Influence of Serotonin Transporter Promoter Region Polymorphisms on Hippocampal Volumes in Late-Life Depression

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Context: Polymorphisms in the promoter region of the serotonin transporter gene (5-HTTLPR) influence transcription and may play a role in the pathogenesis and course of depression. Recent research demonstrates that specific polymorphisms may be associated with differences in hippocampal volumes in subjects with depression.

Objective: To examine associations between 5-HTTLPR genotype and hippocampal volumes in elderly control subjects and elderly subjects classified as having early or late onset of depression.

Design: Cohort study examining baseline characteristics.

Participants: Subjects were community dwelling and 60 years or older. Using a definition of early-onset depression as depression first occurring at 50 years or younger, we examined 72 subjects with early-onset depression, 63 subjects with late-onset depression, and 83 healthy control subjects.

Main Outcome Measures: All subjects underwent genotyping for the 5-HTTLPR and underwent brain magnetic resonance imaging. Analyses of hippocampal volumes were controlled for total cerebral volume, age, and sex.

Results: The interaction between diagnosis and 5-HTTLPR genotype was statistically significant for the right hippocampus ($P = .04$). Subjects with late-onset depression who were homozygous for the long (L) allele (L/L genotype) had significantly smaller right hippocampal volumes than did L/L subjects with early-onset depression ($P = .046$) or L/L control subjects ($P = .01$). Post hoc analyses showed that later age of depression onset was associated with smaller hippocampal volumes in subjects with the L/L genotype, but earlier age of onset was associated with smaller hippocampal volumes in subjects who were homozygous for the short (S) allele (S/S genotype).

Conclusions: Subjects with late-onset depression who were homozygous for the L allele exhibited smaller hippocampal volumes than other groups. Genotype also mediated the effect of age of onset on hippocampal volumes. Our findings differ from previous work; however, we examined an older and larger cohort of subjects than previous studies. Possible explanations for these findings include interactions between the serotonergic system and neurotrophic factors or cortisol response to stresses, each of which may affect hippocampal volumes.

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region (5-HTTLPR) have been associated with hippocampal volumes and may explain some of the conflicting results of studies investigating the hippocampus in depression. This polymorphism in the promoter region of the serotonin transporter gene contains a 44-base pair (bp) insertion (long, or L allele) or deletion (short, or S allele). When compared with the L allele, the S allele is associated with a lower level of serotonin uptake and transcriptional efficiency of the serotonin transporter, which may play a role in depression. Although some have found that allelic differences may be susceptibility factors for depression or are associated with depression subtypes, others have found no association between depression and different genotypes. In subjects with depression, there does appear to be an association between subjects with S alleles and poorer response to antidepressants and increased adverse effects to serotonin reuptake inhibitors, and increased risk of suicide attempts. They proposed that reduced hippocampal volumes in this genotype may be a risk factor for rather than a consequence of major depression. We sought to replicate this finding in a cohort of elderly subjects with depression and elderly control subjects without depression and expand it to examine for differences based on age of depression onset.

METHODS

SAMPLE

Subjects with depression were participants in the National Institute of Mental Health Conte Center for the Neuroscience of Depression in Late Life, located at Duke University, Durham, NC. The Conte Center operates in a naturalistic treatment milieu and screens for both incident and prevalent cases. Eligibility was limited to patients 60 years or older with a diagnosis of unipolar major depression. Exclusion criteria included (1) another major psychiatric illness; (2) alcohol or drug dependence; (3) primary neurologic illness, including dementia; (4) illness or medication precluding cognitive testing; and (6) metal in the body precluding magnetic resonance imaging (MRI).

Control subjects were community dwellers recruited from the Aging Center Subject Registry at Duke University. Eligible control subjects had a nonlocal neurologic examination, no self-report of neurologic or depressive illness, and no evidence of a depression diagnosis based on the National Institute of Mental Health Diagnostic Interview Schedule.

A trained interviewer administered the Duke Depression Evaluation Schedule (DDES) to each subject. The DDES, a composite diagnostic interview instrument, includes sections of the National Institute of Mental Health Diagnostic Interview Schedule assessing depression, enriched with items assessing sleep problems and the clinical features of melancholia and psychosis, dysthymia, mania, and alcohol abuse or dependence. The DDES also includes the Montgomery—Asberg Depression Rating Scale and questions on age of depression onset.

The study protocol was approved by the Duke University Medical Center institutional review board. All subjects provided written informed consent before beginning study procedures.

We have previously published data examining hippocampal volumes in a smaller sample than reported herein. To participate in this study, subjects had to have both genetic and MRI data.

MRI ACQUISITION AND ANALYSIS

All subjects were screened for any condition where MRI was contraindicated. Subjects were imaged with a 1.5-T, whole-body MRI system (Signa; GE Medical Systems, Milwaukee, Wis) using the standard head (volumetric) radiofrequency coil. Padding immobilized the head. The scanner alignment light was used to adjust the head tilt and rotation so that the axial-plane lights passed across the canthomeatal line, and the sagittal lights were aligned with the center of the nose. A rapid sagittal localizer scan was acquired to confirm the alignment.

Two sets of dual-echo, fast spin-echo acquisitions were obtained: one in the axial plane for morphometry of cerebral structures and a second in a coronal oblique plane for segmentation of the hippocampus. The pulse sequence parameters were repetition time = 4000 milliseconds, echo time = 30 and 135 milliseconds, 32-kHz (±16-kHz) full-imaging bandwidth, echo train length = 16, a 256 × 256 matrix, 3-mm section thickness, 1 number of excitations, and a 20-cm field of view. The images were acquired in 2 separate acquisitions with a 3-mm gap between sections for each acquisition. The second acquisition was offset by 3 mm from the first so that the resulting data set consisted of contiguous sections. For the near coronal acquisition, the localizer scan was used to identify the anterior commissure–posterior commissure line. Oblique, near-coronal images were prescribed perpendicular to this line, covering the entire brain from just anterior of the temporal lobe to a plane posterior to the lateral ventricles.

The GRID program was used to quantify the hippocampus; methods for delineating the hippocampus have previously been described. This program allows for semiautomated determination of region of interest volumes and was based on a manual point-counting method. The program used a single magnetic resonance contrast, creating a histogram of the image intensities. The analyst moved tissue-type pointers on the histogram to preassign tissue types in the image. A grid of a size chosen by the operator was then superimposed on the image with the tissue type at each intersection preassigned by the adjusted histogram. Working with an original image series, the analyst efficiently readjusted the intensity cutoffs (histogram) for each tissue type, as well as the size of the grid. If any regions were misclassified, individual grid points were changed to the correct tissue type. A combination of this tissue classification with manual tracing allowed for rapid determination of region of interest volumes.

The hippocampus was defined as follows. On each scan, analysts began with the most posterior coronal slice and moved anteriorly. Measurement of the hippocampus on each side began when the pulvinar nucleus of the thalamus obscured the crura fornici. The fimbria, which extends from the superior surface of the hippocampus across the cerebrospinal fluid into the white matter above, was transected at its narrowest point. Along the medial border of the hippocampus, the thin strip of gray matter was cut at its narrowest point, and tracing then continued around the hippocampal body to the starting point. The

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The cohort with depression was subdivided into those with early-onset and late-onset depression, using age older than 50 years as the cutoff (72 subjects with early-onset depression, 63 subjects with late-onset depression) (Table 1). There were no significant differences in genotype frequency or hippocampal volumes between the depressed and control groups.

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### DNA EXTRACTION AND GENOTYPING OF THE 5-HTTLPR

Subjects provided a blood sample for genetic testing. Genomic DNA was extracted by standard procedures (Puregene D-50K DNA Isolation Kit; Gentra, Minneapolis, Minn) from fresh or frozen samples of peripheral blood. Polymerase chain reaction amplification was used to generate a 484- and a 528-bp fragment corresponding to the short and long 5-HTTLPR alleles, respectively. This method and the subsequent modifications have been previously described. Subjects were thus classified as being homozygous for the long (L/L genotype) or short alleles (S/S genotype), or heterozygous (L/S genotype).

### ANALYTIC STRATEGY

For all analyses, early-onset depression was defined a priori as depression developing at 50 years or younger; late-onset depression was defined as developing at 51 years or older. We used t-tests and χ² tests to determine differences in demographic variables, genotype frequency, and hippocampal volumes first between subjects with depression and control subjects, then among the diagnostic categories of control subjects, subjects with early-onset depression, and subjects with late-onset depression. Hippocampal volumes were measured separately as total volume, right hippocampus, and left hippocampus. Analysis of covariance assessed hippocampal volumes using total cerebral volume as a within-subject factor and diagnosis, genotype, current age, and sex as between-subject factors. Because we were particularly interested in the interaction between diagnosis and genotype, an interaction term was included in these models.

As part of our a priori analytic plan to replicate previous research using similar analytic methods, analysis of covariance was used to examine the effect of diagnosis on hippocampal volume for each genotype separately. This was done after ensuring that hippocampal volumes were normally distributed and was only done for hippocampal measurements that were significantly associated with a genotype×diagnosis interaction. These models controlled for total cerebral volume, current age, and sex. For models showing a statistically significant effect, defined as a P<.05, the least squares means procedure was used to assess for differences between diagnostic groups. Finally, in a post hoc analysis, subjects with depression were examined in general linear models where the association between hippocampal volumes and age of onset was examined as a continuous variable, while including an age of onset by genotype interaction and also controlling for total cerebral volume, sex, and current age. Separate models were examined within each genotype, controlling for the same predictors.

### RESULTS

### SAMPLE CHARACTERISTICS

There were 135 subjects with depression and 83 control subjects; these groups did not differ on demographic variables (Table 1). There were no significant differences in genotype frequency or hippocampal volumes between the depressed and control groups.

The cohort with depression was subdivided into those with early-onset and late-onset depression, using age older than 50 years as the cutoff (72 subjects with early-onset depression, 63 subjects with late-onset depression) (Table 2). Subjects with early-onset and late-onset de-
pressions in hippocampal volumes, genotype, and diagnosis, including age, sex, and total cerebral volumes as covariates. A genotype × diagnosis interaction term was also included in the models. Separate models were created for left, right, and total hippocampal volumes (Table 3). The interaction of genotype by diagnosis was statistically significant only in the right hippocampal volume model. There was a statistical trend for an association between genotype and total and left hippocampal volumes, but there were no statistically significant associations with diagnosis in these models.

In these models, the diagnosis variable trichotomized subjects into those with early-onset depression, late-onset depression, and control subjects. Multivariate models were also created wherein subjects were simply examined as subjects with depression or control subjects. In these models, neither genotype, diagnosis (depression or control), nor the ratio of subjects with early-onset depression to subjects with late-onset depression was statistically significant.

### The Effect of Diagnosis on Right Hippocampal Volumes for Individual 5-HTTLPR Genotypes

Analysis of covariance models next examined right hippocampal volume as the outcome within each genotype.
type, so separate models were created for subjects with L/L, L/S, and S/S genotypes. These examined the influence of diagnostic status on right hippocampal volumes while controlling for total cerebral volume, current age, and sex. Similar models were not created for left and total hippocampal volumes because full models (Table 3) did not show a significant interaction between genotype and diagnosis.

Depression status only significantly influenced right hippocampal volumes in subjects with the L/L genotype (F2,134 = 3.64; P = .03). The least squares means analysis found that subjects with late-onset depression had smaller right hippocampal volumes than did either subjects with early-onset depression (adjusted mean difference, 0.20 mL; P = .046) or control subjects (adjusted mean difference, 0.26 mL; P = .01). There was not a statistically significant difference between subjects with early onset depression and control subjects (adjusted mean difference, 0.06 mL; P = .51).

In subjects with the S/S genotype, there was a statistical trend for an association between diagnostic status and right hippocampal volumes (F2,134 = 2.61; P = .09). There was no such association in subjects with the L/S genotype (F2,134 = 0.15; P = .86).

**MODELS FOR EACH 5-HTTLPR GENOTYPE USING AGE OF ONSET AS A CONTINUOUS PREDICTOR**

Previous models dichotomized subjects based on age of depression onset. As a post hoc analysis, we examined if age of onset itself was associated with hippocampal volumes for the different genotypes while controlling for current age, sex, and total cerebral volume. Only subjects with depression were included in these analyses because control subjects had no history of depression.

Initial models examined the effect of genotype and age of depression onset as predictors while including an interaction term for these 2 predictors and controlling for age, sex, and total cerebral volume. The age of onset by genotype interaction was statistically significant in all models (right hippocampal volume, F2,134 = 4.10; P = .02; left hippocampal volume, F2,134 = 3.29; P = .04; total hippocampal volume, F2,134 = 4.09; P = .02). Additionally, total cerebral volume was statistically significant in all models (right hippocampal volume, F2,134 = 5.54; P = .02; left hippocampal volume, F2,134 = 6.94; P = .01; total hippocampal volume, F2,134 = 7.56; P = .007). No other predictor was significantly associated with hippocampal volumes in any model.

The relationship between age of depression onset and hippocampal volumes was next considered within each genotype, while also controlling for age, sex, and total cerebral volume (statistical results for age of onset variables presented in **Table 4**). For subjects with depression with the L/L genotype, age of onset was negatively associated with total and right hippocampal volumes but not left. This would mean that later age of onset was associated with smaller total and right hippocampal volumes. There was no significant influence of age of onset on hippocampal volumes for subjects with depression with the L/S genotype.

For subjects with depression with the S/S genotype, age of onset was positively associated with total and right hippocampal volumes and exhibited a statistical trend for a positive association with the left hippocampal volume. This would indicate that an earlier onset of depression is associated with smaller hippocampal volumes.

**5-HTTLPR GENOTYPE AND HIPPOCAMPAL VOLUMES IN SUBJECTS WITHOUT DEPRESSION**

The control population without depression was examined separately in a post hoc analysis. It was thought that if an association between 5-HTTLPR genotype and hippocampal volumes was found in this population it would indicate a general genetic susceptibility rather than a phenomenon specific to depression. When controlling for age, sex, and total cerebral volume, there was no association between genotype and any hippocampal measurement (total hippocampal volume, F2,82 = 0.23; P = .79; left hippocampal volume, F2,82 = 1.28; P = .28; right hippocampal volume, F2,82 = 0.16; P = .85).

**COMMENT**

Although others have examined the relationship between 5-HTTLPR genotype and hippocampal volumes in depression,21 to our knowledge this is the first study examining that question in an elderly population. We found no association between 5-HTTLPR and hippocampal volumes when the depressed cohort was examined as 1 group; however, we did find a significant interaction between diagnosis and genotype for the right hippocam-

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**Table 4. Association of Age of Depression Onset With Hippocampal Volumes by 5-HTTLPR Genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>L/L Genotype</th>
<th>F Value</th>
<th>P Value</th>
<th>L/S Genotype</th>
<th>F Value</th>
<th>P Value</th>
<th>S/S Genotype</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>4.59</td>
<td>0.04</td>
<td></td>
<td>0.62</td>
<td>0.43</td>
<td></td>
<td>6.29</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>2.62</td>
<td>0.11</td>
<td></td>
<td>1.73</td>
<td>0.19</td>
<td></td>
<td>4.35</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>5.48</td>
<td>0.02</td>
<td></td>
<td>0.03</td>
<td>0.87</td>
<td></td>
<td>6.10</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: L, long allele; S, short allele.

*Models included only subjects with depression and examined for the relationship between hippocampal volumes and age of depression onset while controlling for current age, sex, and total cerebral volume. Analyses for the L/L genotype had 1,46 df; models for the L/S genotype had 1,66 df; models for the S/S genotype had 1,20 df.
pal volume when subjects with depression were dichotomized based on age of depression onset. After controlling for age, sex, and total cerebral volume, subjects with a late onset of depression and L/L genotype exhibited smaller right hippocampal volumes than did control subjects or subjects with early-onset depression with that genotype. This finding is similar to a previous report associating smaller hippocampal volumes with the L/L genotype, although that report did not exclusively examine older subjects dichotomized based on age of onset.21

Post hoc analyses in subjects with depression examining age at onset as a continuous variable resulted in broader findings. The interaction between age of onset and genotype was statistically significant for all hippocampal volume measurements. When individual genotypes were examined, later age at onset in subjects with depression with the L/L genotype was associated with smaller right and total hippocampal volumes, while earlier age at onset was associated with smaller hippocampal volumes in subjects with depression with the S/S genotype. No relationship between age at onset and hippocampal volumes was found in subjects with the heterozygous genotype.

These findings are important because they expand our understanding of the pathophysiological features of depression and the interaction between genetic and neuroanatomical contributors to depression. This association is critical, given how smaller volumes are associated with poorer outcomes of antidepressant therapy19,44 and may contribute to the cognitive dysfunction seen in geriatric depression.

This report highlights the relevance of age at onset in an elderly population with depression. Elderly individuals with depression may have had multiple episodes of depression, starting earlier in life; alternatively, they may have had no psychiatric history, becoming depressed for the first time later in life. Several distinctions between these groups have been identified, such that subjects with early-onset depression are more likely to have a family history of psychiatric illness,45,46 while subjects with late-onset depression are more likely to have greater subcortical ischemic disease on MRI.47,48 These findings have been interpreted to be signs of different causes or risk factors for depression based on age at depression onset.

If there are different risk factors for depression based on age at depression onset, there may be different risk factors for smaller hippocampal volumes based on age at depression onset. Subjects with an earlier onset of depression who have a greater lifetime depression duration may have reduced hippocampal volumes through repeated stress-induced increases in glucocorticoid levels, which have neurotoxic effects on hippocampal tissue.49 Alternatively, depression occurring for the first time in later life may be related to subcortical ischemia or may be a prodrome of dementia.20,51 The hippocampus could thus be experiencing atrophy, as seen in Alzheimer disease.22 It may also be smaller because of ischemia impairing its cortical and subcortical connections; an association between subcortical ischemia and smaller hippocampal volumes has not been established in depressed but has been seen in subcortical ischemic vascular dementia.32

Subcortical ischemic disease is one potential explanation for our finding of smaller hippocampal volumes in subjects with late-onset depression with the L/L genotype. The L/L genotype has been associated with greater platelet activation and greater increases in heart rate and blood pressure in response to stress.54 These factors may contribute to development of subcortical ischemic disease that is commonly seen in late-life depression,50 which could result in disconnection of the hippocampus from afferent and efferent structures, thus increasing the risk of depression.

Alternatively, the association between hippocampal volumes, depression, and the L/L genotype may be related to preclinical Alzheimer disease. Although there is debate about the role of 5-HTTLPR as a susceptibility factor for Alzheimer disease,55-57 an association between the L/L genotype and psychosis and agitation in Alzheimer disease has been reported.58,59 It is possible that the efficient synaptic removal of serotonin as a result of more efficient transcription of the serotonin transporter provided by the L/L genotype would result in behavioral problems or possible depression in the preclinical population.

It has also been hypothesized that the higher reuptake of serotonin resulting from the L/L genotype may cause a distinct vulnerability in the hippocampus to stress-induced neurotoxic effects of glucocorticoid.21 Our findings associating L/L genotype and smaller hippocampal volumes with a later onset of depression, thus possibly a shorter duration of depression, argues against this hypothesis. However, as age of depression onset does not necessarily equate with duration of depression, this hypothesis may still be correct.

The report of an earlier age at onset being associated with smaller hippocampal volumes in subjects with depression with the S/S genotype should be viewed with caution. The post hoc analyses of this genotype found an association between earlier age at onset and smaller hippocampal volumes but were complicated by a relatively small number of subjects exhibiting the S/S genotype. This finding is biologically plausible because the S/S genotype moderates the influence of stressful life events on the development of depression.60 Thus, individuals with the S/S genotype who underwent multiple stressful life events would be expected to have a greater lifetime duration of depression, which may impact hippocampal volume through elevated glucocorticoid levels, decreases in brain-derived neurotrophic factor,61 and decreases in neurogenesis.62 Replication of this finding is needed because this theory is dependent on depression duration and history of antidepressant therapy.

The primary limitation of this study is its case-control design, which is sensitive to population stratification. This may be seen in genotype frequency; although there was no difference in genotype frequency among diagnostic groups, there was a comparatively small number of subjects with the S/S genotype. It could be argued that a superior study design would have included equal numbers of subjects across genotypes, but this would involve increasing the sample size significantly. Another limitation is our age at onset variable, which was provided by subject self-report. This variable is not a surrogate for depression duration or treatment, which would
include the number, duration, and severity of past episodes and history of treatment and is a subjective recollection rather than data supported by the medical record. It also does not ensure that such first episodes would meet diagnostic criteria for major depression. Future studies should include better measures for duration of total depressive episodes. Finally, although all subjects met criteria for a current diagnosis of active major depression, there was no information on how long subjects had taken antidepressants prior to the MRI.

It is also possible other factors not examined herein contributed to smaller hippocampal volumes. The apolipoprotein E (APOE) genotype, a risk factor for Alzheimer disease, is one possible contributing factor; however, previous reports have failed to demonstrate a cross-sectional association between APOE status and hippocampal volumes in late-life depression. Given the potential association between L/L genotype and subcortical ischemia, it may be reasonable to study the relationship between subcortical lesions and hippocampal volumes. Other contributors, including more complex gene–gene interactions are also possible.

In summary, this study reports an association between the L/L genotype of the 5-HTTLPR and smaller hippocampal volumes in late-onset depression and a tentative association between the S/S genotype and earlier age of depression onset. Further research is needed to unravel the association between genotype and psychosocial stressors in the elderly population and also to determine how genotype and brain morphological characteristics may contribute to treatment outcomes in elderly individuals with depression.

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