

# Reduced Hippocampal Volumes Associated With the Long Variant of the Serotonin Transporter Polymorphism in Major Depression

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**Background:** Substantial evidence supports a role for dysfunction of the serotonin transporter in the pathogenesis of major depression. Several studies have found reciprocal interactions between the serotonergic system and both brain-derived neurotrophic factor and glutamate, which are known to modulate or affect hippocampal morphologic characteristics.

**Objective:** To examine the influence of a polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene on hippocampal volumes in patients with major depression and healthy controls.

**Design:** Baseline investigation of a prospective magnetic resonance imaging study with a 4-year follow-up period.

**Patients:** We examined 40 inpatients with major depression as well as 40 healthy controls matched for age, sex, and handedness.

**Main Outcome Measures:** Subjects underwent high-resolution magnetic resonance imaging. Furthermore, genotyping for the 5-HTTLPR biallelic polymorphism was

performed, which consists of a 44-base pair insertion (L allele) or deletion (S allele).

**Results:** Patients with the L/L homozygous genotype had significantly smaller hippocampal gray matter (left hemisphere:  $P = .003$ ; right hemisphere:  $P = .01$ ) and white matter volumes (left hemisphere:  $P = .001$ ; right hemisphere:  $P = .002$ ) than controls with this genotype. No significant differences were found between patients and controls with the L/S or S/S genotype. Moreover, patients with the L/L genotype had significantly smaller hippocampal white matter volumes than those with the L/S or S/S genotype ( $P = .03$ ).

**Conclusions:** These findings suggest that homozygosity for the L allele is associated with decreased hippocampal volumes in patients with major depression but not in healthy controls. A possible explanation is that the interaction between the serotonergic system and neurotrophic factors as well as excitatory amino acid neurotransmission may affect hippocampal morphologic characteristics.

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ONE OF THE MAJOR BIOLOGICAL substrates in the pathogenesis of major depression is the serotonergic system.<sup>1</sup> Serotonin is a widespread neurotransmitter in the central nervous system. Serotonergic neurons are mainly found in different raphe nuclei, from which they project to numerous brain regions such as cortical areas, the hippocampus, and the basal ganglia.<sup>2</sup> The fine-tuning of serotonin neurotransmission seems to be critically influenced by the serotonin transporter (5-HTT) via clearing synaptic serotonin.

A polymorphism (5-HTTLPR) in the promoter region of the 5-HTT gene on chromosome 17q11.2 was identified with a 44-base pair insertion (L allele) or deletion

(S allele).<sup>3</sup> The relationship of this polymorphism to major depression is unequivocal; one study found a relationship,<sup>4</sup> whereas others did not.<sup>5,6</sup> Cultured human lymphoblast cell lines homozygous for the L allele were associated with nearly 2-fold increased 5-HTT expression and increased serotonin reuptake.<sup>3</sup> Moreover, in mice with targeted inactivation (knock-out) of the 5-HTT gene, the modulation of serotonergic system activity was found to depend on the 5-HTTLPR genotype.<sup>7</sup> In vivo studies also support these findings. A significant association between the L/L genotype and the depressive response to tryptophan depletion showed that patients homozygous for the L/L genotype who were in remission from a major depressive episode had rapid serotonin reuptake com-

**Table 1. Demographic and Clinical Data of Patients With Major Depression and Healthy Controls\***

	Patients (n = 40)	Controls (n = 40)	P Value
Age, y	44.4 (11.7)	41.7 (12.1)	.32
Sex (F/M), No. of patients†	19/21	19/21	>.99
Handedness (right/left), No. of patients†	38/2	38/2	>.99
Height, cm	171.0 (7.7)	175.9 (8.1)	.007
Weight, kg	71.6 (17.1)	73.2 (14.2)	.66
Alcohol, g/d	8.0 (14.7)	11.2 (11.3)	.29
Age at onset, y	37.1 (11.4)	NA	NA
Illness duration, y	7.9 (10.1)	NA	NA
Genotype distribution, No. (%)†			
L/L	16 (40)	13 (32.5)	.76
L/S	16 (40)	17 (42.5)	
S/S	8 (20)	10 (25)	
Allele distribution, No. (%)†			
L	16 (40)	13 (32.5)	.49
S	24 (60)	27 (67.5)	
Hamilton Depression Rating Scale score	22.7 (6.7)	NA	NA

Abbreviation: NA, not applicable.

\*Data are presented as mean (SD) unless otherwise indicated. P values were determined using the t test.

†Determined using the  $\chi^2$  test.

bined with decreased brain serotonin availability.<sup>8</sup> Furthermore, in 8 healthy controls homozygous for the L allele, a significant association with increased iodine 123-labeled 2 $\beta$ -carbomethoxy-3 $\beta$ -(4-iodophenyl) tropane ([<sup>123</sup>I] $\beta$ -CIT) binding to 5-HTT was seen, which may indicate a greater amount of 5-HTT.<sup>9</sup> However, these findings need clarification because 16 healthy controls did not show a relationship between the 5-HTTLPR polymorphism and [<sup>123</sup>I] $\beta$ -CIT binding.<sup>10</sup>

Interestingly, it has been demonstrated that serotonergic signaling is an important regulator of both early central nervous system development<sup>11</sup> and adult neurogenesis.<sup>12</sup> In addition, because a high density of 5-HTT has been found in the hippocampus by autoradiographic<sup>13,14</sup> and immunocytochemical investigations,<sup>15</sup> there may be a relationship between serotonergic function and hippocampal morphologic characteristics.

Aside from the well-documented contribution to learning and memory, the hippocampal formation plays a critical role in the regulation of motivation and emotion.<sup>16</sup> The hippocampus is a core region in the limbic system and has widespread connections to diverse cortical areas such as the prefrontal cortex, anterior thalamic nuclei, amygdala, basal ganglia, and hypothalamus,<sup>17</sup> all of which constitute the neuroanatomical network of mood regulation.<sup>18</sup> Investigations of patients with recurrent depressive episodes have demonstrated structural changes in the hippocampal formation,<sup>19-22</sup> which may decline with longer illness duration.<sup>19</sup> One study investigating first-episode patients and those with multiple episodes found that only patients with multiple episodes had hippocampal volume reduction, which was associated with the duration of their illness.<sup>22</sup> These findings extend those of experimental studies that suppose stress toxicity<sup>23</sup> or a lack of neurotrophic factors<sup>24</sup> to be the cause of these well-

known structural abnormalities of the hippocampus. However, postmortem analysis of the human hippocampus in patients with major depression and in glucocorticoid-treated patients did not show any major morphologic changes associated with neuronal cell death,<sup>25</sup> so it remains unclear whether and to what extent hippocampal changes occur in major depression.

Recently, studies have begun to address the issue that structural changes may predispose patients to depression; hippocampal size has been found to be strongly genetically determined,<sup>26</sup> and even first-episode patients with major depression showed a diminished volume of 6% in the left side of the hippocampus.<sup>27</sup> An association between heritability and brain size is supported by twin studies<sup>28</sup> and by studies from our laboratory showing that methionine homozygosity at codon 129 on the prion protein was associated with white matter reduction as well as enlargement of cerebrospinal fluid volume in healthy controls,<sup>29</sup> and that the allele 2 within the promoter region of the interleukin 1 $\beta$  gene was related to bifrontal temporal gray matter volume deficits and reduced overall white matter in patients with schizophrenia.<sup>30</sup> A genetic influence on brain volumes was also found in patients with geriatric depression, in whom at least 1 APOE  $\epsilon$ 4 allele showed significant decline in the right hippocampus.<sup>31</sup>

The aim of our study was to investigate the influence of functional polymorphisms in the 5-HTT gene on hippocampal morphologic characteristics in patients with major depression and healthy controls.

## METHODS

### SUBJECTS

We recruited 40 inpatients with major depression from the Department of Psychiatry at Ludwig-Maximilians-University, Munich, Germany (age range, 18-65 years; mean  $\pm$  SD age, 44.4  $\pm$  11.7 years) (Table 1). Psychiatric diagnoses based on DSM-IV criteria and on the Structured Clinical Interview for DSM-IV were determined by a consensus of at least 2 psychiatrists. Mean  $\pm$  SD illness duration was 7.9  $\pm$  10.1 years, and number of hospitalizations was 2.2  $\pm$  3.0. Moreover, mean  $\pm$  SD cumulative illness duration as described by Sheline et al<sup>19</sup> (27.7  $\pm$  35.2 months) and total previous duration of antidepressant medication use (25.5  $\pm$  55.8 months) were obtained retrospectively according to the anamnesis. Clinical variables were documented using the 21-item Hamilton Depression Rating Scale. Fourteen patients received selective serotonin reuptake inhibitors (4 sertraline hydrochloride, 7 citalopram, 2 paroxetine, and 1 fluvoxamine maleate), 7 were taking tricyclic antidepressants (2 amitriptyline hydrochloride, 2 doxepin hydrochloride, and 1 trimipramine), and 16 received other new antidepressants (5 venlafaxine hydrochloride, 4 reboxetine, and 7 mirtazapine). Four patients were not taking antidepressant medication at the time of magnetic resonance imaging.

For comparison, 40 healthy control subjects were matched with respect to age (age range, 19-58 years; mean  $\pm$  SD age, 41.7  $\pm$  12.1 years), sex, and handedness. A structured interview was used to assess medical history, trauma, and other exclusion criteria. Neither the healthy controls nor their first-degree relatives had a history of neurologic or mental illness. Exclusion criteria for patients and controls were previous head injury with loss of consciousness, cortisol medication use in the medical history, previous alcohol or substance abuse, or neurologic diseases. Comorbidity with

other mental illnesses or personality disorders was also excluded. No subject had received previous electroconvulsive therapy. Hypertension was excluded using blood pressure measures, and handedness was determined with the Edinburgh inventory.<sup>32</sup>

After an extensive description of the study to the patients with major depression and healthy controls, written informed consent was obtained. The study design was approved by the local ethics committee and was prepared in accordance with the ethical standards in the Declaration of Helsinki.

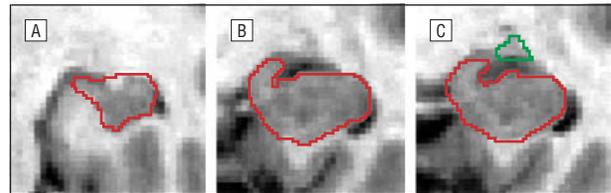
#### MAGNETIC RESONANCE IMAGING PROCEDURES

Magnetic resonance images were obtained (1.5-T Magnetom Vision; Siemens, Erlangen, Germany) using a coronal T2- and proton density-weighted dual echo sequence (repetition time, 3710 milliseconds; echo time, 22/90 ms; total acquisition time, 9 minutes; number of acquisitions, 1; field of view, 230 mm; matrix, 240 × 256; section thickness, 3 mm) and a 3-dimensional magnetization prepared rapid acquisition gradient-echo sequence (repetition time, 11.6 milliseconds; echo time, 4.9 milliseconds; total acquisition time, 9 minutes; number of acquisitions, 1; field of view, 230 mm; matrix, 512 × 512; section thickness, 1.5 mm). A commercial software package was used (Analyze; Biomedical Imaging Resource, Mayo Foundation, Rochester, Minn) for further image processing, with size reduction from 16 to 8 bit and transformation to a uniform matrix of 256 × 256 on 192 sections each 1.0 mm thick. All datasets were realigned and re-sampled 3-dimensionally for the anterior commissure to posterior commissure line according to Talairach coordinates with the software program BRAINS (Brain Research: Analysis of Images, Networks and Systems; developed by Andreasen et al).<sup>33</sup> This program allowed control of the regions of interest for the sagittal and transverse sections simultaneously as well as of the segmentation for calculating the intracranial content and the gray and white matter volumes (cubic centimeters) within the defined region of interest.

#### DEFINITION OF THE HIPPOCAMPAL FORMATION

We used the definition of the hippocampus (**Figure 1**) according to Niemann et al.<sup>34</sup> The hippocampal-amygdala border was detected using the description by Convit et al<sup>35</sup>; in addition, Frodl et al<sup>27</sup> provide a detailed description of this area. The evaluation staff was blind to each subject's status. On each magnetic resonance image, we started with the most posterior coronal section where the hippocampus was clearly detectable. The posterior hippocampal body and intralimbic sulcus can be seen in Figure 1A. In Figure 1B, the shape of the hippocampus may be compared with that of a rabbit with the head directed vertically; the medial ambient cistern is separated from the temporal horn of the lateral ventricles. The amygdala-hippocampal transition zone appears as a diffuse area of gray matter between the anterior portion of the hippocampus and the posterior portion of the amygdala. This structure can be identified most reliably in the axial plane. The boundary between the hippocampus and amygdala is clearly detectable in the sagittal plane (Figure 1C). The anterior part of the hippocampus ends where the cornu inferius of the lateral ventricle becomes vertically oriented.

For determination of interrater reliability, 10 brains were randomly chosen, and regions of interest were independently determined by 2 raters. The intraclass correlations for the interrater reliability of hippocampal gray matter ( $r_0=0.97$ ;  $P<.001$ ) and hippocampal white matter ( $r_0=0.82$ ;  $P=.008$ ) were high. For the intrarater reliability, 10 brains were obtained 4 weeks



**Figure 1.** Magnetic resonance image of coronal sections running in the occipitrostral direction. A, The hippocampal body. B, The shape of the hippocampus may be compared with that of a rabbit with the head directed vertically. C, The amygdala-hippocampal transition area.

apart by 1 rater (T.F.) (hippocampal gray matter:  $r_0=0.96$ ;  $P<.001$ ; hippocampal white matter:  $r_0=0.93$ ;  $P<.001$ ).

#### LABORATORY METHOD

The DNA was extracted from of a 5-mL blood sample using a kit (QIAamp Blood Isolation Kit; QIAGEN GmbH, Hilden, Germany) following the prescription of the supplier. Genotyping was carried out applying polymerase chain reaction amplification using the primers and methods described earlier by Heils et al.<sup>36</sup> The final volume was 25  $\mu$ L consisting of 50 ng of DNA, 1  $\mu$ mol/L of each primer, 200  $\mu$ M of deoxynucleotide triphosphate, 100  $\mu$ M of 7-deaza-guanosine triphosphate, 5% dimethyl sulfoxide, 10mM of Tris hydrochloride (pH, 8.3), 50mM of potassium chloride, 1.5mM of magnesium chloride, and 2.5 U of DNA polymerase (AmpliQ Gold; PerkinElmer, Langen, Germany). The polymerase chain reaction products were separated on a 3% agarose gel (FMC NuSieve, 3:1; Biozym Diagnostic GmbH, Oldendorf, Germany) and visualized with ethidium bromide staining.

#### STATISTICAL ANALYSES

Morphometric measurements in both groups were tested for normal distribution and homogeneity of variance. All statistical tests were considered to be significant at  $P<.05$ . We used  $t$  tests and analysis of variance to test for differences in demographic variables between patients and controls as well as between genotypes. We used  $\chi^2$  tests to compare the genotype frequencies between patients and controls. Hippocampal volumes underwent analysis of covariance (ANCOVA) assessing the main and interaction effects of the within-subject factor of hemisphere (left or right) and the between-subject factors of diagnosis (patients with depression or controls) and genotype ( $L/L$ ,  $L/S$ , or  $S/S$ ) using total cranial volume as the cofactor. Brain lobe volumes also underwent ANCOVA with the within-subject factors of hemisphere (left or right) and region (frontal, temporal, parietal, or occipital) and the between-subject factors of diagnosis (patients with depression or controls) and genotype ( $L/L$ ,  $L/S$ , or  $S/S$ ). Furthermore, hippocampal volumes of individuals homozygous for the  $L/L$  genotype were compared with those carrying the  $L/S$  or  $S/S$  genotype in each diagnostic group by ANCOVA design. Posthoc analyses were carried out with ANCOVA and  $t$  tests to test hippocampal volumes for differences between genotypes. Effect sizes regarding the association between genotype and hippocampal volumes were presented as Pearson correlation coefficients according to a method described by Rosenthal.<sup>37</sup>

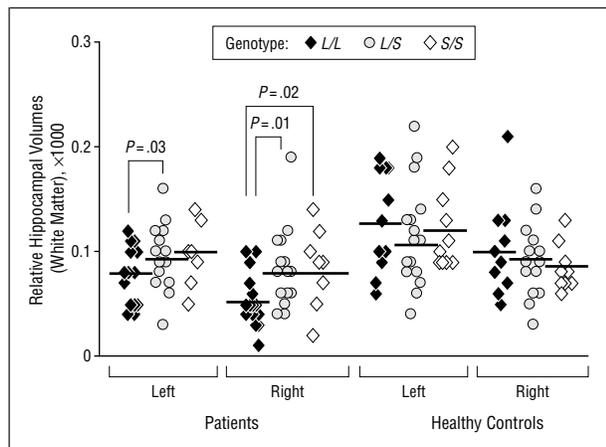
#### RESULTS

Patients and controls did not differ with regard to demographic variables (Table 1), and these variables were not

**Table 2. Repeated-Measures Analysis of Covariance for Hippocampal Gray and White Matter\***

Variable	Gray Matter			White Matter		
	F	df	P Value	F	df	P Value
Hemisphere	0.47	1, 73	.50	0.15	1, 73	.70
Diagnosis	10.3	1, 73	.002	12.2	1, 73	.001
Genotype	1.4	2, 73	.25	0.34	2, 73	.71
Intracranial content	27.2	1, 73	<.001	14.2	1, 73	<.001
Diagnosis by genotype	0.88	2, 73	.42	3.0	2, 73	.05
Diagnosis by hemisphere	0.87	1, 73	.36	2.3	1, 73	.14
Diagnosis by genotype by hemisphere	0.02	2, 73	.98	0.13	2, 73	.88

\*Diagnosis and genotype were between-subject factors, hemisphere was a within-subject factor, and intracranial content was a cofactor.



**Figure 2.** Scattergrams of left and right hippocampal white matter showing serotonin transporter polymorphisms in patients with depression. Bars depict the mean for each group.

different between genotypes (*L/L*, *L/S*, and *S/S*). Illness duration ( $F_{2,39}=0.15$ ;  $P=.86$ ), age at onset ( $F_{2,39}=0.40$ ;  $P=.67$ ), number of hospitalizations ( $F_{2,39}=0.98$ ;  $P=.39$ ), and depression severity measured with the Hamilton Depression Rating Scale ( $F_{2,39}=0.27$ ;  $P=.77$ ) did not differ between genotypes (*L/L*, *L/S*, and *S/S*). Furthermore, antidepressant medication use (selective serotonin reuptake inhibitors, tricyclic antidepressants, and other antidepressants;  $\chi^2_{38}=3.4$ ;  $P=.50$ ) and total previous duration of antidepressant treatment ( $F_{2,39}=0.68$ ;  $P=.51$ ) were not significantly different between genotypes. The allele and genotype frequencies are indicated in Table 1; they did not differ between patients and controls ( $\chi^2_{39}=0.56$ ;  $P=.76$ ).

The analysis of variance for frontal, temporal, parietal, and occipital lobes did not show significant effects. There were neither significant main effects for diagnosis and genotype nor significant interactions of diagnosis and genotype; genotype and region; genotype, diagnosis, and region; or genotype, diagnosis, hemisphere, and region.

The results of ANCOVA for the gray and white matter of the hippocampus appear in **Table 2**. A significant main diagnosis effect was found, indicating smaller hippocampal gray and white matter volumes in patients

with major depression as compared with healthy controls. Moreover, there was a significant association between genotype and hippocampal volumes, with a significant diagnosis and genotype interaction for hippocampal white matter ( $F_{2,73}=3.0$ ;  $P=.05$ ) but not hippocampal gray matter ( $F_{2,73}=0.88$ ;  $P=.42$ ).

Post hoc ANCOVA revealed a significant effect of genotype for hippocampal white matter in patients ( $F_{2,36}=3.8$ ;  $P=.03$ ) (**Figure 2**), whereas no significant effect was found for healthy controls ( $F_{2,36}=0.58$ ;  $P=.56$ ). Furthermore, significantly smaller right hippocampal white matter volumes ( $F_{1,39}=8.0$ ;  $P=.008$ ;  $r_{39}=0.42$ ) and a trend toward smaller left hippocampal white matter volumes ( $F_{1,39}=3.7$ ;  $P=.06$ ;  $r_{39}=0.30$ ) were found in patients with the *L/L* genotype as compared with those who carried the *L/S* or *S/S* genotype.

Post hoc *t* tests showed significantly smaller right hippocampal white matter volumes in patients homozygous for the *L/L* genotype compared with the *L/S* ( $t_{30}=2.6$ ;  $P=.02$ ; effect size:  $r_{29}=0.41$ ) and *S/S* ( $t_{22}=2.7$ ;  $P=.01$ ;  $r_{21}=0.43$ ) genotypes, as well as smaller left hippocampal white matter volumes compared with the *S/S* genotype ( $t_{22}=2.3$ ;  $P=.03$ ; effect size:  $r_{21}=0.36$ ) but not compared with the *L/S* genotype ( $t_{30}=1.5$ ;  $P=.15$ ; effect size:  $r_{29}=0.24$ ).

The results of ANCOVA to assess differences between patients and controls in each genotype group are presented in **Table 3**. Significantly smaller hippocampal gray matter (**Figure 3**) and white matter volumes were detected in patients homozygous for the *L/L* genotype as compared with controls who carried this genotype. On the other hand, no significant differences were observed between patients heterozygous for the *L/S* genotype and controls with this genotype or between those homozygous for the *S/S* genotype and controls who carried that genotype.

There were no significant correlations between hippocampal volumes and illness duration (left hippocampal gray matter:  $r_{29}=-0.17$ ;  $P=.30$ ; right hippocampal gray matter:  $r_{29}=-0.17$ ;  $P=.29$ ; left hippocampal white matter:  $r_{29}=-0.18$ ;  $P=.26$ ; right hippocampal white matter:  $r_{29}=0.06$ ;  $P=.73$ ). There was also no significant correlation between severity of depression as measured by the Hamilton Depression Rating Scale and hippocampal volumes (left hippocampal gray matter:  $r_{29}=-0.17$ ;  $P=.30$ ; right hippocampal gray matter:  $r_{29}=-0.17$ ;  $P=.29$ ; left hippocampal white matter:  $r_{29}=-0.18$ ;  $P=.26$ ; right hippocampal white matter:  $r_{29}=0.06$ ;  $P=.73$ ). Furthermore, including illness duration as a cofactor in the ANCOVA did not change the effect of genotype on hippocampal volumes.

When we excluded from analysis the patient and control subject who appeared to be outliers in the scattergrams, these results did not change. Because 1 study reported brain abnormalities in psychotic depression as compared with nonpsychotic depression,<sup>38</sup> the analysis was also conducted without the 5 patients who had psychotic features, but again the results did not change.

#### COMMENT

Our study investigates the contribution of functional genetics to hippocampal volumes in patients with major de-

**Table 3. Analysis of Covariance for Hippocampal Gray and White Matter\***

Variable	L/L Genotype		L/S Genotype		S/S Genotype	
	F <sub>1,26</sub>	P Value	F <sub>1,30</sub>	P Value	F <sub>1,15</sub>	P Value
Gray matter						
Hemisphere	0.004	.95	1.9	.18	0.21	.65
Diagnosis	9.8†	.004	0.83	.37	0.13	.73
Intracranial content	16.6	<.001	13.3	.001	0.66	.43
Diagnosis by hemisphere	0.45	.51	0.47	.50	0.72	.41
White matter						
Hemisphere	0.31	.58	0.39	.54	0.41	.53
Diagnosis	17.1‡	<.001	0.54	.47	0.30	.59
Intracranial content	9.2	.005	5.5	.03	0.54	.48
Diagnosis by hemisphere	0.26	.61	1.4	.25	0.23	.64

\*Diagnosis was a between-subject factor, hemisphere was a within-subject factor, and intracranial content was a cofactor for each genotype.

†Effect size:  $r = 0.52$ .

‡Effect size:  $r = 0.63$ .

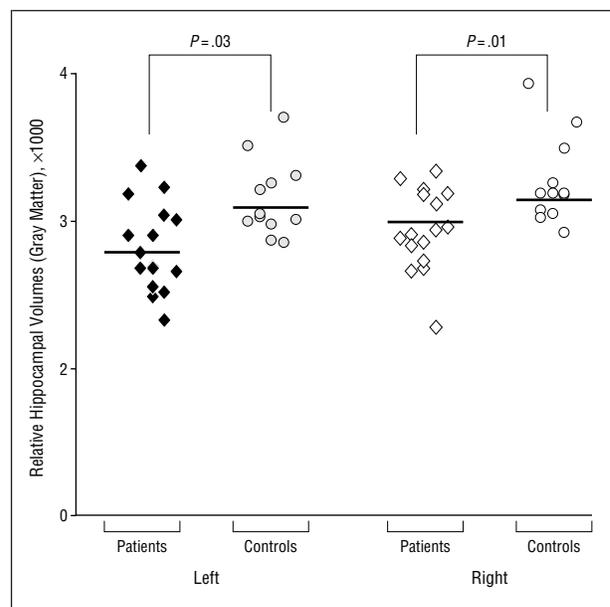
pression and healthy controls. In line with previous results,<sup>20,25-27</sup> hippocampal volumes were found to be significantly smaller in patients as compared with healthy controls. The main new finding of this study was that the L/L genotype was associated with reduced gray and white matter of the hippocampal formation in patients vs healthy controls.

To our knowledge, this is the first study of the relationship between the 5-HTTLPR polymorphism and hippocampal volumes in major depression. Therefore, our findings deserve discussion, particularly with respect to limitations. The study was not designed to test an association of the 5-HTTLPR polymorphism with major depression because the sample size was too small to detect modest contributions. Nevertheless, this result was similar to that in recent investigations with larger samples.<sup>5,6</sup>

Case-control associations are known to be sensitive to population stratification. Patients who have recurrent depression may have a longer duration of exposure to antidepressant medication as compared with those with a first episode. However, because patients and controls with the 5-HTTLPR genotype did not differ with respect to age, sex, illness duration, age at onset, medication use, or national origin, the population stratification in this study was likely not a problem.

Our patients were currently receiving medication. We are aware of no studies on the influence of antidepressants on brain structures in depressed patients, so the medications' effects are unknown. A preliminary investigation in 10 pediatric patients with obsessive-compulsive disorder showed enlarged thalamic volumes before treatment and a decrease in thalamic volumes after 12 weeks of treatment with paroxetine hydrochloride; it is unclear if this effect is due to the medication or to changes in symptoms during treatment.<sup>39</sup> In neural cell lines, tricyclic antidepressants and selective serotonin reuptake inhibitors were found to be neurotoxic.<sup>40</sup> In the future, it would be interesting to study patients with no previous medication use to overcome the bias that hippocampal volumes are influenced by medication.

Because the L allele is more common in the population than the S allele, it could be argued that an equal num-



**Figure 3.** Scattergrams of left and right hippocampal gray matter for subjects with the L/L genotype. Patients and healthy controls did not differ significantly in age, sex, or handedness. The sample size was 16 patients and 13 controls. Bars depict the mean for each group.

ber of individuals for each genetic subtype should have been sampled. However, this would have meant including an extremely large number of patients and controls.

The results of our study indicate that hippocampal volume is genetically determined.<sup>19</sup> Concerning the functional importance of the neurotransmitter serotonin on brain morphologic characteristics, it has been demonstrated that serotonergic signaling is an important regulator of early central nervous system development.<sup>11</sup> Hippocampal volumes were already found to be reduced early in the disease<sup>20</sup>; thus, reduced hippocampal volumes in patients with the L/L genotype might be a risk factor rather than a consequence of major depression.

The effects we saw of genotype on the hippocampus could also be linked to central nervous system development, modulation of certain effects of the ill-

ness, different vulnerability to stress, or different response to antidepressant treatment. If the higher reuptake of serotonin in subjects with the *L/L* genotype modulates the course of the disease, the hippocampal volumes might be affected as a result of depression or stress-related neurotoxic processes. The *L/L* genotype might cause a distinct vulnerability to the stress reaction. A hypothesis is that stress and increased glucocorticoids may contribute to hippocampal volume loss via glutamatergic toxic effects.<sup>29</sup> Stress decreases the expression of brain-derived neurotrophic factor in the hippocampus,<sup>41</sup> which was found to have trophic effects on serotonergic neurons.<sup>42</sup> Brain-derived neurotrophic factor and the serotonergic system may regulate reciprocal functions; the 5-HTT function is modulated by brain-derived neurotrophic factor,<sup>43</sup> which in turn was found to be elevated in the hippocampus and frontal cortex after antidepressant treatment.<sup>42</sup> However, our study did not show a significant correlation between the hippocampus and illness duration in contrast to that by Sheline et al,<sup>19</sup> who investigated elderly patients. Thus, a hippocampal decline during the course of depression must be further evaluated in prospective studies.

Furthermore, the short variant of the 5-HTTLPR polymorphism was associated with a poor response to antidepressant treatment.<sup>44</sup> Therefore, particular patients with the *L/L* genotype may benefit from antidepressant drugs. Adaptation of the cyclic adenosine monophosphate second-messenger pathway, including up-regulation of the cyclic adenosine monophosphate response element binding protein as a result of therapy, increases the expression of brain-derived neurotrophic factor,<sup>45</sup> which has neurotrophic and possibly protective effects on hippocampal neurons.

In summary, this is the first study that investigates genetic contributions of the 5-HTTLPR polymorphism to hippocampal volume reduction in major depression. Patients with the *L/L* genotype may have a higher vulnerability to hippocampal changes. It is unclear whether these changes occur before the beginning of the disease or are triggered from a variety of factors, such as stress or emotional trauma during the depressive episode. We hope that our study may stimulate further research into the influence of functional genetics on brain structure in major depression.

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