Association of GSK3β Polymorphisms With Brain Structural Changes in Major Depressive Disorder

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Context: Indirect evidence suggests that the glycogen synthase kinase-3β (GSK3β) gene might be implicated in major depressive disorder (MDD).

Background: We evaluated 15 GSK3β single-nucleotide polymorphisms (SNPs) to test for associations with regional gray matter (GM) volume differences in patients with recurrent MDD. We then used the defined regions of interest based on significant associations to test for MDD × genotype interactions by including a matched control group without any psychiatric disorder, including MDD.

Design: General linear model with nonstationary cluster-based inference.

Setting: Munich, Germany.

Participants: Patients with recurrent MDD (n = 134) and age-, sex-, and ethnicity-matched healthy controls (n = 143).

Main Outcome Measures: Associations between GSK3β polymorphisms and regional GM volume differences.

Results: Variation in GM volume was associated with GSK3β polymorphisms; the most significant associations were found for rs6438552, a putative functional intronic SNP that showed 3 significant GM clusters in the right and left superior temporal gyri and the right hippocampus (P < .001, P = .02, and P = .02, respectively, corrected for multiple comparisons across the whole brain). Similar results were obtained with rs12630592, an SNP in high linkage disequilibrium. A significant SNP × MDD status interaction was observed for the effect on GM volumes in the right hippocampus and superior temporal gyri (P < .001 and P = .01, corrected, respectively).

Conclusions: The GSK3β gene may have a role in determining regional GM volume differences of the right hippocampus and bilateral superior temporal gyri. The association between genotype and brain structure was specific to the patients with MDD, suggesting that GSK3β genotypes might interact with MDD status. We speculate that this is a consequence of regional neocortical, glial, or neuronal growth or survival. In considering core cognitive features of MDD, the association of GSK3β polymorphisms with structural variation in the temporal lobe and hippocampus is of particular interest in the context of other evidence for structural and functional abnormalities in the hippocampi of patients with MDD.

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**Table 1. Demographic and Clinical Details for Patients With MDD and Healthy Controls for rs6438552**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Controls (n=143)</th>
<th>MDD Cases (n=132)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDD diagnosis, DSM-IV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>worst episode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild depression</td>
<td>6 (5)</td>
<td></td>
</tr>
<tr>
<td>Moderate depression</td>
<td>45 (34)</td>
<td></td>
</tr>
<tr>
<td>Severe without psychotic features</td>
<td>80 (61)</td>
<td></td>
</tr>
<tr>
<td><strong>MDD diagnosis, DSM-IV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>second-worst episode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild depression</td>
<td>13 (10)</td>
<td></td>
</tr>
<tr>
<td>Moderate depression</td>
<td>61 (46)</td>
<td></td>
</tr>
<tr>
<td>Severe without psychotic features</td>
<td>54 (41)</td>
<td></td>
</tr>
<tr>
<td><strong>Patients taking medication in preceding 6 mo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>106 (80)</td>
<td></td>
</tr>
<tr>
<td>SSRIs, NaSSAs, SSNRIs</td>
<td>89 (64)</td>
<td></td>
</tr>
<tr>
<td>TCAs</td>
<td>31 (29)</td>
<td></td>
</tr>
<tr>
<td>BDZ</td>
<td>20 (15)</td>
<td></td>
</tr>
<tr>
<td>Lithium</td>
<td>10 (9)</td>
<td></td>
</tr>
<tr>
<td>Mood stabilizers</td>
<td>9 (8)</td>
<td></td>
</tr>
<tr>
<td>Atypical antipsychotics</td>
<td>7 (7)</td>
<td></td>
</tr>
<tr>
<td>Non-BDZ hypnotics</td>
<td>4 (4)</td>
<td></td>
</tr>
<tr>
<td>Typical antipsychotics</td>
<td>1 (1)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BDZ, benzodiazepine; ellipses, no data collected; MDD, major depressive disorder; NaSSAs, noradrenergic and specific serotonergic antidepressants; SSNRIs, selective serotonin or norepinephrine reuptake inhibitors; TCAs, tricyclic antidepressants; SSRIs, selective serotonin reuptake inhibitors.

n=275. For all remaining single-nucleotide polymorphisms, 108 of 134 (80%) of the patients with MDD were taking at least 1 form of antidepressant medication in the preceding 6 months; the 2 additional patients available for these single-nucleotide polymorphisms were taking SSRIs.15

sures are highly heritable,11 suggests that neuroimaging is a useful endophenotype for genetic association studies of psychiatric disorders.7,12,13

In this study, we tested for associations between regional gray matter (GM) volume estimated from magnetic resonance imaging (MRI) and 15 GSK3β single-nucleotide polymorphisms (SNPs) using a nonstationary cluster-based morphometric analysis in a large sample of well-characterized patients with recurrent MDD and matched controls. We then tested for disease × genotype interactions within the masks derived from significant association clusters in the MDD group and age-, sex-, and ethnicity-matched healthy controls to identify structural variation distinguishing the MDD group.

**METHODS**

**SUBJECTS**

The subjects used for the imaging study were a subset of a larger cohort of 1022 patients with recurrent MDD and 1000 healthy controls recruited for a genetic association study. A detailed description of the recruitment procedure has been previously described.14 Briefly, subjects were recruited and clinically assessed at the Max Planck Institute of Psychiatry, Munich, Germany, except for 2 patients, who were recruited at satellite clinical sites (Bezirkskrankenhaus, Augsburg, and Klinikum, Ingolstadt, Germany). The recruiting hospital obtained approval by the research ethical board to conduct the study, and all individuals gave written informed consent. Patients were diagnosed according to the DSM-IV or International Statistical Classification of Diseases and Related Health Problems (ICD-10), which was established after the administration of the semistructured interview Schedules for Clinical Assessment in Neuropsychiatry. This interview was administered by experienced research assistants who received training at World Health Organization Training and Research Centres. All patients included in the study had a diagnosis of recurrent MDD. Patients with bipolar disorder, mood-incongruent psychotic symptoms, a lifetime history of intravenous drug use, a diagnosis of drug dependency, depression secondary to alcohol, or substance abuse or depression as consequence of medical illnesses or use of medication were not included in the study. Each subject who participated in this study completed a questionnaire regarding their demographics, family, individual, and medical history, and ethnicity. All patients and controls referred to themselves as white with parents of Northern European origin. All control subjects filled in the questionnaires while supervised by a study nurse. The demographic and main clinical characteristics for all subjects included in this study are summarized in Table 1.

High-resolution T1-weighted MRIs were collected at the Max Planck Institute of Psychiatry, for an initial study population of 392 participants (193 patients with MDD and 199 healthy controls). Magnetic resonance images were screened for quality control and analysis at the Clinical Imaging Centre, GlaxoSmithKline. After quality control procedures for both imaging and genetics, 278 subjects were entered in the final voxel-based morphometry (VBM) and genetic analysis (eTable; http://www.archgenpsychiatry.com). Details on subjects excluded for phenotypical-, imaging-, and gene-related criteria are described in the eTable. In brief, subjects were excluded due to (1) detection of gross brain pathology (2 patients with MDD; 3 controls); (2) problems involving data transfer (12 patients with MDD; 5 controls); (3) poor image quality due to head coil instability, excessive motion artifact, or failure to acquire a full image (22 patients with MDD; 3 controls); (4) anatomical deviations (eg, enlarged ventricles) that prevented appropriate cortical segmentation or spatial normalization (7 patients with MDD; 3 controls), poor segmentation in the basal ganglia region or misclassification of dura mater as GM (5 patients with MDD; 2 controls); (5) genotyping exclusions (see below) (eTable).

**GENOTYPING**

All GSK3β SNP genotypes were extracted from genotyping obtained using the HumanHap550 BeadChip platform (Illumina Inc, San Diego, California). The whole-genome scan genotyping, which was available for the full sample of cases and controls collected for genetic studies, followed extensive quality control procedures, as described in detail previously.16 For the individuals available with imaging data, 23 subjects (2 patients with MDD; 21 controls) were excluded because DNA samples were of poor quality and were not sent for genotyping. Of the total DNA samples sent for genotyping, 30 subjects (11 patients with MDD; 19 controls) for SNP rs6438552 and 27 subjects (9 patients with MDD; 18 controls) for all remaining SNPs failed to produce genotyping data owing to low-concentration or poor-quality DNA (eTable). The whole-genome association analysis of the full sample of cases and controls produced a genomic control17 of λ=1.002,18 which suggested the ab-
ence of major population structure and that our cases and
controls were relatively homogenous in terms of genetic structure. There-
fore, any large distortion in our results caused by residual substructure
can be excluded with reasonable confidence.

Two-channel signal intensity data corresponding to the 2
alleles at each SNP were evaluated using the software Beadstudio-
3.1 (Illumina Inc). The initial genotype calls were gener-
ated using the cluster file provide by Illumina. None of the
GSK3β genotypes deviated from Hardy-Weinberg equilib-
rium for patients with MDD and healthy controls (all \( P > 0.13 \)).
The genotypes for the most significant SNP (rs6438552) asso-
ciation showed a cluster separation of 0.3. The raw intensity
data for rs6438552 genotypes from the full sample used in the
whole-genome scan analysis were visually inspected to fur-
ther ensure accuracy (eFigure 1).

**STRUCTURAL BRAIN IMAGING**

**MRI Acquisition**

High-resolution T1-weighted MRIs were acquired on a 1.5-T General
Electric scanner (Signa; later upgraded to Signa Excite; Wauke-
sha, Wisconsin); inversion recovery spoiled gradient echo
recalled with a field of view of \( 22 \times 22 \text{ cm}^2 \), a matrix of 256 \times 256,
124 sagittal slices, and a resulting voxel size of \( (1.2 - 1.4) \times 0.9 \times 0.9 \text{ mm}^3 \) (depending on brain size) (time to repeti-
tion, 10.3 milliseconds; echo time, 3.4 milliseconds; flip angle, 20°).

**Voxel-Based Morphometry Preprocessing**

Simultaneous segmentation and combined linear/nonlinear
intersubject registration and normalization to the Montreal Ne-
urological Institute (MNI) atlas was performed using statistical
parametric mapping 5 (SPM5 [http://www.fil.ion.ucl.ac.uk
/spm, version 573, last updated 25-07-2006]; Wellcome Trust
matter segmented images in atlas space were modulated to gen-
erate maps with volume per voxel of atlas space. Isometric
10-mm Gaussian smoothing was applied.

**Image Quality Control**

All MRI scans were subjected to full radiological reporting su-
ervised by board-certified neuroradiologists to detect relevant
brain pathology. Furthermore, image acquisition artifacts, in-
cluding motion artifact and anatomical factors preventing auto-
mated morphometry, were assessed prior to analysis (eTable). Fol-
lowing this, GM segmented data were assessed for accuracy,
especially in subcortical areas and for dura that was misclassi-
fied as GM. The atlas space GM data were checked to assure good
cortical and ventricular alignment. After a basic model was fit with
no genetic variables, subject outlier detection was performed with
SPMd (http://www.sph.umich.edu/ni-stat/SPMd; Wellcome Trust
Center for Neuroimaging). Data that showed uncorrectable VBM
segmentation-related preprocessing errors in 2 subjects due to en-
larged ventricles and severe cortical atrophy were excluded
(eTable). For the final subjects included in all analyses, there
were no significant differences in total GM volume between healthy
controls (mean [SD], 0.61 [0.07] L) and patients with MDD (mean
[SD], 0.61 [0.07] L).

**Mass Univariate Modeling**

A separate SPM analysis was performed for each SNP. A general
linear model was performed at each voxel and included as a group
effect and a group-specific SNP effect as well as nuisance effects
of age, sex, total GM volume (used to discount global variation
in GM affected by head size), medication, and timing with re-
spect to scanner upgrade. The group effect had 3 levels for con-
trols and for patients with MDD with and without comorbid anxi-
ety. This subdivision of MDD was used because evidence for GM
volume differences involving comorbid anxiety was found in a
previous study (Becky Inkster, DPhil, et al, unpublished data, No-
vember 2008). However, when testing for interactions, we aver-
aged the 2 MDD subgroups, as there was no interest in the anxi-
ety effect specifically. The SNP effect was split by the 3 groups,
fitting SNP × group interactions. Unlike the approach of most ge-
netic studies that fit an additive effect of allele dose, in our study
for SNPs with more than 10% of subjects with the rarest geno-
type (ie, minor allele frequency >0.10=0.3162), we used a ge-
notypic model parameterized with orthogonal polynomials, pro-
viding inference on an additive effect while providing a full 2-df\(^{18}\)
fit. For example, if there were equal numbers of subjects with the
2 homozygote genotypes, the additive predictor would have values
−1, 0, 1 for the 3 genotypes and the nonadditive predictor
would have values −1/2, 1, −1/2. More generally, the 2 predic-
tors begin coded as above (−1|0|−1, −1/2|1|1/2), but the additive
predictor is centered and the nonadditive predictor is centered
and orthogonalized with respect to the additive predictor. For SNPs
with a minor allele frequency less than 0.3162, a recessive model
was used, merging the rare homozygous and heterozygous groups.

**Cluster-Based Inference**

Standard cluster-based methods13,14 assume stationary noise (con-
stant smoothness throughout the image), and VBM data has been
observed to have variable smoothness.20,21 While most studies avoid
using cluster-based inference owing to problems with VBM’s vari-
able smoothness, we used a novel approach of nonstationary cluster-
size inference that allows for valid inference on clusters while ac-
counting for heterogeneous smoothness (ie, as spatially extended
effects were expected, cluster-based inference was used to obtain
optimal sensitivity22). We therefore used nonstationary cluster-size
inference\(^23\) with a cluster-defining threshold of \( \alpha = 0.01 \). We report
familywise error (FWE)–corrected cluster \( P \) values, which control
for the chance of 1 or more false positives. \( P \) values corrected for
searching over image space are denoted \( P_{\text{FWE}} \), and the minimum
nonstationary cluster \( P \) value corrected for searching over imaging
space was used as a summary \( F \) statistic, reflecting the overall strength
of association. Anatomical locations of clusters were established
using the MRCro AAL (Anatomical Automatic Labeling) template
(http://www.sph.sc.edu/comd/orden/micro.html).\(^2\) Cluster loca-
tions are defined in MNI standard space (as defined by the MNI-
space image shipped with SPM).

**Inference on GM Association Modulated by Group**

Major depressive disorder \( \times \) rs6438552 genotype interactions
(where the group is either control or MDD) were conducted using a
1-df \( F \) test for any direction of SNP association between groups.
First, interaction testing was restricted to the region of interest\(^24\)
mask defined by the \( F \)-test contrast of rs6438552 genotype effect
on GM volume differences in patients with MDD only. Because
cluster inference can have reduced sensitivity in small search re-
regions, voxelwise inference (FWE-corrected) was used within the
3 region of interest (ROI) masks.

**Inference Over Multiple GSK3β SNPs**

Each SNP was calculated in a separate model and Bonferroni
correction was applied for testing multiple GSK3β SNPs. The
Bonferroni correction is valid for any set of tests and, though
it is known to be very conservative when there are many tests

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or severe dependence; with only 15 SNPs it should operate satisfactorily. The SPM-corrected cluster $P$ values ($P_{\text{FWE(S)}}$) were then corrected for multiple SNP testing and $P$ values are denoted as $P_{\text{FWE(S,S)}}$. The SNPs tested in this study that were not independent of other SNPs (ie, in high or complete linkage disequilibrium) were still included for analysis because a replication would confirm that no experimental genotyping errors or analysis errors occurred (the extent of linkage disequilibrium between the SNPs is shown in eFigure 2A).

## RESULTS

### ASSOCIATION OF GSK3β SNP rs6438552 WITH REGIONAL GM VOLUME DIFFERENCES IN PATIENTS WITH MDD

We tested 15 SNPs at the GSK3β locus for association with GM variation using VBM in our sample of patients with MDD. Nonstationary cluster-based morphometric analysis revealed significant GM variation associations with 2 SNPs, rs6438552 and rs12630592, that are highly correlated ($r^2=0.951$, which is similar to the 0.950 observed for white individuals’ reference data from HapMap data). The most significant GM volume associations were observed for rs6438552. After correcting for multiple comparisons across the whole brain, 3 statistically significant clusters of GM volume differences were identified in the right and left superior temporal gyri (STG) and the right hippocampus ($P_{\text{FWE(S)}} < .001, P = .02$, and $P = .02$, respectively) (Table 2, Figure 1A). The A allele was associated with lower GM volume in all 3 clusters (Figure 1B). Relative decreases in the GM volume of 7% to 11% were found for AA compared with GG homozygotes in the cluster ROIs (Table 3). The SNP rs12630592 showed colocalized GM association clusters (Figure 2A) with similar load-dependent decreases in GM volume for the G allele, with an effect magnitude similar to rs6438552 (Figure 2B). The results for all SNP associations are summarized in eFigure 2B. Post hoc analyses were performed to examine rs6438552 genotype differences with respect to episode severity, age, or sex between genotype groups, but no significant results were revealed ($P = .32$, $P = .99$, and $P = .34$, respectively, data not shown). We also performed a post hoc analysis with all 15 SNPs included as covariates into 1 model and gained no significant result (eFigure 3).

After correcting for whole-brain search and for the number of SNPs tested, the cluster in the right STG remained significant for rs6438552 ($P_{\text{FWE(S)}} < .001$) and a trend was found for rs12630592 ($P = .06$) (Table 2).

### DIFFERENCE BETWEEN rs6438552 SNP-ASSOCIATED STRUCTURAL VARIATION IN PATIENTS WITH MDD AND CONTROLS

To evaluate whether these associations are specific to MDD or brain structural variation more generally, we tested for interaction between GM volumes within masks derived from the 3 significant clusters for rs6438552 SNP across patients with MDD and matched healthy controls. Significant interactions were revealed within the right hippocampus ROI mask (Table 4); the mean (SD) change in modulated GM (at voxel 30, −26, −12) was 2.3%...
(0.037%) lower in MDD AA homozygotes relative to healthy controls’ AA homozygotes (Figure 3A). When searching within the right STG ROI mask, significant interactions were also revealed (Table 4); the mean (SD) change in modulated GM (at voxel 48, −16, 0) was 5.5% (0.038%) lower in MDD AA homozygotes relative to healthy control AA homozygotes (Figure 3B). No significant interactions were revealed when searching within the left STG cluster. The interaction effects in the right hippocampus and STG remained significant after correcting for searching across multiple masks (Table 4). There were no significant genotype effects when restricting the ROI analysis to controls only.

Here we have shown that variation in GM volume in the right hippocampus and bilateral STG is associated with a common intronic polymorphism (rs6438552) at the GSK3β locus in patients with MDD. Previous in vitro data has demonstrated that this intronic polymorphism regulates the selection of splice acceptor sites and thus alters GSK3β transcription. The polymorphism might therefore define functional differences in GSK3β expression. An additional similar association between genotype and local GM volume was also detected for rs12630592, an SNP in high linkage disequilibrium with rs6438552. The regional specificity of our findings, particularly in the right hippocampus, is notable when considering

**COMMENT**

Here we have shown that variation in GM volume in the right hippocampus and bilateral STG is associated with a common intronic polymorphism (rs6438552) at the GSK3β locus in patients with MDD. Previous in vitro data has demonstrated that this intronic polymorphism regulates the selection of splice acceptor sites and thus alters GSK3β transcription. The polymorphism might therefore define functional differences in GSK3β expression. An additional similar association between genotype and local GM volume was also detected for rs12630592, an SNP in high linkage disequilibrium with rs6438552. The regional specificity of our findings, particularly in the right hippocampus, is notable when considering
meta-analytical evidence from MRI studies for a role of hippocampal integrity in depression.20 In addition, GSK3β is highly expressed in the hippocampus and temporal cortex in the human and mouse brain (Allen Mouse Brain Atlas: http://www.brain-map.org). Furthermore, STG abnormalities have been described in subjects with bipolar disorder who are not taking medication relative to healthy controls,27 and our group has reported higher STG volumes in a subset of the same group of patients with MDD with comorbid anxiety.

There is growing evidence to suggest that inhibition of GSK3β activity might play a role in the therapeutic effects of antidepressants and lithium in patients with refractory MDD.28 Lithium inhibits the enzyme encoded by GSK3β and has been shown to regulate GSK3β expression in human peripheral blood mononuclear cells.29 The activity of GSK3β is inhibited by the antidepressants fluoxetine and imipramine in mice.3 Pharmacogenetic support from a recent study showed that several common GSK3β polymorphisms were associated with selective serotonin reuptake inhibitor therapeutic effects in a large sample of patients with MDD.30 Furthermore, a postmortem study that examined GSK3β activity in the ventral prefrontal cortical tissue of patients with MDD compared with matched controls found increased GSK3β activity in patients with MDD.31 The potential role that GSK3β plays in bipolar disorder has also been examined extensively. Associations have been described between a functional GSK3β polymorphism, −50 T/C (rs334558) and age of onset,32,33 therapeutic response to lithium salts,34 response to lithium augmentation treatment,35 and therapeutic response to total sleep deprivation.36 In a combined sample of patients with bipolar disorder and patients with MDD, an association between the −50 T/C promoter SNP and delusional symptoms and personality traits related to delusions was observed.36 There are, however, some studies that have failed to replicate associations between GSK3β and age of onset36 as well as response to lithium.37 No evidence of association with lithium resistance was revealed for another GSK3β promoter polymorphism that was examined.38 A recent study reported that a copy number variation disrupts several exons of GSK3β, and could therefore have adverse effects on GSK3β expression, was more frequent in bipolar patients.39

Table 4. Interaction Effects When Searching Within the 3 Significant Clusters Based on the Significant Effect of rs6438552 on GM Volume Differences in the MDD Group

<table>
<thead>
<tr>
<th>ROI Mask</th>
<th>F</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>P* (Corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right hippocampus</td>
<td>21.28</td>
<td>30</td>
<td>−26</td>
<td>−12</td>
<td>.001 (&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>17.54</td>
<td>28</td>
<td>−32</td>
<td>−6</td>
<td>.001 (.003)</td>
</tr>
<tr>
<td>Right STG</td>
<td>14.86</td>
<td>48</td>
<td>−16</td>
<td>0</td>
<td>.01 (.04)</td>
</tr>
<tr>
<td></td>
<td>12.96</td>
<td>46</td>
<td>−30</td>
<td>22</td>
<td>.03 (.09)</td>
</tr>
<tr>
<td></td>
<td>12.78</td>
<td>58</td>
<td>−30</td>
<td>2</td>
<td>.03 (.10)</td>
</tr>
</tbody>
</table>

Abbreviations: FWE, familywise error; GM, gray matter; MDD, major depressive disorder; ROI, region of interest; STG, superior temporal gyrus.

Genotyping for the −50 T/C promoter SNP as well as other SNPs previously described in the literature (except rs6438552) were not available for our study. The functional promoter polymorphism (−50 T/C) has not been genotyped in Centre d’Etudes du Polymorphisme Humain (CEPH) families (HapMap), so it was not possible to establish the linkage disequilibrium between this SNP and the SNPs analyzed in our study.

The MDD-specific effect found in our study suggests that MDD status, disease-associated genetic risk factors, or both might be interacting with GSK3β in determining the regional GM differences observed in this study. It remains unclear whether GSK3β genotype-dependent differences in brain morphology develop throughout the course of MDD or if these differences exist premorbidly. Major depressive disorder status is often associated with an hypothalamic-pituitary-adrenal axis dysfunction, which produce consequential effects on growth and resilience of specific neuronal and glial populations.30 Furthermore, environmental factors such as life events that have been suggested to have an effect in increasing the risk for MDD might be, in part, responsible for the hippocampal morphological differences observed in patients with MDD.30 Alternatively, or in conjunction, MDD susceptibility genes may interact with GSK3β to determine the structural associations we have observed in our patients with MDD. Although speculative, the GSK3β associations found in this study may re-
late to previously described genetic associations with hippocampal volume changes in patients with MDD. For example, the association of the 5-HTTLPR polymorphism with hippocampal volume in patients with MDD could be related to the role of GSK3β activity in modulation of serotonergic signaling.

While our results are novel, the VBM methodology used for the between-groups contrast is well established. Identification of significant associations between brain structural variation and single gene polymorphisms is also well preceded. Some brain regions might be more strongly genetically determined than others. For example, there is higher heritability for middle and superior frontal, sensorimotor, paralimbic and, consistent with our results, temporal structures. In principle, therefore, differences in the heritability of different cortical regions might limit the potential for this kind of imaging genetic association study to genes whose variation determines specific brain regions.

There are some additional limitations of our study. The association demonstrated here is robust statistically, but we also have shown considerable variation in the magnitude of the effect across the population of patients with MDD that we studied. This heterogeneity implies that large samples might be required to replicate our findings. In addition, as the size of the effect we have observed is likely to be overestimating the real contribution of GSK3β to brain morphological differences in the overall population of patients with MDD, significantly larger samples than the one used in our study might be required to replicate this finding. We previously reported no significant GM differences in the hippocampus or temporal cortex between MDD cases and controls (unpublished data, November 2008) when genetic factors were not included in the analysis, yet this result was based on a larger cohort than the subset for the GSK3β SNP-GM association analysis. Furthermore, it remains possible that the lack of an effect in the healthy control population, and thus the specificity of the association for MDD, reflects limitations of study power rather than lack of true association. Owing to potential disease heterogeneity and reverse to the mean, replication may demand a substantially larger sample size. Finally, VBM-based GM volume differences cannot be related specifically to neuroanatomical features, as differences can arise from relative changes either in cortical folding or thickness. Cluster detection, in which significance is determined by the spatial contiguity of suprathreshold voxels, is biased toward larger local regions of change. The study might not be adequately powered to detect additional smaller regions. Additional imaging techniques (eg, functional MRI) will provide further tests for the observed associations. Furthermore, these associations need to be examined in other relevant psychiatric diseases such as schizophrenia and bipolar disorder. Additionally, there is an opportunity to test additional candidate genes, but this is beyond the scope of the current investigation.

Our results suggest that in MDD, a proportion of bilateral STG and right hippocampal structural variation can be explained by genetic variation at the GSK3β locus in ways distinguishable from healthy controls. Based on our results, however, it is not possible to differentiate whether this GSK3β polymorphism contributes to the risk of developing MDD or occurs as a consequence of major depression. They are equally consistent with a genetic influence on a predisposing brain structural trait, a higher independent risk of developing brain changes in the course of the disease, or development of these changes in interaction with other genetic or environmental risk factors. In support of the growing evidence suggesting a role for GSK3β in MDD, our study is the first to suggest directly that GSK3β has a role in determining any aspect of brain structure or function related to MDD.

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