (16.67)</td>
<td>42 (35.0)</td>
<td>7.482</td>
<td>0.006</td>
<td>0.371</td>
<td>0.180–0.766</td>
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<tr>
<td>S (%)</td>
<td>138 (71.88)</td>
<td>134 (69.79)</td>
<td>60 (83.33)</td>
<td>78 (65.0)</td>
<td>4.800</td>
<td>0.467–49.389</td>
<td>0 (0)</td>
<td>3 (20.00)</td>
<td>/</td>
<td>0.526*</td>
<td>1.250</td>
<td>0.971–1.610</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>192</td>
<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.502–1.612</td>
<td>3 (37.50)</td>
<td>6 (40.00)</td>
<td>1.000*</td>
<td>0.900</td>
<td>0.154–5.258</td>
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<tr>
<td>allelic</td>
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<tr>
<td>LL (%)</td>
<td>3 (13.04)</td>
<td>1 (3.03)</td>
<td>/</td>
<td>0.295*</td>
<td>4.800</td>
<td>0.467–49.389</td>
<td>0 (0)</td>
<td>3 (20.00)</td>
<td>/</td>
<td>0.526*</td>
<td>1.250</td>
<td>0.971–1.610</td>
</tr>
<tr>
<td>SL (%)</td>
<td>9 (39.13)</td>
<td>11 (33.33)</td>
<td>0.198</td>
<td>0.656</td>
<td>1.286</td>
<td>0.425–3.389</td>
<td>3 (37.50)</td>
<td>6 (40.00)</td>
<td>1.000*</td>
<td>0.900</td>
<td>0.154–5.258</td>
<td></td>
</tr>
<tr>
<td>SS (%)</td>
<td>11 (47.83)</td>
<td>21 (63.63)</td>
<td>1.383</td>
<td>0.524</td>
<td>0.524</td>
<td>0.177–1.547</td>
<td>5 (62.50)</td>
<td>6 (40.00)</td>
<td>1.000*</td>
<td>0.900</td>
<td>0.154–5.258</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>33</td>
<td>8</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The allelic frequencies and genotypes for SLEs and HCs were analyzed by the chi square test and Fisher’s exact tests. SLE: systemic lupus erythematous; HC: healthy control; SLE-D: depressive SLE patient; SLE-ND: non-depressive patient; OR: odds ratio; 95% CI: confidence interval.*Fisher’s exact test.
status of 5HTT in HCs as well as SLE patients. The bisulfate-sequencing methods used in the present study make it possible to identify the site-specific methylation of each CpG in a particular length of DNA. The results of this study also revealed specific methylated sites in this region, especially at sites −15101, −15108, and −15119. These three sites were methylated in HCs and SLE patients. However, these sites exhibited a relatively higher percentage of mCs in SLE patients. Whether there is any functional significance of the selective methylation of specific sites needs to be explored in further studies.

It was coincidental that the promoter region we tested partially overlapped with that of the 5HTTLPR polymorphism (Figure 1). Intriguingly, this promoter region of 5HTT might constitute a type of self-regulation, while the polymorphism may constitute the primary means of regulation in the same region. It is possible that 5-HTT was functionally activated but that the risk allele of the 5HTTLPR controlled susceptibility to depression in SLEs and HCs. These results suggest a possible interaction between genetic and epigenetic regulation of the 5-HT transporter.

The relationship between the brain and the immune system is intriguing. The abnormal transmission of 5HT and the number and function of 5HTT/5HT1A can be found in both the CNS and peripheral blood. These synchronous changes may reflect a relationship between serotonin and the CNS and the immune system. These apparently independent systems may share a tight internal connection or may have common pathways. For example, the extracellular regulation kinase/mitogen-activated protein kinase (ERK-MAPK) pathway is important for signal transduction in the CNS and plays a role in the pathology of depression. Deficits in the ERK signaling pathway were also found in SLE and are, to some extent, related to epigenetic factors. The induction of ERK in mouse T-cells leads to the reduction of methyltransferase 1 (DNMT1) expression, to the overexpression of methylation-sensitive genes, and to the production of antibodies to anti-double-stranded DNA (anti-dsDNA). In lupus, certain defects in ERK signaling may have formed because of the activation of protein kinase (PKC) in T cells. Other pathways, such as the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway and MAPK/cAMP responsive element binding protein (CREB) pathway, have been found to be related to the immune system and depression. These pathways may become potential connections between the brain and the immune system.

Our results provided evidence for the genetic regulation of the serotonin system in a mood disorder among SLE patients. These results also confirmed the low methylation state in this region. This is the first study to suggest a role for the 5HTT promoter in SLE and concomitant depression from both genetic and epigenetic perspectives. Studying the relationship between epigenetic and genetic regulation is important for understanding gene-environment interactions in the etiology of complex diseases such as SLE and depression.

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**Conflict of interest**

None declared.

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