STin2 RISK GENOTYPES FOR MAJOR DEPRESSIVE DISORDER

By

SARAH ELISABETH WILLIAMSON

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Approved by:

Francisco Moreno, M.D.
Department of Psychiatry
STATEMENT BY AUTHOR

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Signed: Sarah Williamson
STin2 Risk Genotypes for Major Depressive Disorder

Sarah E. Williamson

Department of Biochemistry and Molecular Biophysics

University of Arizona

Thesis Advisor: Francisco Moreno, M.D.

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Abstract

Major depressive disorder has been shown to have a heritable factor. Gene association studies are one way to establish these genetic risk factors. The serotonin transporter gene (SERT) has shown possible involvement in major depression. A variable number tandem repeat (VNTR) called STin2 on the SERT gene has provided cause for previous study. Known polymorphisms of STin2 include 7, 9, 10, and 12 repeats, with 10 and 12 repeats the major alleles. Previous studies have associated a higher frequency of STin2.10/STin2.10 and STin2.9 and a lower frequency of STin2.12/STin2.12 with major depression. The study consisted of 75 subjects with major unipolar depression and 91 non-depressed controls. DNA samples were amplified and sent for microsatellite analysis. A chi-square analysis with the criterion for significance set at $p \leq 0.05$ was performed for different ethnic groups as well as relative risk calculations. Statistical significance was established between depressed and control samples for gene frequencies in Hispanic, White, European, Western European, and Eastern European populations and for allele frequencies in White, Western European, and Eastern European populations. The relative risk for STin2.10/STin2.10 is 1.01, for not having STin2.12/STin2.12 is 1.20, and for having the STin2.9 allele is 2.45. From our data, we conclude that gene and allele frequencies of STin2 are significantly different between depressed and non-depressed subjects. Also, it appears that STin2.9 can be associated with a risk for depression.
STin2 Risk Genotypes for Major Depressive Disorder

It has been shown that major depression is a highly heritable disease [1, 2]. The heritability of major depressive disorders is 0.33, which means that 33% is accounted for by inherited factors and 67% by environmental factors [3]. Molecular methods have also given support to genetic influence contributing to risk for depression [4]. Gene association studies are one possible method to establish these genetic risk factors.

The serotonin transporter gene (SERT), SLC6A4, which is located on chromosome 17, is possibly involved in affective disorders, including major depression [5]. SERT is a good target for genetic study because it plays a role in the regulation of serotonin and SERT is the primary target for action of antidepressant drugs, including SSRIs. Many previous findings have supported that allelic variation in the SERT gene might lend to susceptibility for major depression.

A 17 base pair variable number tandem repeat (VNTR) has been discovered on the second intron of the SERT gene [6, 7]. The major alleles, formally known as STin2.10 and STin2.12, display 10 and 12 repeats respectively. It has also been determined that there are two more minor alleles, STin2.7 and STin2.9, each displaying 7 and 9 repeats respectively [8]. There is a difference in genotype frequency between depressed patients and controls in various populations; however, some inconsistent findings have been reported [9, 5]. It is possible that STin2.12 is a transcriptional enhancer, with the STin2.12/STin2.12 homozygous genotype lowering serotonin transporter availability in the brain [10, 8]. Consequently, this genotype has proven to be more prevalent among non-depressed Asian populations when compared to depressed Asian populations [11]. Also, the STin2.10/STin2.10 homozygous genotype has been found in higher frequencies in depressed groups compared to controls [5]. Finally, the frequency
of the STin2.9 allele, although rare overall, is greater in depressed populations when compared to non-depressed controls, meaning that 9 repeats can be associated with a risk for depression [12, 7].

In this study, we will be looking at the different frequencies of the STin2 alleles in various depressed populations compared to controls. Our hypothesis is that depressed groups will be genetically distinct from healthy controls. Also, we expect to see a higher frequency of “risk” genotypes (those including STin2.9 as well as STin2.10/STin2.10) as well as a lower frequency of the homozygous genotype STin2.12/STin2.12 in depressed groups when compared to controls.

Methods

Subjects

75 subjects, aged 19 to 71 years and diagnosed with unipolar major depression (DSM-IV)[13] participated in the study. All of them had participated in a research trial for tryptophan depletion. The control group consisted of 91 volunteers, aged 20 to 80 who denied a personal history of mental illness based on the mood disorders section of the Structured Questionnaire Interview of DSM-IV-R (SCID) questionnaire [14]. Subjects were not initially matched for ethnicity.

Genotyping

All DNA was extracted from human blood samples using QIAamp® DNA Blood Mini Kit (Qiagen, USA). All genotyping procedures were carried out in the Molecular Psychiatry Laboratory at the University of Arizona and imaging was conducted at the Genomics and Technology Core facility, also at the University of Arizona.
The STin2 polymorphism was first described by Lesch et al, 1994, and PCR conditions are modeled after Bellivier et al, 2002 [6, 15]. Genomic DNA was amplified for the STin2 VNTR using the primers SERTVNTR-F 5’-fam-GCTGTGGACCTGGGCAATGT-3’ and SERTVNTR-R 5’-GACTGAGACTGAAAAGACATAATC-3’. Amplification of the 290-341 bp region was carried out in a reaction volume of 25 μl consisting of 10 ng DNA, .25 mM dNTPs, .2 μM sense and anti-sense primers, 1X QIAGEN PCR buffer and 1 U Taq DNA Polymerase (Qiagen, USA). Thermocycling conditions consisted of an initial denaturation step of 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 60 s, with a final extension step of 72°C for 5 min. PCR product fragment analysis was analyzed using the ABI 3730 Genetic Analyzer and scored with the ABI PRISM® 3730 DNA analyzer (Applied Biosystems). Allele sizes were called using GeneMarker version 1.71 software. Products were observed of 290 bp, 307 bp and 341 bp corresponding to 9, 10 and 12 numbers of the 17 bp repeat, respectively.

Statistical Analysis

The data presented in this study is the result of a chi-square analysis, which was used to test for differences in the frequency of genotype polymorphisms between the depressive subjects and healthy controls. A standard p-value ≤0.05 was chosen to determine statistical significance. Due to uneven ethnic populations between depressed and control samples, further ethnic analyses were conducted. All sample groups fit Cochran’s criterion before chi-square analysis was conducted.

Also, the relative risk was calculated for the risk genotypes established a priori to testing. These include the increase of STin2.10/Stin2.10, the decrease of STin2.12/STin2.12, and the presence of STin2.9 in the depressed group when compared to controls. Relative risk measures
how many times more likely a depressed patient is to have a given “risk” genotype of allele than a non-depressed control.

Results

Table 1 provides the student’s t-test p-value for genotype and allele frequency comparisons between the depressed and control groups. P-values less than 0.05 were obtained when comparing gene frequencies in Hispanic, White, European, Western European, and Eastern European populations. P-values less than 0.05 were also seen between allele frequencies in White, Western European, and Eastern European populations.

Table 2 shows relative risk for each of the “risk” genotypes previously established. The relative risk associated with the STin2.10/STin2.10 genotype is 1.01, the relative risk for not having the STin2.12/STin2.12 genotype is 1.20, and for the STin2.9 allele the relative risk is 2.45.

Discussion

The difference in gene and allele frequencies are both very statistically significant for many of the populations studied (Table 1). Gene frequency between depressed and control groups is significant for Hispanic, White, European, Western European, and Eastern European populations. Allele frequency between the two groups is significant for White, Western European, and Eastern European populations. Thus, it is likely that polymorphisms of STin2 are associated with depression.

The relative risk for STin2.10/STin2.10 is 1.01, which means that it is unlikely that this homozygous genotype would cause risk for depression. The relative risk for not having the STin2.12/STin2.12 genotype in the depressed group is 1.20. This demonstrates that it is slightly more likely for a depressed patient to not have the STin2.12/STin2.12 genotype. Lastly, the
STin2.9 allele had the highest relative risk of 2.45, which indicates that it is likely associated with depression. Depressed patients are 2.45 times more likely to have the STin2.9 allele than non-depressed patients.

From our data, we conclude that polymorphism frequencies of STin2 are significantly different between depressed and non-depressed subjects. Also, it appears that the allele with 9 repeats demonstrates a major risk for depression. In the future, more diverse demographics should be compared with larger sample sizes to confirm significance.

The shorter repeats may be more of a risk than longer repeats because the longer repeats exponentially enhance transcription of the serotonin transporter. Thus, more serotonin can be transported at once in patients with the longer repeats, including STin2.12/STin2.12, which is why patients with this genotype may be at a lower risk for depression. This same reasoning can be used to explain why the STin2.9 allele, which is typically found with the STin2.10 allele, may lead to a higher risk for depression.
References


Tables

Table 1 – $X^2$ Analysis Results

<table>
<thead>
<tr>
<th>Population</th>
<th>Gene Frequency p-value</th>
<th>Allele Frequency p-value</th>
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<tbody>
<tr>
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<td>Hispanic</td>
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Table 2 – Relative Risk

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<th>Relative Risk</th>
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<tbody>
<tr>
<td>STin2.10/STin2.10</td>
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</tr>
<tr>
<td>Not STin2.12/STin2.12</td>
<td>1.20</td>
</tr>
<tr>
<td>STin2.9</td>
<td>2.45</td>
</tr>
</tbody>
</table>