Serotonin Transporter Gene, Depressive Symptoms, and Interleukin-6

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Background—We explored the relationship of genetic variants of the serotonin transporter gene SLC6A4, a key regulator of the serotonergic neurotransmission, with both depressive symptoms and plasma interleukin-6 (IL-6) levels.

Methods and Results—We genotyped 20 polymorphisms in 360 male twins (mean age, 54 years) from the Vietnam Era Twin Registry. Current depressive symptoms were measured with the Beck Depression Inventory II. IL-6 was assessed using a commercially available ELISA kit. Genotype associations were analyzed using generalized estimating equations. To study how SLC6A4 genetic vulnerability influences the relationship between depressive symptoms and IL-6, bivariate models were constructed using structural equation modeling. Of the 20 polymorphisms examined, the effective number of independent tests was 6, and the threshold of significance after Bonferroni correction was 0.008. There were 6 single-nucleotide polymorphisms significantly associated with Beck Depression Inventory (P≤0.008), including rs8071667, rs2020936, rs25528, rs6354, rs11080122, and rs8076005, and 1 single-nucleotide polymorphism was borderline associated (rs12150214, P=0.017). Of these 7 single-nucleotide polymorphisms, 3 were also significantly associated with IL-6 (P<0.008), including rs25528, rs6354, and rs8076005, and the other 4 were borderline associated (P=0.009 to 0.025). The subjects with 1 copy of the minor allele of these 7 single-nucleotide polymorphisms had higher Beck Depression Inventory scores and IL-6 levels. Further bivariate modeling revealed that ≈10% of the correlation between Beck Depression Inventory and IL-6 could be explained by the SLC6A4 gene.

Conclusions—Genetic vulnerability involving the SLC6A4 gene is significantly associated with both increased depressive symptoms and elevated IL-6 plasma levels. Common pathophysiological processes may link depression and inflammation, and implicate the serotonin pathway in neural-immune interactions. (Circ Cardiovasc Genet. 2009;2:614-620.)

Key Words: atherosclerosis ■ epidemiology ■ genetics ■ inflammation ■ depression

The relationship between depression and the incidence of coronary artery disease (CAD) is well established.1 Adverse lifestyle behaviors, lower heart rate variability, and enhanced platelet activation have long been considered potential explanations for this association.2 Of more recent interest is the role of inflammation in the development and progression of atherosclerosis3 and its potential association with depression.4 Major depression and depressive symptoms are associated with higher levels of inflammatory biomarkers such as interleukin-6 (IL-6).5 However, the causal direction of this association remains unclear and may be bidirectional, such that neurobiological correlates of depression, or physical illness, may result in enhanced inflammation, and the latter, in turn, may increase the risk of depression.6

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It is also plausible that a common genetic vulnerability accounts for the observed association between depression and inflammation, because both phenotypes are heritable.7,8 We have recently reported evidence for a common genetic pathway linking depressive symptoms and IL-6 using twins; specifically, we found that more than two thirds of the covariance between these 2 phenotypes could be explained by the genes they have in common.8 Potential candidates for this shared genetic substrate are genetic variants involved in neurobiological pathways of the stress response, which are associated with both depression and inflammation. Among these, the serotoninergic pathway is of particular interest.
Serotonin (5-HT) is released by human mast cells and platelets after antigen challenge. This neurotransmitter is well known for its role in depression, but there is also evidence that serotonin modulates immune responses and proinflammatory cytokine synthesis. The serotonin transporter (5-HTT) is a key protein of the serotonergic system, which regulates serotonin concentration in the synaptic cleft and extrasynaptic sites. The human serotonin transporter gene (SLC6A4) is mapped to chromosome 17q11.1 to 17q12, and several polymorphisms within the SLC6A4 gene have been described. One of the most frequently studied functional polymorphisms of the SLC6A4 gene is the serotonergic transporter-linked polymorphic region (5-HTTLPR) located in the promoter region, which is a 44-bp insertion or deletion polymorphism producing 2 common alleles, long (L) and short (S) alleles. The homozygous or heterozygous carriage of the S allele is associated with lower transcription of 5-HTT and therefore with increased serotonin binding and re-uptake. Some studies have found an association between 5-HTTLPR and depression, whereas others have not. Only a few studies investigated the relationship of other polymorphisms within the SLC6A4 gene and depression. Furthermore, no study has investigated the association of variants of the SLC6A4 gene with plasma IL-6 levels.

In this study, we investigated the association between polymorphisms of the SLC6A4 gene on depressive symptoms and IL-6 levels in a well-characterized sample of middle-aged male twins. The study of twins allowed us to quantify the role of the specific genotype of interest in explaining shared genetic effects using bivariate structural equation models (SEM).

Methods

Study Populations

The Twins Heart Study (THS) is an investigation of psychological, behavioral, and biological risk factors for subclinical cardiovascular disease using twins. Twins were selected from the Vietnam Era Twin Registry, which includes 7369 middle-aged male-male twin pairs both of whom served in the US military during the time of the Vietnam war.

The THS included 180 twin pairs (102 monozygotic and 78 dizygotic) who were born between 1946 and 1956. The methods of construction of this sample were described in the online-only Data Supplement and were also described previously. Twin pairs were examined together at the Emory University General Clinical Research Center between March 2002 and March 2006 and maintained an identical schedule during examination. All twins had a comprehensive physical examination and were queried about previous diagnoses of cardiovascular diseases. We also administered the Structured Clinical Interview for DSM-IV-SC to classify subjects based on a lifetime history of major depressive disorder (MDD). The final data included 105 twin pairs where neither twin had a history of MDD, 68 twin pairs discordant for history of MDD, and 7 twin pairs where both had a history of MDD. Compared with the normal twin pairs, the discordant twin pairs and the concordant-depressed twin pairs were younger, had higher prevalence of diabetes, more depressive symptoms, and more likely to use antidepressants. For other study variables, there were no significant differences among these twin pairs. Because few twins (n=8) had a current major depressive episode, we focused on current levels of depressive symptoms measured with the continuous Beck Depression Inventory II (BDI-II) score. Therefore, we used all the twins in the analysis rather than study the twins based on discordant and concordant for history of MDD or current major depressive episode. This protocol was approved by the Institutional Review Board at Emory University, and informed consent was obtained from all subjects.

Assessment of Depression

Lifetime history of MDD was assessed by means of the Structured Clinical Interview for DSM-IV, which yields a clinical diagnosis of major depression based on a lifetime history of major depressive episodes. We also collected information on current level of depressive symptoms using the BDI-II, a standardized scale providing a continuous measure of depressive symptoms. It has been used extensively in community samples and has satisfactory test-retest and internal consistency reliabilities.

Assessment of IL-6

Plasma level of IL-6 was assessed using commercially available high-sensitivity ELISA kits obtained from R&D Systems (Minneapolis, Minn). All samples were run in duplicate. Inter- and intraassay variability were reliably <10%. The values of IL-6 were log transformed to improve distribution. We were also able to evaluate the multiplicative changes due to genetic variants, ie, percent change of IL-6 for twins with the risk allele compared with those without the risk allele.

Other Measurements

We measured weight, height, blood pressure, high- and low-density lipoprotein (HDL and LDL) cholesterol, and fasting glucose, as reported previously. Physical activity was assessed with a modified version of the Baecke Questionnaire of habitual physical activity; this is a 16-question instrument documenting level of physical activity at work, during sports, and nonsports activities. The global physical activity score was used in the analysis. Cigarette smoking was classified into current versus never or past smoker. A history of coronary heart disease occurring since 1990 was defined as a previous diagnosis of myocardial infarction or angina pectoris, or previous coronary revascularization procedures. Diabetes mellitus was defined as having a fasting glucose level >126 mg/dL or being treated with antidiabetic medications. Framingham risk score, a commonly used summary index of CAD risk, was also calculated to incorporate information on presence and severity of the following risk factors: age, LDL cholesterol, HDL cholesterol, blood pressure, diabetes mellitus, and current smoking. Information on current use of antidepressants was also collected.

Genotyping the SLC6A4 Polymorphisms and Multiple Testing

5-HTTLPR Polymorphism Genotyping

Genotyping of the 5-HTTLPR length polymorphism was carried out using polymerase chain reaction (PCR). PCR was performed in 384-well plates in a 10-µL volume with 10 ng of genomic DNA. The PCR products were mixed with a ROX 500 sizing ladder (Applied Biosystems, Foster City, Calif) then separated using an Applied Biosystems 3100 genetic analyzer and analyzed with Applied Biosystems GenMapper 4.0 software. Fragment lengths for the L allele and S allele were 291 and 247 bp, respectively. The primers and PCR reaction conditions can be obtained by request.

Single-Nucleotide Polymorphisms Genotyping

We used the HapMap Caucasian panel (CEPH, release 24, build 36) to select SLC6A4 single-nucleotide polymorphisms (SNPs). Twenty-three SNPs with minor allele frequency (MAF) >0.05 were selected. An additional SNP (rs6355) with MAF of 0.03 was also selected because it was located in the codon region. A total of 24 SNPs were genotyped in our sample using the Beckman GenomeLab SNPstream Genotyping System (Beckman Coulter, Inc, Fullerton, Calif), whereas 5 SNPs failed to perform well in genotyping assays and were therefore discarded from further analysis. However, these 5 SNPs were in strong linkage disequilibrium (LD) with other 19 SNPs (pairwise r² range from 0.85 to 1). Genotyping accuracy for all 19 SNPs was 99% as assessed by inclusion of duplicates (pairs of
monzygotic twins) in the arrays, and negative controls (water blanks) were included on each plate.

**Multiple Testing**

We used the method developed by Gao et al.\(^2^8\) for multiple testing correction because there were 20 polymorphisms being studied. Briefly, this method uses composite LD among SNPs to capture the correlation and derives the effective number of independent tests using a principal component approach. Then the Bonferroni correction can be implemented based on the number of independent tests. The method of Gao et al.\(^2^8\) is accurate and efficient and is comparable with the permutation test. By using this method, we performed 6 independent tests from 20 correlated polymorphisms. To achieve a nominal significance of 0.05, the threshold after Bonferroni correction was 0.05/6 = 0.008.

**Statistical Analyses**

The main purpose of our analyses was to test the associations between each single polymorphism and haplotype variations in the *SLC6A4* gene with depressive symptoms and IL-6 plasma levels. We further investigated to what extent the covariance between depressive symptoms and IL-6 could be explained by variations in the *SLC6A4* gene.

We used Haplovie 4.0 software for computing Hardy-Weinberg equilibrium, MAF, and pair-wise LD between the studied polymorphisms, where 1 twin was randomly selected from each pair for the calculation.\(^2^9\) Association analyses were performed using generalized estimating equations (GEE), which corrects for the relatedness between twins. The exchangeable correlation structure was used for the GEE. Analyses were first performed separately for each of the studied polymorphisms and followed up by haplotype analysis. For individual polymorphism analysis, we tested the additive model of polymorphisms on depressive symptoms and IL-6 levels, respectively, where the polymorphisms were coded as 0, 1, or 2 depending on the carrier status of the minor allele. In the presence of a significant association, we further adjusted for a priori specified covariates, including body mass index, physical activity, history of coronary heart disease, use of antidepressants, and Framingham risk score. Using GEE, we also examined the association between *SLC6A4* polymorphisms and MDD as a binary outcome variable.

The haplotype trend regression approach was adapted to test for associations of statistically inferred haplotypes with depressive symptoms and IL-6, respectively, as outlined by Zaykin et al.\(^3^0\), where linear regression was replaced with the GEE procedure to take into account the relatedness between twins. Briefly, haplotype trend regression is based on the regression of a trait on a design matrix that includes the expected proportions of haplotypes. The contributions of haplotypes are weighted with the design matrix such that unambiguous pairs of haplotypes are coded 1 for the haplotypes of homozygotes, 0.5 for each of the haplotypes of a heterozygote, and 0 for all other haplotypes. However, the contributions of ambiguous pairs of haplotypes in the design matrix are based on the probabilities of haplotype pairs (divided by 2) as estimated by PHASE 2.0.\(^3^1\) The most frequent haplotype was used as the reference with which the other haplotypes were contrasted. Haplotypes with estimated frequencies \(< 5\%\) were pooled together. The analyses were repeated after adjusting for the CAD risk factors and use of antidepressants.

We further constructed bivariate SEM, which models and takes account of residual twin covariance, to study the interplay of depressive symptoms and IL-6 with the *SLC5A4* genetic vulnerability. Details of the approach for incorporating the measured genotypes in SEM have been described previously.\(^2^2\) Compared with univariate analysis, bivariate modeling has the advantage of allowing genetic effects on the means, variances, and relations between depressive symptoms and IL-6. The models were fitted with Mx software.\(^3^2\)

Most of the twins from the THS are whites (94%). Further adjustment for race did not change the results. To avoid the potential influences of race/ethnicity, we repeated all the analyses after exclusion of blacks. The results were virtually identical and are not reported.

### Results

Of the 360 THS twins, 82 twins had a lifetime history of MDD. The mean duration of major depressive episodes was 8.3 months. However, the majority of twins with MDD were in remission, with only 8 subjects meeting DSM-IV criteria for a current major depressive episode and 77% having had the last depressive episode \(> 1\) year before examination. Of the 82 MDD twins, 27 (33%) were on antidepressants. There was no association between *SLC6A4* polymorphisms and lifetime history of MDD. Further adjustment for the covariates did not change the results (data not shown). Table 1 shows the demographic characteristics and CAD risk factors of the THS twins. The mean age (±SD) was 54 years (±2.89), with a range of 47 to 60 years. Twenty percent of the participants were current smokers and \(\sim 10\%\) had a history of coronary heart disease. Fifty-two subjects (14.4%) were using antidepressants.

A total of 20 polymorphisms in the *SLC6A4* gene were genotyped, including an ins/del polymorphism located in the promoter region (5-HTTLPR), a missense mutation in the codon region (rs6355), 2 SNPs in untranslated regions (UTR, rs6354 and rs1042173), and 16 SNPs in introns. The location of these polymorphisms is shown in supplemental Figure I. Mutation types on all polymorphisms and MAF are presented in Table 2, along with the Hardy-Weinberg equilibrium tests. Sixteen polymorphisms were common with MAF \(> 10\%\), 2 SNPs had MAF \(< 5\%\) but \(> 5\%\), and 2 SNPs had MAF \(< 5\%\). No significant deviation from Hardy-Weinberg equilibrium was found for any polymorphism after the multiple testing corrections.

Pairwise LD coefficients of the 20 polymorphisms are displayed in supplemental Figure I. There was strong LD among rs8071667, rs12150214, rs2020936, rs25528, rs6354, rs11080122, and rs8076005 (pairwise \(r^2\) of 0.72 to 0.95). Two pairs of SNPs, rs2066713 and rs2020942 (\(r^2 = 0.96\)) as well as rs4325622 and rs1042173 (\(r^2 = 0.98\), showed almost perfect

### Table 1. Characteristics in the Twins Heart Study Subjects

| N  | Age, yr | Systolic blood pressure (mm Hg) | Diastolic blood pressure (mm Hg) | LDL cholesterol (mg/dL) | HDL cholesterol (mg/dL) | Diabetes | Body mass index | Current smoker | Framingham risk score | Physical activity (Baecke) score | Prior coronary heart disease | Posttraumatic stress disorder | Current antidepressant use | IL-6 (mg/dL) | Depressive symptoms (BDI-H) score |
|----|---------|---------------------------------|---------------------------------|-------------------------|-------------------------|-----------|----------------|--------------|---------------------|--------------------------|-----------------------------|------------------------|-----------------------------|--------------------------|--------------|-------------------------------|
| 360| 54.4 ± 2.89 | 129.4 ± 15.8                  | 80.7 ± 10.7                    | 122.6 ± 33.8            | 38.6 ± 9.69             | 33 (9.19) | 29.2 ± 4.86 | 70 (20.0)   | 5.26 ± 2.07        | 7.43 ± 1.59             | 35 (9.72)                  | 23 (6.4)              | 52 (14.4)                   | 2.86 ± 6.33 | 4.94 ± 6.62                     |

Data are expressed as mean ± SD or n (%).
LD. Strong LD was also found among rs2020939, rs140701, and rs2054847 ($r^2 = 0.85$ to 0.9). The association of each SLC6A4 polymorphism with depressive symptoms and IL-6 is shown in Table 2. No significant association was observed between 5-HTTLPR and depressive symptoms. However, 6 SNPs in other locations were significantly associated with BDI-II scores ($P < 0.008$), including rs8071667, rs2054847, rs25528, rs6354, rs11080122, and rs8076005, and 1 SNP was borderline associated (rs12150214, $P = 0.017$). Of these 7 SNPs, 3 were also significantly associated with IL-6 ($P < 0.008$), including rs25528, rs6354, and rs8076005, and the other 4 were borderline associated ($P = 0.009$ to 0.025). For these 7 SNPs, the minor allele was associated with higher BDI-II scores and IL-6 levels. Adjustment for covariates did not change the results.

The haplotype analysis using these 7 SNPs reflected the single SNP analysis because they were highly correlated. Approximately 94% of the genetic variability of the haplotypes consisting of these 7 SNPs was explained by 2 common haplotypes, including G-G-T-A-A-C-A (Hap1) and A-C-C-C-C-T-G (Hap2) with frequencies of 81% and 13%, respectively (supplemental Table I). Haplotype-based association tests are shown in Table 3. The $\beta$ coefficient represents the effect size for haplotype homozygote. Compared with the subjects homzygous for Hap1, those homzygous for Hap2 had, on average, a 98% higher BDI-II score ($P = 0.006$) and a

### Table 2. Summary Data on 20 Polymorphisms in the Serotonin Transporter Gene and Their Associations With Depressive Symptoms and IL-6 Levels

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Alleles*</th>
<th>MAF</th>
<th>HWT†</th>
<th>$\beta$‡</th>
<th>SE</th>
<th>$P$§</th>
<th>$\beta$¶</th>
<th>SE</th>
<th>$P$‡</th>
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<tr>
<td>5-HTTLPR</td>
<td>L:S</td>
<td>0.431</td>
<td>0.08</td>
<td>-0.14</td>
<td>0.50</td>
<td>0.78</td>
<td>-0.02</td>
<td>0.06</td>
<td>0.80</td>
</tr>
<tr>
<td>rs16965628</td>
<td>G:C</td>
<td>0.065</td>
<td>0.28</td>
<td>0.76</td>
<td>0.99</td>
<td>0.44</td>
<td>0.18</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>rs8071667</td>
<td>G:A</td>
<td>0.135</td>
<td>1.00</td>
<td>2.36</td>
<td>0.75</td>
<td>0.002</td>
<td>0.22</td>
<td>0.09</td>
<td>0.016</td>
</tr>
<tr>
<td>rs4251417</td>
<td>G:A</td>
<td>0.124</td>
<td>0.84</td>
<td>-0.10</td>
<td>0.79</td>
<td>0.90</td>
<td>0.03</td>
<td>0.09</td>
<td>0.79</td>
</tr>
<tr>
<td>rs2066713</td>
<td>C:T</td>
<td>0.350</td>
<td>0.89</td>
<td>-0.58</td>
<td>0.55</td>
<td>0.29</td>
<td>-0.10</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>rs12150214</td>
<td>G:C</td>
<td>0.172</td>
<td>0.95</td>
<td>1.59</td>
<td>0.66</td>
<td>0.017</td>
<td>0.18</td>
<td>0.08</td>
<td>0.025</td>
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<tr>
<td>rs2020936</td>
<td>T:C</td>
<td>0.153</td>
<td>0.38</td>
<td>2.00</td>
<td>0.69</td>
<td>0.004</td>
<td>0.22</td>
<td>0.08</td>
<td>0.009</td>
</tr>
<tr>
<td>rs2020939</td>
<td>C:T</td>
<td>0.484</td>
<td>0.16</td>
<td>-0.63</td>
<td>0.50</td>
<td>0.20</td>
<td>-0.04</td>
<td>0.06</td>
<td>0.46</td>
</tr>
<tr>
<td>rs25528</td>
<td>A:C</td>
<td>0.146</td>
<td>0.24</td>
<td>2.06</td>
<td>0.69</td>
<td>0.003</td>
<td>0.23</td>
<td>0.08</td>
<td>0.005</td>
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<tr>
<td>rs6354</td>
<td>A:C</td>
<td>0.143</td>
<td>0.97</td>
<td>2.07</td>
<td>0.73</td>
<td>0.005</td>
<td>0.24</td>
<td>0.09</td>
<td>0.006</td>
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<tr>
<td>rs6355</td>
<td>G:C</td>
<td>0.026</td>
<td>1.00</td>
<td>-0.92</td>
<td>1.61</td>
<td>0.57</td>
<td>-0.15</td>
<td>0.19</td>
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<tr>
<td>rs11080122</td>
<td>C:T</td>
<td>0.145</td>
<td>1.00</td>
<td>2.09</td>
<td>0.73</td>
<td>0.004</td>
<td>0.20</td>
<td>0.09</td>
<td>0.019</td>
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<tr>
<td>rs8076005</td>
<td>A:G</td>
<td>0.170</td>
<td>0.40</td>
<td>1.75</td>
<td>0.66</td>
<td>0.008</td>
<td>0.23</td>
<td>0.08</td>
<td>0.003</td>
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<tr>
<td>rs2020942</td>
<td>G:A</td>
<td>0.337</td>
<td>0.90</td>
<td>-0.30</td>
<td>0.55</td>
<td>0.58</td>
<td>-0.07</td>
<td>0.06</td>
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<tr>
<td>rs140700</td>
<td>A:G</td>
<td>0.062</td>
<td>0.57</td>
<td>-0.08</td>
<td>1.10</td>
<td>0.94</td>
<td>0.20</td>
<td>0.14</td>
<td>0.15</td>
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<td>rs140701</td>
<td>C:T</td>
<td>0.481</td>
<td>0.02</td>
<td>-0.41</td>
<td>0.49</td>
<td>0.41</td>
<td>-0.004</td>
<td>0.06</td>
<td>0.95</td>
</tr>
<tr>
<td>rs2054847</td>
<td>C:T</td>
<td>0.487</td>
<td>0.03</td>
<td>-0.39</td>
<td>0.49</td>
<td>0.43</td>
<td>-0.003</td>
<td>0.06</td>
<td>0.96</td>
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<tr>
<td>rs1165736</td>
<td>G:A</td>
<td>0.019</td>
<td>1.00</td>
<td>-0.75</td>
<td>1.94</td>
<td>0.70</td>
<td>0.17</td>
<td>0.23</td>
<td>0.46</td>
</tr>
<tr>
<td>rs4325622</td>
<td>C:T</td>
<td>0.471</td>
<td>0.14</td>
<td>0.65</td>
<td>0.49</td>
<td>0.19</td>
<td>0.04</td>
<td>0.06</td>
<td>0.49</td>
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<tr>
<td>rs1042173</td>
<td>C:A</td>
<td>0.466</td>
<td>0.26</td>
<td>0.55</td>
<td>0.50</td>
<td>0.27</td>
<td>0.05</td>
<td>0.06</td>
<td>0.39</td>
</tr>
</tbody>
</table>

HWT indicates Hardy-Weinberg equilibrium Test; Ln, logarithm transformation.

*Major allele vs minor allele.
†$P$ values of $\chi^2$ testing for Hardy-Weinberg equilibrium.
‡Regression coefficient and SE using generalized estimating equations.
§$P$ values derived from generalized estimating equations.
¶$P$ values reached the nominal significance after multiple testing correction ($P = 0.008$) for both depressive symptoms and IL-6 or at least one of them.
¶¶$P$ values < 0.05 for both depressive symptoms and IL-6 but did not reach significance after multiple testing correction ($P = 0.008$).

### Table 3. Haplotype Association Tests for Depressive Symptoms and Interleukin-6

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Frequency</th>
<th>BDI-II Scores</th>
<th>Ln IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGTACA</td>
<td>0.81</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>ACCCTG</td>
<td>0.13</td>
<td>4.29</td>
<td>1.56</td>
</tr>
<tr>
<td>Other haplotypes§</td>
<td></td>
<td>0.06</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Ln indicates logarithm transformation; IL-6, interleukin-6 SNPs in the order of rs8071667→rs12150214→rs2020936→rs25528→rs6354→rs11080122→rs8076005.

*Regression coefficient and SE using GEE.
†$P$ values derived from GEE.
‡Most frequent haplotype chosen as the reference.
§Haplotypes with estimated frequencies $< 5\%$ pooled together.
56\% higher IL-6 level (P=0.01). Adjustment for covariates did not change the results. In addition, we performed a full haplotype analysis using all 20 polymorphisms. No significant associations were found (data not shown).

To avoid fitting multiple SNP models, we used the haplotypes consisting of these 7 SNPs for the bivariate SEM construction instead of using each single SNP. The bivariate SEM effects of the SLC6A4 risk haplotype (Hap2) on BDI-II scores (β=4.299, P=0.02) and IL-6 levels (β=0.469, P=0.01) were comparable with the β values obtained from GEE as shown in Figure 6. Moreover, the bivariate SEM provided the estimations of SLC6A4 haplotypes contributing to the correlation between BDI-II and IL-6. As shown in the Figure, the explained proportions of the phenotypic variance by SLC6A4 haplotypes were 2.1\% and 2.4\% for BDI-II and IL-6, respectively. The overall phenotypic correlation between BDI-II and IL-6 was 0.19 (P<0.001). After taking into account the effect of SLC6A4 haplotypes, the residual correlation was estimated as 0.17 (P<0.001; see Figure), indicating that ≈10\% of this correlation could be explained by the SLC6A4 haplotypes. As we have previously reported, more than two thirds of this correlation is due to the genetic factors. This implies that the SLC6A4 gene contributes ≈15\% of the shared genetic correlation between these 2 phenotypes.

Discussion

We report for the first time that genetic variations in the serotonin transporter gene SLC6A4 are associated with both depression and inflammation in middle-aged men. Participants who carried a specific haplotype consisting of 7 SNPs in the SLC6A4 gene had both significantly increased depressive symptoms and elevated IL-6 plasma levels. This risk haplotype explained ≈10\% of correlation between these 2 phenotypes. Our results indicate that the SLC6A4 gene underlies a shared pathophysiological pathway linking depression and inflammation. Our data also suggest that other genetic pathways, yet to be discovered, are involved in the link between depression and inflammation.

Growing evidence has implicated the serotonergic neurotransmission pathway, and its related genes, in the pathophysiology of depression. The gene encoding the serotonin transporter is a fundamental regulator of serotonin signaling and therefore has been the focus of many previous studies. Several studies have investigated the relationship of a common functional polymorphism in the promoter region of this gene (5-HTTLPR) and depression, but the results are inconsistent. In this study, we found no association between 5-HTTLPR and depressive symptoms. It is possible that the previously reported inconsistent results may be due to an interaction between stressful life events and 5-HTTLPR, whereby carriers of the S allele are more likely to suffer from MDD but only if they had experienced multiple traumatic events.

Only a few studies examined the relationship of other SNPs within the SLC6A4 gene and depression. A recent study examined the 5-HTTLPR and additional 4 SNPs in this gene in relation to depressive symptoms. For these 4 SNPS, no significant main effects were observed, although an interaction with stressful life events was found. Three of the 4 SNPs examined in this previous study (rs2020942, rs140700, and rs1042173) were also genotyped in our study. The SNP rs3794808 was not genotyped, but 3 SNPs (rs2020939, rs140701, and rs2054847) in strong LD with it (based on HapMap data) were studied in this study. No significant associations of these 6 SNPs with depressive symptoms were observed in our study.

In contrast, we found that 6 additional SNPs in the SLC6A4 gene were significantly associated with depressive symptoms and 1 SNP was borderline, where each minor allele was associated with increased BDI-II scores. These 7 SNPs were in strong LD with each other. Small to moderate LD was observed between these 7 SNPs and other polymorphisms. Six of the 7 SNPs were located in introns, and 1 SNP was in 5′ UTR (rs6354). Our study is the first one to report an association between these SNPs and depressive symptoms. Further study is necessary to explore whether these SNPs affect SLC6A4 gene expression.

We found that these same 7 SNPs were also significantly or borderline associated with IL-6 plasma levels. Substantial previous data suggest that serotonin plays a role in immune function and inflammation. An in vivo study showed that autologous serotonin is required for optimal T-cell activation and that the activation of suboptimally stimulated T cells can be augmented with low doses of exogenously added serotonin. In addition, serotonin induces NF-κB activation in human vascular smooth muscle cells and enhances IL-6 synthesis via the 5-HT2A receptor. Despite this evidence, no previous study examined the association between genetic variants in the serotonergic system and IL-6 levels.

In a previous twin study using SEM, we reported that depression and inflammation share a common genetic substrate. More than two thirds of the correlation between depressive symptoms and IL-6 plasma levels was explained by shared genetic effects. This study extends our previous work and shows that the SLC6A4 gene plays a fundamental role in the relationship between depression and inflammation.
role in the shared genetic liability to both depressive symptoms and inflammation. We found that variants in this gene explained $\approx 10\%$ of the shared phenotypic correlation of depressive symptoms and inflammation and 15% of their shared genetic correlation. These results suggest that the serotonin system is pathophysiologically implicated in both depression and inflammation, and that these 2 phenotypes may be the expression of a common pathophysiological mechanism involving neural-immune dysregulation. Our data also indicate that the remaining portion (85%) of the shared genetic correlation between depressive symptoms and IL-6 is still unexplained. Future studies should evaluate other genetic pathways that are involved in the link between depression and inflammation.

Our study is cross-sectional, thus limited in the ability to discern the temporal order between depression and inflammation. However, based on our results, the covariation of these 2 phenotypes is due in large part to a common genetic precursor rather than being a cause-effect relationship. Another limitation is that few twins met the criteria for a current major depressive episode, preventing us from examining current clinical depression. Because of this low prevalence, our analysis focused on current depressive symptoms, which are also related to CAD risk and which showed a graded effect with increasing depressive symptoms in this sample as previously described. Finally, because our twins were all middle-aged male military veterans, caution should be used in generalizing our results to women or older individuals. Our study provides the foundation for future investigations including diverse sociodemographic groups.

In conclusion, we have discovered a specific genetic vulnerability involving the serotonin transporter gene that is shared between depressive symptoms and elevated IL-6 plasma levels. Our results are consistent with the hypothesis that common pathophysiological processes link depression and inflammation and provide important insight into the role of serotonin in the pathophysiology of depression and the modulation of neural-immune interactions. Our data suggest that combining genetic and psychiatric profiling may ultimately prove useful in identifying depressed patients at highest risk for cardiovascular consequences and potentially aid in the design of novel drug targets, which may benefit clinical treatments.

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Disclosures
None.

References
CLINICAL PERSPECTIVE

There is growing evidence that depression is associated with increased levels of inflammatory markers, but whether this link is causal remains speculative. We sought to determine whether a common genetic vulnerability may be an alternative explanation for the observed association between depression and inflammation. Consistent with this hypothesis, in a study of middle-age male twins, we found that the serotonin transporter gene (SLC6A4) is a common precursor of both depressive symptoms and circulating interleukin-6 levels. Approximately 10% of the correlation between these 2 phenotypes can be explained by the SLC6A4 genetic variants. Our results provide important insight into the role of serotonin in the pathophysiology of both depression and inflammation.
Serotonin Transporter Gene, Depressive Symptoms, and Interleukin-6
Shaoyong Su, Jinying Zhao, J. Douglas Bremner, Andrew H. Miller, Weining Tang, Mark Bouzyk, Harold Snieder, Olga Novik, Nadeem Afzal, Jack Goldberg and Viola Vaccarino

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Subjects

The Twins Heart Study (THS) is an investigation of psychological, behavioral and biological risk factors for subclinical cardiovascular disease using twins. Twins were selected from the Vietnam Era Twin (VET) Registry, \(^1\) which includes 7,369 middle-aged male-male twin pairs both of whom served in the United States military during the time of the Vietnam War.

The THS included 360 twins from the VET Registry all born between 1946 and 1956 (>90% of the twins in the VET registry fall into this range). The methods of construction of this sample were described before. \(^2\) Briefly, the twins were free of a self-reported previous diagnosis of cardiovascular disease based on survey data collected in 1990 \(^3\), including a previous diagnosis of myocardial infarction, coronary heart disease, angina, congestive heart failure or stroke, or previous coronary angioplasty or coronary bypass surgery. From this group, we randomly sampled two groups of twin pairs: one group included twin pairs discordant for major depressive disorder (MDD), where one member of the pair had a lifetime history of MDD, assessed with the Diagnostic Interview Schedule around the same time as the cardiovascular survey, and the other did not; the second group of twins included pairs where neither had a history of MDD. Once selected, twin pairs came together but were examined separately at the Emory University General Clinical Research Center between March 2002 and March 2006, where the twins had a comprehensive physical exam and were queried again about previous diagnoses of cardiovascular diseases that might have occurred since the initial screen in 1990. We also administered the Structured Clinical Interview for DSM IV (SCID) \(^4\) to classify subjects based
on a lifetime history of MDD. The final data included 105 twin pairs where neither had a history of MDD, 68 twin pairs discordant for history of MDD and 7 twin pairs where both had a history of MDD. Compared to the normal twin pairs, the discordant twin pairs and the depressed twin pairs were younger, had higher prevalence of diabetes, more depressive symptoms and more likely to use antidepressants. For other study factors, there were no significant differences among these twin pairs. Of the 360 twins, 82 twins had a lifetime history of MDD. The mean duration of major depressive episodes was 8.3 months. However, the majority of twins with MDD were in remission, with only 8 subjects meeting DMS-IV criteria for a current major depressive episode and 77% having had the last depressive episode > 1 year before examination. Of the 82 MDD twins, 27 (33%) were on antidepressants. This protocol was approved by the Institutional Review Board at Emory University and informed consent was obtained from all subjects.
**Supplemental Table 1.** Haplotype estimations consisting of 7 single nucleotide polymorphisms

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<th>rs25528</th>
<th>rs6354</th>
<th>rs11080122</th>
<th>rs8076005</th>
<th>Freq&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
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<td>C</td>
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<tr>
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<tr>
<td>Rare&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>3.2%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Frequencies estimated using PHASE 2.0 software

<sup>b</sup> Rare haplotypes with frequency<1% grouped together
**Supplemental Figure 1.** Position and linkage disequilibrium (LD) map of genotyped polymorphisms in serotonin transporter gene. Boxes represent exons. Pairwise LD statistics ($r^2$) in examined genes were calculated with Haploview. Squares are colored darker if the $r^2$ value is high, that is, LD is strong.
Supplemental References


