A longitudinal study of SLC6A4 DNA promoter methylation and poststroke depression

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Abstract

Serotonin transporter gene (SLC6A4) has been shown to play an important role in the pathophysiology of mood disorders including poststroke depression (PSD). SLC6A4 expression is influenced by DNA methylation status and the SLC6A4 linked promoter region (5-HTTLPR) polymorphism. This study aimed to investigate whether SLC6A4 methylation status was associated with depression ascertained at two weeks and one year after stroke taking into account the 5-HTTLPR polymorphism. A total of 286 patients were evaluated two weeks after stroke, and 222 (78%) were followed one year later. Depression was diagnosed according to DSM-IV criteria, and depression severity was assessed by the Hamilton Depression Rating Scale (HAMD) at each evaluation point. The effects of SLC6A4 methylation status on PSD status and HAMD scores were investigated using multivariate logistic regression models and partial correlation tests, respectively. Analyses were repeated after stratification by 5-HTTLPR genotype groups (‘l/l’ or ‘l/s’ and ‘s/s’). Higher SLC6A4 promoter methylation status was independently associated with PSD both at 2 weeks and more prominently at 1 year after stroke, and was significantly associated with the worsening of depressive symptoms over one year. These findings were significant only in the presence of the 5-HTTLPR s/s genotype. SLC6A4 methylation profile was supported as a potential diagnostic and prognostic biomarker for PSD; associations with SLC6A4 methylation status may represent a target for drug development.

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1. Introduction

Depression is a common and significant complication following stroke (Robinson, 2003). Recent genetic etiologies of post-stroke depression (PSD) have been suggested (Spalletta et al., 2006), and the serotonin transporter gene (SLC6A4), located on chromosome 17q11.1–17q12, has received particular attention. SLC6A4 expression is significantly regulated by epigenetic chromatin remodelling, including DNA methylation of cytosines in cytosine-guanine (CpG) dinucleotides. An increase in CpG methylation at promoter regions on SLC6A4 has been found to be correlated with decreased SLC6A4 mRNA levels (Philibert et al., 2007) and brain serotonin synthesis (Wang et al., 2012). Higher methylation status of the SLC6A4 promoter has also been associated with increased liability to major depression (Olsson et al., 2010; Philibert et al., 2008). The SLC6A4 expression is also regulated by the biallelic polymorphism in the SLC6A4 linked promoter region (5-HTTLPR) with long (l) and short (s) alleles. The s allele reduces SLC6A4 expression and therefore has been hypothesised to be a risk factor for depression (Anguelova et al., 2003). Furthermore, SLC6A4 methylation status has been reported to be dependent on the 5-HTTLPR polymorphism, and consequently with differential effects on depression (Kinnally et al., 2010; Philibert et al., 2007). The 5-HTTLPR s allele has found to be significantly associated with PSD specifically (Kim et al., 2012a; Kohen et al., 2008; Ramasubbu et al., 2006), although this has not been found in all studies (Choi-Kwon et al., 2012). To our knowledge, there has been no previous evaluation of SLC6A4 DNA methylation status in relation to PSD. Using data from a longitudinal study of a post-stroke cohort, we investigated the role of SLC6A4 promoter methylation status in PSD at two weeks and one...
year after the index stroke, taking into account the 5-HTTLPR polymorphism.

2. Methods

2.1. Study overview

This analysis was carried out as a component of a larger parent investigation of mental disorders in stroke survivors with a naturalistic prospective design, which has been previously described in detail (Kim et al., 2012b). Participants were consecutively recruited from all patients with recent ischaemic stroke hospitalized within the Department of Neurology of Chonnam National University Hospital, Gwangju, South Korea. Assessments are made at 2 weeks and 1 year after the stroke to investigate consequences of stroke at both acute and chronic stages. The recruitment period for the initial 2 week assessment was from 2006 to 2010 and for the follow-up evaluation was one year thereafter. The mean (SD) interview times were 12.3 (3.0) days post-stroke at baseline and 13.2 (3.6) months post-stroke at follow-up.

2.2. Participants

All patients with acute stroke hospitalized at the study site were considered for participation. Inclusion criteria were: i) confirmed ischaemic stroke by brain magnetic resonance imaging (MRI) (or computed tomography (CT) if MRI was contraindicated); ii) ability to complete the necessary investigations and questionnaires; and iii) capacity to understand the objective of the study and provide informed consent. Exclusion criteria were: i) severe physical illnesses which were life-threatening or interfering with the recovery from stroke; ii) communication difficulties due to dysphasias or dysarthria precluding informed consent and questionnaire completion; iii) any of the following comorbid neuropsychiatric conditions: dementia, Parkinson’s disease, brain tumour, epilepsy, psychosis, alcohol and substance dependence; iv) severe physical illnesses limiting movement prior to stroke; and v) Mini-Mental State Examination (Folstein et al., 1975) score of <16 at baseline evaluation. All participants gave written informed consent and the study was approved by the Chonnam National University Hospital Institutional Review Board.

2.3. Evaluations for depression

Diagnoses of depressive disorders after stroke were determined by applying DSM-IV diagnostic criteria using the Mini International Neuropsychiatric Interview (MINI), a structured diagnostic psychi- atric interview for DSM-IV defining major or minor depression categories as outputs (Sheehan et al., 1988). According to these criteria, patients were diagnosed as having major depression if they had at least one core symptom (i.e., depressed mood or loss of interest) and at least four other symptoms of depression. A diagnosis of minor depression was made if patients had at least one core symptom and at least two others but less than five symptoms in total. Patients were further re-categorised into ‘any PSD’ (both major and minor depression) and major PSD. Depression severity was assessed by the Hamilton Depression Rating Scale (HAMDS) (Hamilton, 1960), consisted of 17 items with a total score ranging 0–52, with higher scores indicating more severe depressive symptoms.

2.4. SLC6A4 analysis

Blood samples were obtained in a subsample who agreed to this. DNA was extracted from venous blood, and DNA methylation and genotying analysis of SLC6A4 promoter were conducted using standard procedures. Serotonin has a critical role in the association between early life experience and increased susceptibility to lifetime risk for depression (Jans et al., 2007), and SLC6A4 is a key regulator of serotonergic neurotransmission. Hypermethylation of the gene promoter is recognised to reduce respective gene expression (Devlin et al., 2010). Increased methylation levels of SLC6A4 promoter measured from blood cell were associated with decreased levels of SLC6A4 RNA (Philibert et al., 2007) and brain serotonin synthesis (Wang et al., 2012).

SLC6A4 promoter region for analyzing methylation status is presented in Fig. 1. In the SLC6A4 promoter region, there are several CpG rich islands in 799bp with 81 CpG sites (Philibert et al., 2007). Because of resource constraints, we chose one CpG-rich region of the promoter between −479 and −350 relative to the transcriptional start site, including seven CpG sites, on the basis that it was recognized to be associated with changes in SLC6A4 mRNA expression (Mortensen et al., 1999; Philibert et al., 2007), and had been investigated in relation to antenatal depressed mood as a marker of SLC6A4 methylation status in a recent study (Devlin et al., 2010). These data have been deposited in GenBank (accession number: BankIt1577778 SLC6A4 KC106430). Genomic DNA (1 μg) was extracted from leukocytes using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer’s suggested protocol, and was bisulfite-treated using the EpiTech Bisulfite Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s protocol. A 130bp fragment of SLC6A4 promoter was amplified by PCR from bisulfite-treated DNA using the forward and reverse primers designated in Fig. 1. PCR conditions were 95 ℃ for 15 min, followed by 45 cycles of 95 ℃ for 15 s, 57 ℃ for 30 s, and 72 ℃ for 15 s, with a final extension of 5 min at 72 ℃. PCR products were sequenced using the PSQ 96M Pyrosequencing system (Biotage) according to the manufacturer’s protocol with the following sequencing primers designated in Fig. 1. The methylation percentage at each CpG region was quantified using the Pyro Q-CpG software, version 1.0.9 (Bio- tage). For genotyping, polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism assays were performed. A PCR product was amplified using primers (5’-GGCGTGGCGCCTCCTGAAATGC-3’, 5’-GAGGACTAGCTGGACCAACCA-3’) flanking the region containing the gene variation. The PCR conditions consisted of a 5 min denaturation step at 94 ℃, 40 cycles of 30 s denaturation at 94 ℃, 30 s annealing at 63 ℃ and 60 s extension at 72 ℃, and a final 10 min extension step at 72 ℃. PCR products were separated by electrophoresis in a 3% agarose gel.

Fig. 1. SLC6A4 promoter regions for analyzing methylation status. Figure legends: The CpGs are underlined and numbered. Forward and backward primers and sequencer are designated. Numbering of the gene sequence is relative to the transcriptional start site.
stained with ethidium bromide and visualized by UV transillumination. The different genotypes were defined by the specific bands. The SLC6A4 promoter region for analyzing methylation status is illustrated in Fig. 1. These data have been deposited in GenBank (accession number: BankIt1577778 SLC6A4 KC106430). The individual methylation percentages at seven CpG sites of a promoter region and their average values were estimated.

For the 5-HTTLPR genotype analysis, the ‘l/l’ genotype was rare, and therefore the genotype was categorized as ‘l/l or l/s’ and ‘s/s’.

2.5. Demographic and clinical covariates

Characteristics potentially associated with PSD were considered as covariates in this analysis. Age, gender, year of education, and previous histories of depression or stroke were recorded according to information obtained from the participant or their caregiver, as appropriate. Stroke severity was measured using the National Institutes of Health Stroke Scale (NIHSS) (Kasner et al., 1999), and the score at the time of admission was used. According to brain imaging data, stroke location by hemisphere was divided into left, right, and bilateral with further sub-division into anterior, posterior, and both.

2.6. Statistical analyses

The dependent variables were any PSD and major PSD both at 2 weeks and at 1 year after stroke. Demographic and clinical characteristics including SLC6A4 genotype were compared between no PSD and any PSD at 2 weeks after stroke using t-tests or \( \chi^2 \) tests as appropriate. SLC6A4 methylation percentages were compared between no PSD and any PSD, and between no PSD and major PSD at both examination points using t-tests. Individual effects of SLC6A4 methylation percentages on PSD outcomes were examined using pairwise logistic regression models after adjustment for those baseline demographic and clinical characteristics which were associated with PSD (\( p \)-value < 0.1). To investigate the potential effects of 5-HTTLPR genotype on the associations between the methylation percentages and PSD outcomes, the following analyses were carried out: i) SLC6A4 methylation percentages were compared between genotypes using ANOVA; ii) two way interactions between SLC6A4 methylation percentages and genotype were tested using multivariate logistic regression models; iii) associations between the average SLC6A4 methylation percentage and PSD outcomes were recalculated stratified by genotype groups (‘l/l or l/s’ and ‘s/s’); iv) associations of SLC6A4 methylation percentages with baseline and change scores (values at one year minus values at two weeks) in the HAMD were evaluated using partial correlation tests in the total and genotype groups. Since eight comparisons (CpG sites 1~7 and the average value) were carried out for the SLC6A4 methylation percentages analyses, Bonferroni corrections were applied to maintain an overall type I error rate of 0.05 taking into account the multiple comparisons. Statistical analyses were carried out using SPSS 13.0 software.

3. Results

3.1. Recruitment

Having applied inclusion and exclusion criteria, of 465 potentially eligible and consecutively enrolled stroke patients, 423 (91%) consented to participate in the study. Of these 286 (68%) agreed to provide blood samples for genetic tests, and formed the baseline sample. There were no significant differences between participants and non-participants with respect to any demographic and clinical characteristics (all \( p \)-values > 0.1). Of the 286 participants, any and major PSD were diagnosed in 80 (28%) and 32 (11%), respectively. At one year follow-up, 222 (78%) were re-examined. Those present or not at follow-up were not significantly different at baseline with respect to any demographic and clinical characteristics including depression status (all \( p \)-values > 0.1). Any and major PSD were present in 53 (24%) and 21 (9%), respectively, of all 222 followed-up participants.

3.2. Demographic and clinical characteristics

Overall distributions of sample characteristics are summarized in the first column of online supplement Table A, and are compared by baseline PSD status in the 2nd–4th columns. Compared to unaffected participants, those with any PSD group were older, were more likely to have previous histories of depression and stroke, had higher stroke severity (NIHSS) scores, were more likely to have an anterior stroke location, and had a higher s allele frequency at \( p \)-values < 0.1, and these factors were therefore chosen as covariates for later analyses.

3.3. Associations between SLC6A4 promoter methylation percentages and PSD status

SLC6A4 promoter methylation percentages are compared by PSD status in Table 1. Statistical power estimates (assuming two-tailed tests and alpha 0.05) for comparisons of methylation percentages between with and without PSD were 93~96%. After applying Bonferroni corrections, significant findings were as follows: any PSD at baseline was associated with a higher methylation percentage at CpG site 1; major PSD at baseline was associated with higher methylation percentages at CpG sites 1 and 7; any PSD at follow-up was associated with higher methylation percentage at all CpG sites and a higher average value; and major PSD at follow-up was associated with a higher methylation percentage at CpG site

<table>
<thead>
<tr>
<th>Methylation site</th>
<th>Baseline sample (N = 286)</th>
<th>Followed-up sample (N = 222)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No PSD (N = 206)</td>
<td>Any PSD (N = 80)</td>
</tr>
<tr>
<td>Cpg 1</td>
<td>13.4 (9.2)</td>
<td>17.2 (10.6)*</td>
</tr>
<tr>
<td>Cpg 2</td>
<td>18.8 (12.6)</td>
<td>22.9 (15.5)*</td>
</tr>
<tr>
<td>Cpg 3</td>
<td>6.8 (10.3)</td>
<td>9.2 (12.9)</td>
</tr>
<tr>
<td>Cpg 4</td>
<td>10.1 (7.1)</td>
<td>11.6 (8.5)</td>
</tr>
<tr>
<td>Cpg 5</td>
<td>13.5 (13.6)</td>
<td>18.5 (18.6)*</td>
</tr>
<tr>
<td>Cpg 6</td>
<td>14.5 (13.7)</td>
<td>18.9 (18.0)</td>
</tr>
<tr>
<td>Cpg 7</td>
<td>12.4 (13.3)</td>
<td>16.6 (18.3)</td>
</tr>
<tr>
<td>Cpg average</td>
<td>12.8 (10.3)</td>
<td>16.4 (13.9)*</td>
</tr>
</tbody>
</table>

* \( p \)-value<0.05; \( ^\dagger \) \( p \)-value<0.01; \( ^\ddagger \) \( p \)-value<0.001 versus no PSD using t-tests.

Values in bold type show statistical significance after Bonferroni correction.

Table 1: Mean (SD) SLC6A4 methylation percentages by post-stroke depression (PSD) status.
Values in bold type show statistical significance after Bonferroni correction.

4. In adjusted analyses, the strengths of these associations were generally increased (Table 2). After applying Bonferroni corrections, significant findings were as follows: any PSD at baseline was associated with a higher methylation percentage at CpG site 1; major PSD at baseline was associated with higher methylation percentages at CpG sites 1, 4, 5, and a higher average value; any PSD at follow-up was associated with higher methylation percentage at all CpG sites and a higher average value; and major PSD at follow-up was associated with higher methylation percentages at CpG sites 5, 6, 7, and a higher average value.

3.4. Associations between SLC6A4 promoter methylation percentages and PSD status by genotype

SLC6A4 methylation percentages were significantly higher in s/s genotype compared to l/l or l/s genotypes (online Table B). There were no interactive effects of SLC6A4 methylation percentages and genotype on PSD status in the multivariate logistic regression models (online Table C). However, the strength of the associations between SLC6A4 methylation average percentage and PSD status differed by genotype, being strongest in the presence of s/s genotype (online Table D).

3.5. Associations between SLC6A4 promoter methylation percentages and HAMD scores

The mean (SD) baseline HAMD was 7.9 (6.3) and the mean (SD) change in HAMD score from baseline to follow-up was −1.5 (6.5). Partial correlations of SLC6A4 methylation percentages with baseline scores and changes at follow-up for HAMD are summarised in Table 3. After applying Bonferroni corrections, baseline scores on HAMD were not generally associated with methylation status in adjusted analyses, being only significantly correlated with a higher methylation percentage at CpG site 1 in the presence of s/s genotype. However, worsening HAMD scores over the follow-up period were significantly correlated with higher individual methylation percentages at all CpG sites except for CpG site 4, and with a higher average methylation percentage, these associations being consistently stronger in the s/s genotype subgroup in the same adjusted models.

4. Discussion

Principal findings in this longitudinal study of a post-stroke cohort were that higher SLC6A4 promoter methylation status showed some independent association with PSD at 2 weeks after stroke, but was more prominently associated with both PSD and worsening depressive symptoms over the 1 year after stroke. These findings were consistently stronger in participants with the 5-HTTLPR s/s genotype, although no individually significant methylation—genotype interactions were found.

The serotonin transporter is a key regulator of serotonergic neurotransmission, which itself is critically involved in the pathophysiology of depressive disorders (Lesch et al., 1995). Serotonin transporter function is influenced by epigenetic profiles, one of which is the DNA promoter methylation in SLC6A4. Higher SLC6A4 methylation has been found to be correlated with decreased SLC6A4 mRNA levels (Philibert et al., 2007) and brain serotonin synthesis (Wang et al., 2012), and has been associated with increased liability to major depression (Olsson et al., 2010; Philibert et al., 2008). However, as far as we are aware, there has been no study to test this hypothesis in relation to PSD. Our findings support previous results

Table 2
Multivariate analyses of examining the individual effects of SLC6A4 methylation percentages on post-stroke depression (PSD).

<table>
<thead>
<tr>
<th>Methylation site</th>
<th>Baseline sample (N = 286)</th>
<th>Followed-up sample (N = 222)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any PSD</td>
<td>Major PSD</td>
</tr>
<tr>
<td></td>
<td>Wald OR (95% CI)</td>
<td>Wald OR (95% CI)</td>
</tr>
<tr>
<td>CpG1</td>
<td>10.36 1.05 (1.02–1.08)</td>
<td>9.02 1.06 (1.02–1.10)</td>
</tr>
<tr>
<td>CpG2</td>
<td>6.68 1.03 (1.01–1.05)</td>
<td>7.14 1.04 (1.01–1.07)</td>
</tr>
<tr>
<td>CpG3</td>
<td>3.03 1.02 (0.99–1.04)</td>
<td>3.94 1.03 (1.00–1.07)</td>
</tr>
<tr>
<td>CpG4</td>
<td>4.61 1.04 (1.01–1.08)</td>
<td>8.41 1.09 (1.03–1.15)</td>
</tr>
<tr>
<td>CpG5</td>
<td>6.56 1.02 (1.01–1.04)</td>
<td>7.79 1.03 (1.01–1.06)</td>
</tr>
<tr>
<td>CpG6</td>
<td>6.28 1.02 (1.01–1.04)</td>
<td>6.11 1.03 (1.01–1.06)</td>
</tr>
<tr>
<td>CpG7</td>
<td>5.94 1.02 (1.01–1.04)</td>
<td>6.90 1.03 (1.01–1.06)</td>
</tr>
<tr>
<td>CpG average</td>
<td>7.02 1.03 (1.01–1.05)</td>
<td>8.00 1.05 (1.01–1.08)</td>
</tr>
</tbody>
</table>

All analyses are adjusted for age, previous history of depression and stroke, National Institutes of Health Stroke Scale score, stroke localization, and 5-HTTLPR genotype.

Table 3
Partial correlations between SLC6A4 promoter methylation percentages and scores on Hamilton depression rating scale by polymorphism. Data are Pearson's correlation coefficients.

<table>
<thead>
<tr>
<th>Methylation site</th>
<th>Baseline score</th>
<th>Change in score over follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N = 286)</td>
<td>l/l or l/s (N = 128)</td>
</tr>
<tr>
<td>CpG1</td>
<td>+0.126*</td>
<td>+0.042</td>
</tr>
<tr>
<td>CpG2</td>
<td>+0.091</td>
<td>+0.028</td>
</tr>
<tr>
<td>CpG3</td>
<td>+0.082</td>
<td>+0.058</td>
</tr>
<tr>
<td>CpG4</td>
<td>+0.091</td>
<td>+0.079</td>
</tr>
<tr>
<td>CpG5</td>
<td>+0.114</td>
<td>+0.057</td>
</tr>
<tr>
<td>CpG6</td>
<td>+0.109</td>
<td>+0.079</td>
</tr>
<tr>
<td>CpG7</td>
<td>+0.093</td>
<td>−0.011</td>
</tr>
<tr>
<td>CpG average</td>
<td>+0.109</td>
<td>+0.068</td>
</tr>
</tbody>
</table>

All data are adjusted for age, previous history of depression and stroke, National Institutes of Health Stroke Scale score, and stroke localization.

* p-value<0.05; 0.05<p-value<0.01.

Values in bold type show statistical significance after Bonferroni correction.
for major depression (Olsson et al., 2010; Philibert et al., 2008) in that higher SLC6A4 methylation status was significantly associated with PSD. It has been suggested that DNA methylation might mediate responses to stress (McGowan et al., 2009), and our positive finding may therefore be related to the particular situation of the study participants, since stroke is a significant life stress with potentially wide-ranging implications for physical function and quality of life.

The association between higher SLC6A4 methylation status and PSD was more prominent at 1 year than at 2 weeks after stroke. Furthermore, higher methylation status predicted the worsening of depressive symptoms over the 1 year follow-up. It has been suggested that the aetiology of depression may differ according to the time elapsed after stroke, with biological factors more important at an acute stage and psychosocial factors increasing in salience later on (Bhogal et al., 2004). Of relevance, SLC6A4 methylation has been proposed to modify the effect of stress on chronic rather than acute consequences (Koenen et al., 2011). Taken together, the modifying effect of SLC6A4 methylation status on associations between stroke (as a life stress) and depression might be strengthened over a more extended period, and this should be borne in mind for future investigations.

Associations of SLC6A4 methylation status with depression diagnoses and depressive symptoms over 1 year period were generally stronger for the presence of 5-HTTLPR s/s genotype. In addition, methylation percentages at all CpG sites were significantly higher in participants with higher numbers of s alleles. These findings were largely consistent with previous studies with have reported that SLC6A4 DNA methylation and gene expression are modified by the 5-HTTLPR polymorphism (Philibert et al., 2007; Vijayendran et al., 2012), which has been found to have differential effects on depression risk (Kinnally et al., 2010; Olsson et al., 2010; Philibert et al., 2008). The s allele has also been found to be associated with reduced serotonin transporter function (Heils et al., 1995), and therefore may have synergistic effects in combination with a higher SLC6A4 DNA methylation status.

Our study has several strengths, as well as being the first to report on associations between SLC6A4 methylation status and PSD. Depression was ascertained using a structured diagnostic interview and depressive symptoms were evaluated with a well-validated assessment scale. The sample size provided sufficient power to detect differences in promoter methylation percentages between the groups. Depression and other features were assessed at a similar time point (two weeks and one year after stroke) in all participants, reducing potential heterogeneity and error arising from time-dependent influences on PSD aetiology (Bhogal et al., 2004). Participants were recruited consecutively from all eligible patients with a recent stroke at the study hospital, which reduced the likelihood of selection bias and increased the potential generalizability. Finally, a range of covariates were considered in the analyses, and the follow-up rates were reasonable and not apparently differential with respect to risk factors of interest.

Our study also has some limitations which should be borne in mind. Blood samples for genetic assays was obtained only in 68% of the total stroke patients in the parent study, although there were no significant differences in demographic and clinical characteristics between those with and without samples. Second, due to resource constraints, the methylation status could only be investigated for one CpG island of the SLC6A4 gene. Methylation status measured for this single island will only have been a proxy for the status of the whole gene which will have biased associations towards the null, and thus obscured true group differences; however, such measurement error would not account for those associations which were observed. Studies of other CpG islands for the gene, other genes, and genome-wide DNA methylation are clearly indicated. Third, childhood adversities, found to be associated with increased SLC6A4 DNA methylation status (Beach et al., 2010; Vijayendran et al., 2012), were not evaluated in the present study, and it remains to be established whether these had any role in the observed associations between SLC6A4 methylation status and PSD. Finally, the SLC6A4 promoter methylation profile could be tissue specific. Until now, it has been unclear whether SLC6A4 methylation status in genomic DNA isolated from leukocytes was related with that in the brain. However, increased levels of SLC6A4 promoter methylation measured from blood cell were associated with decreased levels of SLC6A4 RNA (Philibert et al., 2007) and brain serotonin synthesis (Wang et al., 2012).

In conclusion, our findings support a role for SLC6A4 in PSD and have several potential implications. The DNA promoter methylation profile of SLC6A4 might be a diagnostic biomarker for PSD vulnerability and a prognostic biomarker for long-term depression risk, possibly particularly in conjunction with 5-HTTLPR polymorphism. Based on our findings, we suggest a novel ‘epigenetic hypothesis’ in the pathogenesis of PSD; however, as with any first report further independent replication is needed. There are also potential treatment implications. PSD is difficult to treat, and the effectiveness of conventional antidepressants remains inconclusive (Hackett et al., 2008). Regulation of DNA promoter methylation might be helpful to increase the successful treatment of PSD, and deserves at least some consideration and evaluation. Overall, epigenetics is in its infancy but remains a potentially promising area of enquiry in the field of stroke and mood disorders. We believe that our study represents an important first step to elucidate the role of epigenetic mechanisms in the aetiology of PSD, and as such is a reference point for future research.

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Contributors

J-M Kim conducted the data analysis and draft conceived and set up the study, conducted the data analysis and drafted the article. Stewart interpreted the data and revised the article critically. Kang, S-W Kim, I-S Shin, H-R Kim, M-G Shin, Yoon helped to analyze the data and to draft the article. J-T Kim, Park and Cho took charge of data collection and study coordination. All authors approved the final version of manuscript to be published. The authors have no conflicts of interest to declare with respect to this report.

Conflict of interest statement

No potential conflict of interest is disclosed.

Acknowledgement

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2013.04.010.
References


