Association of Serotonin Transporter Gene Polymorphisms With Poststroke Depression

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Context: Polymorphisms of the serotonin transporter gene (SERT) have been associated with mental illness. In people with long-term medical conditions, variants of the 5-HTTLPR and STin2 VNTR polymorphisms of SERT have been shown to confer a heightened vulnerability to comorbid depression.

Objective: To determine whether the 5-HTTLPR, STin2 VNTR, and rs25531 polymorphisms of SERT are associated with poststroke depression (PSD) in stroke survivors.

Design: A case-control study in which stroke survivors were screened for depressive symptoms and assigned to either a depressed group or a nondepressed group.

Setting: Outpatient clinic.

Participants: Seventy-five stroke survivors with PSD and 75 nondepressed stroke survivors.

Approximately 33% of patients experience major depression after stroke. The etiology of poststroke depression (PSD) is thought to be multifactorial, involving psychosocial and biological mechanisms. A premorbid history of depression and high levels of disability increase the risk of depression in stroke survivors, yet these factors are poor predictors of who will become depressed after stroke and who will not.

The goal of this study is to investigate the role of polymorphisms of the serotonin transporter gene (SERT) (GenBank L05568) in the etiology of PSD. The SERT protein is localized on the presynaptic membrane of serotonergic neurons, where it controls the intensity and duration of serotonergic signaling through reuptake of the neurotransmitter into the synapse. Because SERT is the target of selective serotonin reuptake inhibitors, variations in the SERT gene have been widely studied as possible risk factors for psychiatric illness.

The SERT gene is located on chromosome 17q11.1-17q12 and is organized into 14 exons spanning approximately 31 kilobases. Its most frequently studied variant, 5-HTTLPR, located in the promoter region, is subdivided into a short (s) and a long (l) allele based on the presence or absence of a 43–base pair (bp) insertion/deletion polymorphism. The short (s) variant has been associated with depression in response to stressful life events and with PSD in a small sample. rs25531 is a single nucleotide polymorphism, present in either a common (A) or a rare (G) variant, located immediately upstream of 5-HTTLPR in the SERT gene. It was included in this study despite its low minor allele frequency because it is a functional polymorphism in which the rare al-
lele (G) lowers SERT transcription. rs25531 and 5-HTTLPR have been shown to jointly affect SERT expression levels, resulting in high (I-A), intermediate (I-G), and low-expressing (s-G) genotypes. A third SERT polymorphism, STin2 VNTR, is located in intron 2 and consists of a variable number (usually 9, 10, or 12) of nearly identical 17-bp segments, with the 9-repeat allele (STin2.9) conferring increased odds of major depression and bipolar disorder in one study. The 12-repeat allele (STin2.12) has been associated with enhanced affective disorder and schizophrenia. In people with long-term medical conditions, the 5-HTTLPR s/s and STin2 9/12 genotypes were more frequent in patients with comorbid depression. The study goal is to determine whether the odds of PSD are similarly heightened in carriers of alleles previously associated with mental illness: the 5-HTTLPR s allele and the STin2.9 and STin2.12 alleles.

**METHODS**

**PARTICIPANTS**

The case-control study presented herein is a supplement to a larger parent study, the randomized clinical trial Living Well With Stroke, which investigates the role of behavioral intervention in PSD. Participants in the parent study were recruited from among 287 ischemic stroke survivors discharged from acute care hospitals in the Puget Sound region of Washington State who were within 4 months of an ischemic stroke and had signed informed consent forms to be screened for PSD. On intake, participants were screened for the presence of depressive symptoms using the 30-item Geriatric Depression Scale (GDS) and were classified as having PSD or not depending on whether they scored above (≥11) or below (<11) the depression cutoff level on the GDS. This cutoff point of a GDS score of 11 or greater has previously been validated with a sensitivity of 96% and a specificity of 69% for major depression using DSM-IV criteria. Screened patients with a GDS score of 11 or greater were invited to enroll in the parent study of a brief psychosocial intervention for depression. Patients with hemorrhagic stroke, receptive or global aphasia, a reduced level of consciousness (Glasgow Coma Scale score <15), inability to understand and follow directions, or psychosis were excluded from recruitment.

Enrollment in the parent study began October 10, 2002, and ended April 9, 2007. Enrollment in this supplemental genetic study occurred between December 13, 2005, and December 13, 2006. Eligible stroke survivors who consented to be screened for the parent study during this time were invited to co-enroll in the genetic study beginning January 1, 2006. Simultaneously, all eligible individuals previously screened for the parent study were contacted and invited to enroll in the genetic study. Participants consented separately for the supplemental genetic study and provided blood or saliva samples for analysis of SERT genetic polymorphisms. This process of inviting enrollment from newly and previously screened participants in the parent study continued until the predetermined group size of 75 patients with depressive symptoms and 75 nondepressed individuals was reached (December 13, 2006). All the study procedures were reviewed and approved by the University of Washington institutional review board.

Race was assessed in this study because the distribution of genetic polymorphisms and their associations with medical illness or treatment response have been shown to differ among population groups. Study participants classified themselves as belonging to 1 or more of the race options defined by the investigators. Participant disability was assessed using the National Institutes of Health Stroke Scale (NIHSS).

Sixty-two of the 75 patients with a GDS score of 11 or greater in the genetic association study described herein chose to enter the behavioral intervention trial. In these patients, a DSM-IV diagnosis of major depression (n = 58) or a non-DSM-IV diagnosis of minor depression (n = 4) was established using a structured diagnostic interview, the Depression Interview and Structured Hamilton. Of these 62 patients, 46 (74%) had a history of major depression by self-report, with the reported number of episodes ranging from 1 to 8 (median, 2). The remaining 13 patients with a GDS score of 11 or greater consented to genetic testing only but declined to be part of the intervention trial. Because the Depression Interview and Structured Hamilton and psychiatric history were obtained only as part of the intervention trial, a more detailed depression history was not obtained in these individuals or in the control group (n = 75).

**SAMPLE COLLECTION AND GENOTYPING**

From participants who were mobile, a 10-µL sample of EDTA-anticoagulated blood was collected at a local laboratory. Individuals for whom travel to a laboratory would have been a burden donated saliva samples instead. Saliva samples were obtained by asking participants to hold their saliva for 2 minutes and subsequently spit into sterile 50-µL polypropylene tubes. Samples were identified by participant number only. DNA isolation and genotyping was performed by investigators masked to any participant information.

DNA was isolated from blood using buffy coat preparations in a modification of the procedure by Miller et al using Puregene DNA Purification Kits (Gentra Systems, Minneapolis, Minnesota) and following the manufacturer’s instructions. DNA was isolated from saliva using QiAamp DNA Blood Mini Kits (Qiagen, Valencia, California) and using the manufacturer’s protocol for isolation of genomic DNA from saliva. For genotyping of 5-HTTLPR, 0.5µM oligonucleotide primers flanking the 5-HTTLPR (forward: 5’-ATGCAGCACCTAACCCCCTAATGT-3’ and reverse: 5’-GGACGCGAAGGTGCGCGGAG-3’) were used in 10-µL polymerase chain reactions containing 5 µL of HotStar Taq Master Mix (Qiagen), 2.5 µL of betaine (Sigma-Aldrich Chemical Co, St Louis, Missouri), and 100 ng of genomic DNA from each participant. Polymerase chain reactions were run on a PCT-200 DNA Engine (MJ Research, Waltham, Massachusetts) using the following cycling parameters: 15-minute incubation at 95°C, followed by 33 cycles at 95°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes, followed by a 10-minute final extension step of 72°C for 10 minutes. Results were size fractionated on a 3% agarose gel that allowed for easy distinction of the s allele and the l allele, resulting in an approximately 460-bp fragment.

rs25531 was genotyped using an ABI7000 Gene Expression System (Applied Biosystems, Foster City, California). Genomic DNA, 100 ng, was amplified in the presence of gene-specific primers (forward: 5’-CCCTGCAGGGAGCTCCC-3’ and reverse: 5’-GTCTGAGGGCCGTCGCA-3’) and allele-specific fluorescent probes (VIC-CTGCACCCCCCAGCAT-NFQ and FAM-CTGCACCCCCCAGCAT-NFQ) obtained through Applied Biosystems Custom TaqMan single nucleotide polymorphism genotyping assay service and following the manufacturer’s instructions.

For genotyping of STin2 VNTR, 0.5µM oligonucleotide primers flanking the polymorphic site (forward: 5’-GTTCAGTATCAACAGGGAGCTGAG-3’ and reverse: 5’-GTTCAGTATCAACAGGGAGCTGAG-3’) were used in poly-
Table 1. Demographic and Clinical Characteristics of the Depressed and Nondepressed Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Depressed Group (n=75)</th>
<th>Nondepressed Group (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>43 (57)</td>
<td>51 (68)</td>
</tr>
<tr>
<td>Female</td>
<td>32 (43)</td>
<td>24 (32)</td>
</tr>
<tr>
<td>Race, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White only</td>
<td>50 (67)</td>
<td>58 (77)</td>
</tr>
<tr>
<td>African American only</td>
<td>7 (9)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Asian only</td>
<td>4 (5)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>American Indian/Alaskan native only</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Hawaiian native/Pacific Islander only</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;1 Race b</td>
<td>12 (16)</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Stroke hemisphere, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>30 (40)</td>
<td>33 (44)</td>
</tr>
<tr>
<td>Right</td>
<td>37 (49)</td>
<td>37 (49)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>8 (11)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Stroke location, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>38 (51)</td>
<td>45 (60)</td>
</tr>
<tr>
<td>Posterior</td>
<td>22 (29)</td>
<td>20 (27)</td>
</tr>
<tr>
<td>Anterior to posterior</td>
<td>15 (20)</td>
<td>10 (13)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>56.8 (12.5)</td>
<td>62.6 (14.2)</td>
</tr>
<tr>
<td>NIHSS score, mean (SD), y</td>
<td>5.6 (4.4)</td>
<td>3.6 (3.0)</td>
</tr>
</tbody>
</table>

Abbreviation: NIHSS, National Institutes of Health Stroke Scale.

a Differences between the depressed and nondepressed groups were significant for age (2-sample t test, P=.009) and NIHSS score (2-sample t test, P=.002) only but not for any of the other, categorical variables (Fisher exact test).

b Participants who identified themselves as members of more than 1 race selected all races except Hawaiian native/Pacific Islander.

merase chain reactions using the same reagents and cycling parameters as described previously herein for 5-HTTLPR. Reaction products were size fractionated on a 5% nondenaturing polyacrylamide gel, allowing distinction of the 214-bp STin2.12, 248-bp STin2.9, 263-bp STin2.10, and 299-bp STin2.12 alleles.

DATA ANALYSIS

Comparisons between SERT genotype groups and other categorical variables were made using the generalization of the Fisher exact test. Calculations for deviation from Hardy-Weinberg equilibrium were made using \( \chi^2 \) tests. Continuous variables, such as age and NIHSS score, were compared between the groups of depressed and nondepressed participants using 2-sample \( t \) tests or Mann-Whitney tests when data were nonnormally distributed. Odds ratios (ORs) for depression by genotype were determined by means of logistic regression, controlling for sex, age, and NIHSS score.

RESULTS

Although no deliberate attempt was made to match participants with or without PSD in this study, both groups had similar distributions of sex and race. Likewise, there were no statistically significant differences in stroke location by hemisphere or along the anterior-posterior brain axis between the 2 groups (Table 1). Patients with PSD, however, were significantly younger than nondepressed individuals, with a mean (SD) age of 36.8 (12.5) years for depressed individuals vs 62.6 (14.2) years for nondepressed individuals (P=.009). Also, the mean (SD) NIHSS score was significantly higher in participants with depressive symptoms (5.6 [4.4]) than in nondepressed individuals (3.6 [3.0], P=.002).

The distributions of SERT genotypes for the 5-HTTLPR, rs25531, and STin2 VNTR polymorphisms in all 150 patients with stroke were in Hardy-Weinberg equilibrium (5-HTTLPR: \( \chi^2=5.1, P=.17 \); STin2 VNTR: \( \chi^2=1.6, P=.65 \); and rs25531: \( \chi^2=0.54, P=.46 \)). Allele frequencies are given in Table 2. The rare 5-HTTLPR xl allele was observed in only 1 nondepressed individual with the genotype s/xl. The STin2.9 allele occurred twice, in 2 patients with PSD with the genotype 9/12. To facilitate further analysis, we grouped the xl allele together with the l allele and the STin2.9 allele with STin2.12. This grouping, along with the identification of potential risk alleles (Table 3), was based on previous genetic association studies of 5-HTTLPR or STin2 VNTR and mental illness and on studies suggesting similar functional effects of the STin2.9 and STin2.12 alleles. Analysis results did not differ significantly depending on whether the rare allele carriers were included (alternative results excluding rare allele carriers are not shown).

For the STin2 VNTR polymorphism, genotype frequencies differed significantly between groups, with the STin2.9/12 and 12/12 genotypes being more common in patients with PSD (Table 3). The 5-HTTLPR s/s genotype was also more common in participants with depressive symptoms, yet this effect did not reach statistical significance. The frequency of the rare rs25531 G allele was similar in both groups. We next compared the ORs for depression between individuals carrying different numbers (0, 1, or 2) of potential risk alleles at each of the 2 polymorphic sites, 5-HTTLPR and STin2 VNTR (Table 3). For rs25531, no potential risk allele has been identified, and genotypes were, therefore, compared without risk assignment. Because younger age and higher NIHSS score were significantly associated with depressive symptoms in this study, ORs were adjusted for these 2 variables. In addition, ORs were adjusted for sex because the prevalence of depression is higher in women. For 5-HTTLPR and STin2 VNTR, carrying 2 potential risk alleles significantly raised the odds of PSD.
Genotypes at the 3 polymorphic sites, 5-HTTLPR, STin2 VNTR, and rs25531, were not independent from one another. The 5-HTTLPR’s allele occurred significantly more frequently in combination with the STin2.12 allele and the rs25531 A allele than together with STin2.10 or rs25531 G, indicating the presence of linkage disequilibrium among the 3 sites (Table 4).

As a post hoc analysis, we investigated a possible additive effect of 5-HTTLPR and STin2 VNTR. Although being homozygous for a potential risk allele at either site significantly raised the odds of PSD, the odds were not much higher for individuals who were homozygous at both sites compared with individuals who were homozygous at only 1 of the 2 sites (Table 5).
These results show that the STin2 VNTR and 5-HTTLPR polymorphisms of SERT are associated with PSD in stroke survivors. Homozygous carriers of potential risk alleles for mental illness, the s allele of 5-HTTLPR and the STin2.9 and STin2.12 alleles, had at least 3-fold higher odds of depression compared with individuals with other genotypes. No association between rs25531 and depression was observed in this study. However, given the low minor allele frequency of rs25531, this study did not carry enough statistical power to detect anything but a strong association between PSD and rs25531. The limitations imposed by the sample size are also reflected in the fairly wide confidence intervals on the ORs in Tables 3 and 5.

The 5-HTTLPR s allele has previously been linked to depression moderated by life stress, suicidal behavior, neuroticism, and bipolar disorder but not by unipolar depression. Moreover, the s allele has been associated with lower remission and response rates in depressed white patients treated with selective serotonin reuptake inhibitors. Compared with the 5-HTTLPR polymorphism, STin2 VNTR has been much less intensively investigated. The STin2.12 allele has been associated with schizophrenia and bipolar disorder but not with unipolar depression, whereas the STin2.9 allele has been associated with depression and bipolar disorder in a single study. However, a possible effect of STin2.9 was not confirmed in a subsequent meta-analysis of STin2 VNTR association studies.

To date, few studies have investigated the role of the SERT polymorphism in medical-psychiatric comorbidity. A single study comparing 26 depressed and 25 nondepressed stroke survivors investigated only the 5-HTTLPR polymorphism and found that the s allele was more common in patients with PSD. In a cross-sectional population-based study, Grabe et al found that the s allele predisposed female carriers to higher levels of perceived mental and physical distress in the face of multiple chronic illnesses and unemployment. In a large study of cardiac patients, carriers of at least 1 s allele were more frequently depressed and had an increased risk of subsequent cardiac events. In a study of 138 patients with irritable bowel syndrome, the STin2 VNTR 9/12, along with the 5-HTTLPR s/s genotype, were found to be more common in patients with a history of comorbid depression.

The present results confirm those of previous studies that describe linkage disequilibrium among 5-HTTLPR, rs25531, and STin2 VNTR. For rs25531, the rare G allele has been shown to occur almost exclusively combined with the 5-HTTLPR l allele, a finding confirmed in the present study. Furthermore, we found evidence of an association between the 5-HTTLPR s and the STin2.12 alleles. This agrees with previous population studies indicating linkage disequilibrium between these 2 alleles in European populations. As a result of linkage disequilibrium, potential risk alleles at both polymorphic sites are more likely to occur in combination. Hence, disease associations ascribed to the 5-HTTLPR s allele in studies investigating this polymorphism alone might at least in part be due to an underlying 5-HTTLPR s–STin2.12 association.

It is as yet unknown how SERT polymorphisms might affect the risk of mental illness. The s allele has been associated with lower transcriptional activity in cell culture and slower serotonin uptake in human platelets compared with the l allele. Yet, several positron emission tomography studies in adult human brain have not shown a correlation between 5-HTTLPR genotype and SERT availability. In contrast, laboratory studies of STin2 VNTR have identified the 9- and 12-repeat alleles as transcriptional enhancers, thus defying a simple explanation whereby a global increase or decrease in SERT expression through these polymorphisms might affect the risk of depressive symptoms.

We used the GDS as a screening instrument for depressive symptoms consistent with PSD. Although GDS sensitivity and specificity for major depression are fairly good, as a screening test it does not in itself make a DSM-IV diagnosis of major depression and indicates only the pres-
ence of depressive symptoms. Moreover, a GDS score less than 11, although below the scale’s cutoff value for depression, is not synonymous with a complete absence of depressive symptoms. Hence, the term depressed as used in this article indicates a group of patients carrying either a DSM-IV diagnosis of a mood disorder due to stroke with depressive features or the more stringent DSM-IV diagnosis of a mood disorder due to stroke with major depressive-like episodes (both code 293.83).

This case-control study design did not call for patients with PSD and nondepressed stroke survivors to be matched by demographic or clinical factors. Thus, it is relevant to note that patients with or without depressive symptoms had similar distributions of stroke location and PSD. The present finding that mean NIHSS scores were higher in patients with depressive symptoms confirms the conclusion reached in a previous systematic review,60 of observational studies that showed a correlation between the incidence of PSD and stroke severity. In addition, greater levels of disability, such as those caused by larger strokes, have been linked to an increased risk of PSD in a large, prospective, population-based study.5 The same study identified a history of depression before stroke as risk factor for PSD.3 This is in keeping with the self-report of those study participants from whom we obtained an extensive psychiatric history (62 of the 75 depressed participants), as 74% of these patients reported a history of major depression. The mean age of the present study participants was young compared with the age of the average patient with stroke.41,42 This is interesting in light of results from the Framingham Study,43 in which symptoms of depression have been shown to significantly increase the risk of stroke in patients younger than 65 years. Hence, the present results are consistent with a possible effect where premorbid depression heightens the risk of stroke and PSD in a younger patient group, thereby resulting in the observation that patients with PSD were even younger than their nondepressed counterparts.

This study is the first to characterize an association of the 5-HTTLPR and STIN2VNTR polymorphisms of SERT with PSD. Although these polymorphisms, with the possible exception of STIN2.9, do not seem to be associated with depression in the absence of psychosocial stress, this study shows them to raise significantly the odds of depressive symptoms in the context of medical illness. In the case of stroke, this can lead to a vicious circle, whereby some SERT genotypes lower resilience to psychosocial stress and thereby increase the risk of a depressive illness, which itself raises the risk of stroke at a younger age. Consequently, as stroke survivors, carriers of these genotypes seem to be at increased risk for PSD and further morbidity. This illustrates how genetic factors might participate in forming a vicious circle by increasing medical and psychiatric morbidity in a biopsychosocial framework.

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