Research report

Serotonergic and BDNF genes and risk of depression after stroke

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Abstract

Background: Polymorphisms of serotonin transporter (5-HTT) and brain-derived neurotrophic factor (BDNF) have been investigated as candidate genes for post-stroke depression (PSD). Serotonin 2a receptor (5-HTR2a) genes have not been yet investigated in PSD. This study aimed to investigate whether the 5-HTT, 5-HTR2a, and BDNF genes are associated with PSD independently and/or interactively in a Korean sample with high prevalence of risk alleles.

Methods: In 276 stroke cases, depression was diagnosed using DSM-IV at 2 weeks after stroke, further classified to major PSD (N=29), all (major plus minor) PSD (N=77), and control (N=199) groups. Associations between PSD and 5-HTTLPR, STin2 VNTR, 5-HTR2a 1438A/G, 5-HTR2a 102T/C, and BDNF val66met genotypes were estimated using logistic regression models, and gene–gene interactions were investigated using the generalized multifactor dimensionality reduction method.

Results: 5-HTR2a 1438 A/A genotype was associated with major PSD, while 5-HTTLPR s/s and BDNF met/met genotypes were associated with all PSD. There was a significant interaction between 5-HTR2a 1438A/G and BDNF val66met polymorphisms for major PSD and a borderline significant interaction between 5-HTTLPR and BDNF val66met polymorphisms for all PSD.

Conclusions: In a large cohort, we found evidence for serotonin and BDNF polymorphisms as susceptibility factors and gene–gene interactions between these systems for depression at 2 weeks post-stroke.

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1. Introduction

Depression is common after stroke and has adverse effects on the course and prognosis of disease (Hackett et al., 2005; Robinson, 2003). Both psychosocial and biological factors are important in the etiology of post-stroke depression (PSD) (Spalletta et al., 2006; Whyte and Mulsant, 2002), and recently genetic predispositions have been suggested. The serotonin transporter (5-HTT) gene, located on chromosome 17q11.1–17q12, has received particular attention. The most frequently studied variant is a biallelic polymorphism in the 5-HTT gene linked promoter region (5-HTTLPR) with short (s) and long (l) alleles based on the presence or absence of a 43-base pair insertion/deletion polymorphism. The s allele reduces the transcriptional activity of the 5-HTT gene promoter resulting in decreased 5-HTT expression (Heils et al., 1995), and therefore has been hypothesized to be a risk factor for depression (Anguelova et al., 2003). Ramasubbu et al. (2006) reported that the 5-HTTLPR s allele was associated with PSD in a sample...
of 26 stroke patients with DSM-IV major depression and 25 non-depressed stroke patients, the first genetic study of PSD to our knowledge. Kohen et al. (2008) replicated this finding with a larger sample of 75 depressive and 75 non-depressive stroke patients categorized by the Geriatric Depression Scale 10/11 cutoff. Another common variant in 5-HTT gene is a triallelic polymorphism located in intron 2 and corresponding to 9-, 10-, or 12-variable tandem repeat units of 17-base pair (STin2 VNTR). The 9- and 12-repeat alleles (STin2.9 and STin2.12, respectively) have been shown to enhance transcription of 5-HTT compared with the 10-repeat allele (STin2.10) (Lovejoy et al., 2003), and have been found to be associated with affective disorders (Battersby et al., 1996; Jarrett et al., 2007). Kohen et al. (2008) found that participants with STin2 9/12 or 12/12 genotype had higher risk of PSD compared with STin2 10/10 genotype carriers, although Ramosubbu et al. (2008) found no such associations. As well as this 5-HTT polymorphism, the serotonin 2a receptor (5-HTR2a) gene, located on chromosome 13q14–q21, has also received attention. Two polymorphisms have been reported within this gene: a 1438A/G polymorphism in the promoter region, and an MspI polymorphic site at position 102T/C. The 5-HTR2a 1438A/G A allele has been found to be associated with mood disorder and depressive mood (Bonnier et al., 2002; Enoch et al., 1999); and the 5-HTR2a 102T/CC allele has been found to be associated with major depressive disorder or suicidal ideation (Du et al., 2000; Zhang et al., 1997). However, these 5-HTR2a polymorphisms have not been investigated yet in the context of PSD.

Another more recent candidate for depression and PSD is the brain-derived neurotrophic factor (BDNF) gene. This is located on chromosome 11p14.1 and has several polymorphic markers. These include the single nucleotide polymorphism (SNP) at nucleotide 196G/A, which results in an amino acid substitution (valine to methionine) at codon 66 (val66met) of the proBDNF molecule. This SNP affects intracellular processing and secretion of BDNF, and the met allele is associated with reduced activity-dependent secretion of BDNF (Egan et al., 2003). Previously, we have reported a significantly stronger association between incident stroke and depression in the presence of the met allele in a 2 year prospective study with 500 community elders (Kim et al., 2008). However, this hypothesis has not been tested in a clinical case–control design so far.

Linkage disequilibrium has been found between 5-HTTLPR and STin2 VNTR (Wendland et al., 2006), and between 5-HTR2a 1438A/G and 102T/C polymorphisms (Ono et al., 2001), and there is evidence that serotonin and BDNF systems may be linked at multiple intracellular and intercellular levels (Duman et al., 1997). Therefore, addressing potential gene–gene interactions may be helpful in elucidating the pathogenesis of PSD. This study aimed to investigate whether the 5-HTT, 5-HTR2a, and BDNF genes are associated with PSD independently and/or interactively in a sample of Korean stroke patients.

2. Methods

2.1. Study outline

This analysis was carried out as a component of a larger parent study, which seeks to investigate mental disorders in stroke survivors using a naturalistic prospective design. Participants were consecutively recruited from all patients with recent ischemic stroke hospitalized within the Department of Neurology of Chonnam National University Hospital, Gwangju, South Korea. Assessments are made at 2 weeks and 1 year post-stroke to investigate both acute and chronic outcomes. The recruitment period for the initial 2 week assessment was from 2006 to 2009 and only the 2 week data are considered in the analyses described here.

2.2. Participants

All patients with acute stroke hospitalized at the study site were approached regarding participation. Inclusion criteria were: i) confirmed ischemic 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 dysphasia or dysarthria precluding informed consent and questionnaire completion; iii) any of the following comorbid neuropsychiatric conditions: dementia, Parkinson’s disease, brain tumor, epilepsy, psychoses, 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. All participants gave written informed consent and the study was approved by the Chonnam National University Hospital Institutional Review Board.

2.3. Demographic and clinical characteristics

Age, gender, year of education, and previous histories of depression and 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). All participants underwent brain MRI or CT imaging. Stroke location by hemisphere was divided into left, right, and bilateral with further sub-division into anterior, posterior, and both.

2.4. Diagnoses of PSD

The diagnoses of major and minor depressive disorders after stroke were determined by applying DSM-IV diagnostic criteria using the Mini International Neuropsychiatric Interview (MINI), a structured diagnostic psychiatric interview for DSM-IV (Sheehan et al., 1988) giving rise to major or minor depression categories. 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 but less than five symptoms in total. PSD diagnoses were re-categorized into all PSD (major/minor depression) and major PSD. The MINI has been formally translated and standardized in Korean (Yoo et al., 2006).
2.5. Genotyping

Blood samples for genotyping were obtained in a subsample who agreed to this. DNA was extracted from venous blood using standard procedures. Polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism assays were performed (Table 1). The amplification conditions were pre-denaturation at 95 °C for 5 min, followed by 30 or 40 cycles consisting of denaturation at 95 °C for 35 s, 55 °C or 62 °C for 35 s and 72 °C for 35 s, and post-elongation at 72 °C for 5 min, with a final maintenance step at 4 °C, as required for each polymorphism. For 5-HTTLPR polymorphism, the genotypes were categorized as ‘s/s’, ‘s/l’, and ‘l/l’. For STin2 VNTR polymorphism, the 9 allele was extremely rare (present in only one participant), and therefore the genotypes were categorized as ‘10/12’ and ‘9 or 12/12’. For 5-HTR2a 1438A/G polymorphism, the genotypes were categorized as ‘G/G’, ‘G/A’, and ‘A/A’. For 5-HTR2a 102T/C polymorphism, the genotypes were categorized as ‘C/C’, ‘C/T’, and ‘T/T’. For the BDNF val66met polymorphism, the genotypes were categorized as ‘val/val’, ‘val/met’, and ‘met/met’.

2.6. Statistical analyses

The two dependent variables were all PSD and major PSD. Demographic and clinical characteristics were compared between no PSD and all PSD, and between no PSD and major PSD using t-tests, χ² tests, or Fisher’s exact tests as appropriate. Genotype distributions and allele frequencies for the five polymorphisms were compared between no PSD and all PSD, and between no PSD and major PSD using χ² tests, or Fisher’s exact tests as appropriate. Tests for Hardy–Weinberg equilibrium and linkage dysequilibrium were made using Haploview v.4.2 program. Odds ratios (ORs) for all and major PSD outcomes by genotype were estimated using logistic regression models after adjustment for potential covariates. Finally, gene–gene interactions were investigated using generalized multifactor dimensionality reduction (GMDR) methods, which have been described in detail previously (Lou et al., 2007). The n-dimensional space formed by a given set of SNPs, and the combination with the lowest misclassification error was selected. The GMDR software provides several output parameters including cross-validation consistency, testing balance accuracy, and p-values to assess interactions for each selected combination. The cross-validation consistency value measures the degree of consistency with which the selected combination is identified as the best model among all possibilities considered. The testing balance accuracy measures the degree to which the interaction accurately predicts case vs. control status with scores between 0.5 (indicating the model predicts no better than chance) and 1.0 (indicating perfect prediction). Permutation testing obtains empirical p-values of prediction accuracy as a benchmark based on 1000 shuffles. Potential covariates of PSD were included in the gene–gene interaction analyses. Logistic regression tests were performed additionally to confirm the results from the GMDR analyses. Finally, potential gene by stroke location interactions were investigated using logistic regression. Statistical analyses were carried out using SPSS 13.0 and GMDR software.

Table 1
Polymerase chain reaction (PCR) methods for allele detection.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Forward (F) and reverse (R) primer</th>
<th>Restriction enzyme</th>
<th>PCR product and allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HTTLPR</td>
<td>F: 5′-GGGTTGGCCCTCTGATTGC-3′</td>
<td>–</td>
<td>44 bp 14 copy of a repetitive sequence: s</td>
</tr>
<tr>
<td></td>
<td>R: 5′-GGGGACTGAGGGACAC-3′</td>
<td></td>
<td>44 bp 16, 18, 20 and 22 copies of a repetitive sequence: l</td>
</tr>
<tr>
<td>STin2 VNTR</td>
<td>F: 5′-GTCAGTATACAGCGTTCAG-3′</td>
<td>–</td>
<td>17 bp 9 copy of a repetitive sequence: g</td>
</tr>
<tr>
<td></td>
<td>R: 5′-TGTGTCCTAGCTTGCCCTAG-3′</td>
<td></td>
<td>17 bp 10 copy of a repetitive sequence: 10</td>
</tr>
<tr>
<td>5-HTR2a 1438A/G</td>
<td>F: 5′-ACTCCGACAAACACTTTATTC-3′</td>
<td>MspI</td>
<td>17 bp 12 copy of a repetitive sequence: 12</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CTTGTGCAAATCGCCATTAG-3′</td>
<td></td>
<td>352 bp: 1438A</td>
</tr>
<tr>
<td>5-HTR2a 102T/C</td>
<td>F: 5′-CTCAGCAGTTTCTCAGG-3′</td>
<td>HpaII</td>
<td>342 bp: 102T</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CTCAGCAGTTTCTCAGG-3′</td>
<td></td>
<td>215 and 126 bp: 102C</td>
</tr>
<tr>
<td>BDNF val66met</td>
<td>F: 5′-GGTGAAGACTGATAGACA-3′</td>
<td>BbrII</td>
<td>490 and 208 bp: 66val</td>
</tr>
<tr>
<td></td>
<td>R: 5′-GCCAGCCAATCTCCTCTTTT-3′</td>
<td></td>
<td>698 bp: 66met</td>
</tr>
</tbody>
</table>

| 5-HTTLPR: serotonin transporter gene linked promoter region; STin2 VNTR: serotonin transporter intron 2 variable number tandem repeat; 5-HTR2a: serotonin 2a receptor; BDNF: brain-derived neurotrophic factor. |
126 (45.7%), right hemisphere in 137 (49.6%), and bilateral in 13 (4.7%); and stroke location was anterior in 159 (57.6%), posterior in 86 (31.2%), and both in 31 (11.2%). These characteristics are compared by PSD status in Table 2. Compared to those without PSD, the combined case groups were significantly older, more likely to have previous histories of stroke and/or depression, had higher NIHSS scores, and were more likely to have an anterior stroke location. The major PSD group had higher NIHSS scores and more frequently had experienced anterior stroke location compared to those with no depression.

### 3.3. Genotype distribution and allele frequency

No deviation from the Hardy–Weinberg equilibrium was observed for any genotype (χ² = 3.03, 1.97, 2.83, 0.00, and 3.74 for 5-HTTLPR, STin2 VNTR, 5-HTR2a 1438 A/G, 5-HTR2a 102T/C, and BDNF val66met respectively; all p-values > 0.05). Genotype distributions and allele frequencies are compared by PSD status in Table 3. Cases with any depression had significantly higher 5-HTTLPR s allele, 5-HTR2a 1438 A allele, and BDNF met allele frequencies compared to controls with no depression. The major depression group had significantly higher 5-HTR2a 1438 A allele and BDNF met allele frequencies compared to controls. If Bonferroni corrections were applied, considering multiple comparisons, the strength of these associations remained significant between major PSD and 5-HTR2a 1438 A allele, with borderline significance for the other combinations.

### 3.4. Individual effects of genotypes on PSD risks after adjustment

Associations between genotypes and PSD status adjusted for age, previous depression and stroke, NIHSS score, and stroke location are summarized in Table 4. The 5-HTTLPR s/s and the BDNF met/met genotypes were significantly associated with any depression, but only the 5-HTR2a 1438 A/A genotype was significantly associated with major depression. Although no other significant associations were found, the odds tended to be higher with increasing numbers of the hypothesized risk alleles.

### 3.5. Gene–gene interactions

The genotype distributions between the two 5-HTT genes and between the two 5-HTR genes were not independent. That is, strong linkage disequilibrium was observed between the 5-HTTLPR and STin2 VNTR polymorphisms (D' = 0.522, r² = 0.252, p-value < 0.001); and between the 5-HTR2a 1438 A/G and 5-HTR2a 102T/C polymorphisms (D' = 0.807, r² = 0.345, p-value < 0.001). GMDR analyses for adjusted 2- to 4-way interactions showed significant gene-gene interactions.
5-locus models are summarized in Table 5. There was a significant 2-locus model involving 5-HTR2a 1438 A/G and BDNF val66met polymorphisms for major PSD, and a borderline significant 2-locus model involving 5-HTTLPR and BDNF val66met polymorphisms for any PSD. In a logistic regression analysis re-examining these interactive effects, the Wald statistic (on one degree of freedom) was 6.03 (p=0.033) for a 5-HTR2a 1438 A/G×BDNF val66met interaction, and 3.17 (p=0.063) for a 5-HTTLPR×BDNF val66met interaction. No significant models were found for any other combinations.

### 3.6. Gene–stroke location interactions

The interactive effects of genes by stroke location on PSD status were investigated in logistic regression models but no significant interactions were found for any combinations. The Wald statistics (on one degree of freedom) were 0.03–2.73 (all p>0.1) for the all PSD, and were 0.36–1.53 (all p>0.2) for major PSD.

### Table 4

Adjusted associations of genotypes with post-stroke depression (PSD) status.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>All PSD</th>
<th>Major PSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l/l</td>
<td>Ref</td>
<td>0.024</td>
</tr>
<tr>
<td>l/s</td>
<td>3.14 (0.80–12.3)</td>
<td>1.53 (0.28–8.38)</td>
</tr>
<tr>
<td>s/s</td>
<td>4.39 (1.16–16.5)</td>
<td>2.55 (0.52–12.5)</td>
</tr>
<tr>
<td>STin2 VNTR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/12</td>
<td>Ref</td>
<td>0.857</td>
</tr>
<tr>
<td>9 or 12/12</td>
<td>1.08 (0.49–2.38)</td>
<td>4.07 (0.52–31.9)</td>
</tr>
<tr>
<td>5-HTR2a 1438A/G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>Ref</td>
<td>0.147</td>
</tr>
<tr>
<td>G/A</td>
<td>1.02 (0.50–2.09)</td>
<td>1.56 (0.45–5.42)</td>
</tr>
<tr>
<td>A/A</td>
<td>1.74 (0.81–3.75)</td>
<td>5.59 (1.62–19.3)</td>
</tr>
<tr>
<td>5-HTR2a 102T/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>Ref</td>
<td>0.070</td>
</tr>
<tr>
<td>T/C</td>
<td>1.35 (0.65–2.83)</td>
<td>1.03 (0.33–3.23)</td>
</tr>
<tr>
<td>C/C</td>
<td>2.11 (0.93–4.79)</td>
<td>2.49 (0.77–8.06)</td>
</tr>
<tr>
<td>val/met</td>
<td>Ref</td>
<td>0.036</td>
</tr>
<tr>
<td>val/met</td>
<td>1.37 (0.65–2.91)</td>
<td>0.94 (0.31–2.82)</td>
</tr>
<tr>
<td>met/met</td>
<td>2.45 (1.05–5.75)</td>
<td>2.79 (0.91–8.54)</td>
</tr>
</tbody>
</table>

5-HTTLPR: serotonin transporter gene linked promoter region; STin2 VNTR: serotonin transporter intron 2 variable number tandem repeat; 5-HTR2a: serotonin 2a receptor; BDNF: brain-derived neurotrophic factor.

*Adjusted for age, previous depression, previous stroke, National Institutes of Health Stroke Scale score, and stroke location. p-value for logistic regression likelihood ratio test.

4. Discussion

The principal findings of this genetic association study of depression in the acute period after stroke were that 5-HTTLPR s/s and BDNF met/met genotypes were independently associated with any depression, that 5-HTR2a 1438 A/A genotype was independently associated with major depression, and that 5-HTR2a 1438 A/G and BDNF val66met polymorphisms were interactively associated with major depression.

To our knowledge, this study, although limited in size is one of the largest to date on genes associated with depression following stroke, as well as being the first to report on associations between 5-HTR2a polymorphisms and PSD and the first to report on an interaction between serotonergic and BDNF genotypes for this outcome. It is also one of the first, to our knowledge, to investigate an East Asian sample. One of the strengths of our study was that depression was ascertained using a structured diagnostic interview. Based on DSM-IV, major PSD was found in 10.5% and minor PSD was found in 17.4% (all PSD 27.9%). These were comparable to, although

### Table 5

Optimal gene–gene interaction models for post-stroke depression (PSD) according to the generalized multifactor dimensionality reduction method.*

<table>
<thead>
<tr>
<th>Locus number</th>
<th>Best combination</th>
<th>Cross-validation consistency</th>
<th>Testing balance accuracy</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All PSD</td>
<td>5-HTTLPR, BDNF val66met</td>
<td>10/10</td>
<td>61.35</td>
<td>0.075</td>
</tr>
<tr>
<td>3</td>
<td>5-HTTLPR, STin2 VNTR, BDNF val66met</td>
<td>10/10</td>
<td>59.41</td>
<td>0.126</td>
</tr>
<tr>
<td>4</td>
<td>5-HTTLPR, STin2 VNTR, 5-HTR2a 1438A/G, BDNF val66met</td>
<td>10/10</td>
<td>57.15</td>
<td>0.341</td>
</tr>
<tr>
<td>5</td>
<td>5-HTTLPR, STin2 VNTR, 5-HTR2a 1438A/G, 5-HTR2a 102T/C, BDNF val66met</td>
<td>10/10</td>
<td>49.51</td>
<td>0.963</td>
</tr>
<tr>
<td>Major PSD</td>
<td>5-HTR2a 1438A/G, BDNF val66met</td>
<td>9/10</td>
<td>64.35</td>
<td>0.036</td>
</tr>
<tr>
<td>3</td>
<td>5-HTTLPR, 5-HTR2a 1438A/G, BDNF val66met</td>
<td>10/10</td>
<td>60.15</td>
<td>0.139</td>
</tr>
<tr>
<td>4</td>
<td>5-HTTLPR, 5-HTR2a 1438A/G, 5-HTR2a 102T/C, BDNF val66met</td>
<td>10/10</td>
<td>54.14</td>
<td>0.775</td>
</tr>
<tr>
<td>5</td>
<td>5-HTTLPR, STin2 VNTR, 5-HTR2a 1438A/G, 5-HTR2a 102T/C, BDNF val66met</td>
<td>10/10</td>
<td>56.55</td>
<td>0.666</td>
</tr>
</tbody>
</table>

5-HTTLPR: serotonin transporter gene linked promoter region; STin2 VNTR: serotonin transporter intron 2 variable number tandem repeat; 5-HTR2a: serotonin 2a receptor; BDNF: brain-derived neurotrophic factor.

*Adjusted for age, previous depression, previous stroke, National Institutes of Health Stroke Scale score, and stroke location. p-value based on 1000 permutations.
served between 5-HTTLPR allele status, brain structures, with depression at various time points after stroke. This suggests that the 5-HTTLPR allele might be associated with depression after stroke in previous research (Kohen et al., 2008; Ramasubbu et al., 2006). However, the potentially relevant variant of this SNP is known to be extremely rare in Asian populations (Zhang et al., 2009). A further limitation was that genotyping was only possible in 76% 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 blood samples.

The role of the 5-HTTLPR s allele as a risk factor for depression has been controversial (Levinson, 2006). However, this allele has been associated with depression after stressful life events, replicated in various age groups (Caspil et al., 2003; Kendler et al., 2005; Kim et al., 2007), and linked to depression occurring in combination with medical diseases such as Parkinson’s disease (Mossner et al., 2001), hip fracture (Lenze et al., 2005), coronary disease (Otto et al., 2007), and multi-system chronic ill-health (Kim et al., 2009), which are all potential environmental stressors. In line with these findings, the s allele has also been found to be significantly associated with depression after stroke in previous research (Kohen et al., 2008; Ramasubbu et al., 2006), as well as in our own study. The evaluation points for depression have differed between previous studies, being unclear in the report from one study (Ramasubbu et al., 2006), within 4 months after stroke in another (Kohen et al., 2008), and at 2 weeks in our study. These suggest that the 5-HTTLPR s allele might be associated with depression at various time points after stroke. This association may be explained by underlying relationships observed between 5-HTTLPR allele status, brain structures, and response to antidepressants. For example, depressed older s allele carriers with major depression were found to have lower caudate nucleus volume (Hickie et al., 2007), as well as both microstructural white matter abnormalities and a low remission rate when treated with an SSRI antidepressant than l homozygotes (Alexopoulos et al., 2009). The strength of the association did not reach significance for major PSD in our study which may reflect a lack of power. Previous findings of an association between STin2 VNTR polymorphism and PSD have been inconsistent with one study reporting a significant association between STin2 9 or 12/12 genotype and PSD (Kohen et al., 2008), but another finding no such association (Ramasubbu et al., 2006). In our study we also found no significant association between STin2 VNTR polymorphism and PSD either before or after adjustment.

The serotonergic receptor system has also been implicated in the pathophysiology of mood disorders and in the action of antidepressants. 5-HTR2a polymorphisms have received particular attention but have not been investigated in relation to PSD. A novel finding of the present study was the significant association between the A allele of the 5-HTR2a 1438A/G polymorphism and major PSD. The 5-HTR2a 1438A/G A allele has been found to be associated with mood disorders and depressive mood (Bonnier et al., 2002; Enoch et al., 1999; Jansson et al., 2003) and our findings support this. With respect to the 5-HTR2a 102T/C polymorphism, the C allele was associated with both all PSD and major PSD at borderline significance levels in our study. In a meta-analysis, it was concluded that the 5-HTR2a 102T/CC allele was not directly associated with depressive disorders (Anguelova et al., 2003). However, it has been found to be significantly associated with particular traits related to depression such as suicidal ideation (Du et al., 2000; Zhang et al., 1997) and treatment responses (Minov et al., 2001; Serretti et al., 2000).

Previously, we have reported a significant association between the BDNF val66met met allele and incident PSD in a Korean community (Kim et al., 2008), and were here able to replicate this in a clinical post-stroke sample. The met allele has been associated with reduced activity-dependent secretion of BDNF (Egan et al., 2003), and because BDNF is the most abundant neurotrophin in the brain and has antidepressant neuroprotective effects (Manji et al., 2001), functional deficiency might increase a depressogenic impact of stroke. In addition, BDNF appears to have survival-promoting actions on a variety of CNS structures including hippocampal, cortical, cholinergic, dopaminergic and serotonergic neurons (Manji et al., 2001), and neuronal repair after stroke in brain regions associated with depression might therefore be more delayed in those with the met allele. In addition, relationships between BDNF val/met status, white matter abnormalities and response to antidepressants in late-life depression have been reported (Alexopoulos et al., 2010). However, the relationship between BDNF val/met status and PSD might be more complex than deficient neuroprotection related to inadequate transcription of BDNF. The effects of BDNF in the brain vary depending on the location in which it is expressed. For example, administration of BDNF in the ventral tegmentum has been found to be depressogenic in animals and blockade of BDNF action in the nucleus accumbens has an antidepressant-like effect (Eisch et al., 2003), while BDNF may have an antidepressant effect in the hippocampus (Manji et al., 2001). Therefore this finding requires further exploration and replication in other populations.

The GMVT approach was used to investigate potential gene–gene interactions for PSD. Our findings support evidence that the serotonin and BDNF systems are linked at micro- and macroscopic levels. For example, stress has been associated with reductions in both Raphe serotonin transporter mRNA and hippocampal BDNF mRNA (Vollmayr et al., 2000). We have previously reported a three-way gene–
gene–environment (5-HTTLPR, BDNF, and life stresses) interaction for depression in late-life in an East Asian population (Kim et al., 2007). Novel findings clearly require replication in other populations as well as all genetic association studies, and it should be borne in mind that the present analysis was restricted to the acute stage of stroke. Further studies later after stroke are needed because the etiology of depression cannot be assumed to remain constant.

With respect to allele frequencies, there are known ethnic differences between East Asian and Caucasian populations. Our sample had higher 5-HTTLPR s allele, 72% compared to 43–44% in other settings (Collier et al., 1996; Kohen et al., 2008); for the StIn2 VNTR 9 or 12/12 allele: 84% compared to 63–67% (Collier et al., 1996; Kohen et al., 2008); for the 5-HTR2a 1438A/G A allele: 49% compared to 38–41% (Bonnier et al., 2002; Enoch et al., 1999); and for the BDNF val66met met allele: 49% compared to 17–21% (Kaufman et al., 2006; Zhang et al., 2006). Taken together, the potential risk alleles for PSD (5-HTTLPR s, StIn2 VNTR 9 or 12/12, 5-HTR2a 1438A/G, and BDNF val66met met) were relatively common in our sample as has been found in other East Asian studies (Kato et al., 2005; Kunugi et al., 1997, 2004; Ohara et al., 1998). These results may have public health relevance in East Asian populations in terms of the increased genetic vulnerability to PSD, and it is noteworthy that the prevalence of PSD of the present study was higher than findings from Western studies with similar designs (Aben et al., 2003; Berg et al., 2001).

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Conflict of interest
The authors declare that we have no conflict of interest in relation to this study.

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