Brief report

Analysis of catechol-O-methyltransferase and 5-hydroxytryptamine transporter polymorphisms in patients at risk for suicide

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Abstract

Genotype frequencies of functional polymorphisms in the genes encoding the serotonin transporter (5-HTT) and the enzyme catechol-O-methyltransferase (COMT) were not different in 51 suicidal inpatients compared to 51 control subjects. Within the patient group, increased hopelessness and suicide ideation were associated with homozygosity of the 5-HTT high promotor activity allele.

Keywords: Hopelessness; Serotonin; Major depression

1. Introduction

Suicidal behavior appears to have a genetic component (Mann et al., 1999a; Roy et al., 1999). The 5-hydroxytryptamine (5-HT) transporter (5-HTT, SERT, SLC6A4) may be a candidate gene for suicidal behavior based upon various lines of evidence linking serotonin and suicide (Asberg et al., 1986), and, more specifically, serotonin uptake binding sites in platelets and brain to affective illness and suicide (Roy et al., 1999). The 5-HTT gene has two common alleles which differ in length by 44 base pairs and transcriptional activity; the long allele, L, has higher transcriptional activity than the short allele, S (Heils et al., 1996). The S allele has been associated with affective illness in some studies (Collier et al., 1996; Fur-
long et al., 1998; Gutiérrez et al., 1998), but not in others (Kunugi et al., 1997; Ohara et al., 1998). Ohara et al. (1998) reported that number of suicide attempts was not related to allele frequency. Du et al. (1999), however, recently found that the l allele was more common in 24 depressed suicide victims compared to normal control subjects.

Another gene, catechol-O-methyltransferase (COMT), which is involved in catecholamine degradation, is of potential interest because of data linking catecholamines to suicidal behavior (Castrogiovanni et al., 1994; Ordway et al., 1994; Klinteberg et al., 1987; von Knorring et al., 1987). COMT enzyme activity is governed by a common genetic polymorphism at codon 158 (Weinshilboum and Raymond, 1977; Aksoy et al., 1993; Lotta et al., 1995; Lachman et al., 1996). An allele encoding a valine residue at amino acid 158 is associated with relatively high activity (H allele), whereas the allele encoding a methionine residue is associated with relatively low activity (L allele). Although there is no evidence for an association between this polymorphism and the core diagnosis in patients with either bipolar disorder or schizophrenia (Daniels et al., 1996; Biomed European Bipolar Collaborative Group, 1997; Lachman et al., 1998), it appears that COMT may modify the clinical phenotype of these conditions. Schizophrenic patients homozygous for the L allele, for example, show an increased propensity for violence (Strous et al., 1997b; Lachman et al., 1998).

Given these findings, as well as the data relating aggressive/impulsive behavior to suicide risk (Mann et al., 1999b), we tested the hypothesis that the functional polymorphisms in the COMT and 5-HTT genes may be associated with suicidal ideation and/or behavior.

2. Methods

2.1. Subjects

Fifty-one patients (32 female, 19 male; mean age ± S.D., 38 ± 15; 41 Caucasian, 6 African-American, 2 Hispanic, 1 Indian, 1 Asian) participated in this IRB-approved study. Subjects consisted of newly hospitalized patients who were considered to be at high risk of suicide (principal reason for admission) based on the clinical assessment by the admitting physician. Patients were diagnosed by the admitting physician using DSM-IV criteria (DSM-IV, American Psychiatric Association, 1994). Axis I diagnoses included major depression without psychosis (n = 22), major depression with psychosis (n = 12), bipolar disorder, depressed, without psychosis (n = 9), and bipolar disorder, depressed, with psychosis (n = 3), schizoaffective disorder (n = 4), schizophrenia (n = 3), anxiety disorder (n = 7), substance abuse (n = 7), and post-traumatic stress disorder (n = 3). Twenty-six patients (50%) had more than one Axis I diagnosis. Of the patients 84% had a history of making at least one suicide attempt.

Control subjects were 51 individuals (28 females and 23 males; mean age ± S.D., 41 ± 13) matched for race from the same general geographic area as the clinical sample. These subjects were not formally screened for psychiatric disorders, but did deny a history of serious psychiatric illness.

2.2. Research design

Patients meeting criteria for participation completed interview and self-report instruments 24 h after admission to assess the severity of suicidal ideation [Scale for Suicide Ideation (SSI), Beck et al. (1979), mean ± S.D. = 17.8 ± 8.6], depression [Beck Depression Inventory (BDI), Beck and Steer (1987), mean ± S.D. = 32.4 ± 12.8], hopelessness [Hopelessness Scale (HS), Beck and Steer (1988), mean ± S.D. = 13.2 ± 5.6], and general psychopathology [Brief Psychiatric Rating Scale (BPRS), Overall and Gorham (1962), mean ± S.D. = 29.2 ± 7.5]. Patients were also interviewed to assess factors associated with suicide risk. Given the small sample size, we limited statistical analyses to factors most likely to have genetic contributions: family history of suicide (20 yes, 28 no, 3 unknown) and lifetime number of suicide attempts (mean ± S.D. = 2.5 ± 4.5).
2.3. COMT and 5-HTT genotype determinations

The codon 158 polymorphism was identified by a PCR-based restriction fragment length polymorphism analysis as previously described in detail (Lachman et al., 1996). The 5-HTT promoter polymorphism was detected by PCR using primers CCGCTCTGAATGCCAGCACCTAAC and AGAGGGACTGAGCTGGACAACCAC. The PCR reaction was carried out in a 20-μl volume containing approximately 100 ng of genomic DNA with the Expand Long Template PCR system (Boehringer Mannheim, Germany) in Expand buffer 1. After an initial denaturation step of 94°C for 2 min, the cycling parameters were 30 cycles consisting of 94°C for 15 s, 68°C for 2 min, and then a final extension at 68°C for 5 min. The PCR fragment was radio-labeled by including 5 μCi 32P-dCTP in the reaction mix. The PCR product is a 479/523-bp fragment that was resolved by electrophoresis through a 4% non-denaturing acrylamide gel and visualized by autoradiography.

2.4. Data analysis

COMT and 5-HTT genotype frequencies were compared between groups using χ² analysis. Within the patient group, exploratory χ² analyses, t-tests, and one-way analyses of variance (ANOVA) were performed to examine possible associations between genotype and our clinical measures. We assumed a dominant-recessive effect for the 5-HTT gene and grouped the ss and ls genotypes for statistical analyses. This assumption is based on the work of Lesch et al. (1996) who found that mRNA concentrations, [125I]RTI-55 binding, and [3H]5-HT uptake are significantly higher in lymphoblast cell lines associated with the ll genotype compared to either the ls or ss genotypes. We also performed these analyses without assuming a dominant-recessive effect for the 5-HTT gene. Where appropriate, P values were adjusted using a Bonferroni correction. The genotypes of both control and patient groups were in Hardy–Weinberg equilibrium.

3. Results

There were no significant differences between patient and control groups with respect to COMT or 5-HTT genotype frequencies (Table 1). However, the genotype frequency for 5-HTT was significantly related to HS and SSI scores. The HS score for the ll genotype (16.6 ± 2.3) was significantly higher than for the grouped ss and ls genotypes (11.8 ± 5.9; t(46) = 2.9, P = 0.005, 95% CI = 1.5–8.1). Likewise, the SSI score for the ll

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Genotype frequencies for COMT and 5-HTT gene polymorphisms, patient vs. control groupsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT</td>
<td>Patients (n = 49)</td>
</tr>
<tr>
<td>LL</td>
<td>9</td>
</tr>
<tr>
<td>LH</td>
<td>28</td>
</tr>
<tr>
<td>HH</td>
<td>12</td>
</tr>
<tr>
<td>L frequency</td>
<td>0.47</td>
</tr>
<tr>
<td>H frequency</td>
<td>0.53</td>
</tr>
<tr>
<td>5-HTT</td>
<td>Patients (n = 51)</td>
</tr>
<tr>
<td>ss</td>
<td>9</td>
</tr>
<tr>
<td>ls</td>
<td>28</td>
</tr>
<tr>
<td>ll</td>
<td>14</td>
</tr>
<tr>
<td>s frequency</td>
<td>0.45</td>
</tr>
<tr>
<td>l frequency</td>
<td>0.55</td>
</tr>
</tbody>
</table>

aL, COMT low enzyme activity allele; H, COMT high enzyme activity allele; s, low promotor activity allele; l, 5-HTT high promotor activity allele.
genotype (22.6 ± 7.1) was significantly higher than for the grouped ss and ls genotypes (16.0 ± 8.5; \( t(28) = 2.7, P = 0.01, 95\% CI = 1.7–11.4 \).)

Without assuming a dominant-recessive effect for the 5-HTT gene, there continued to be a significant group effect for the HS score (ANOVA, \( F_{2,47} = 4.2, P = 0.021 \)). The HS score for the ll genotype was significantly higher compared to the ls genotype (11.7 ± 5.9, \( P = 0.02, 95\% CI = 0.6–9.2 \)), but not compared to the ss genotype (12.2 ± 6.5, \( P = 0.20, 95\% CI = –1.4–10.1 \)). In addition, significant group effects were observed for SSI (\( F_{2,50} = 3.9, P = 0.03 \)) and BPRS scores (\( F_{2,50} = 3.1, P = 0.05 \)). Mean scores for the ll, ls, and ss genotypes for the SSI were 22.6 ± 7.1, 15.2 ± 8.5, and 18.6 ± 8.6, respectively (ll vs. ls, \( P = 0.03, 95\% CI = –0.7 to –14.0, \) other post hoc comparisons, minimum \( P = 0.76 \)). Mean scores for the ll, ls, and ss genotypes for the BPRS were 30.4 ± 7.2, 27.1 ± 6.0, and 33.7 ± 10.2, respectively (ss vs. ls, \( P = 0.065, 95\% CI = –13.4–0.3, \) other post hoc comparisons, minimum \( P = 0.58 \)).

4. Discussion

Although suicidal patients did not differ from normal control subjects with respect to the frequency of functional polymorphisms in the COMT and 5-HTT genes, the observed relationship between the 5-HTT ll genotype and increased mean HS and SSI scores is of interest. Hopelessness is among the most consistently observed factors associated with long-term suicide risk in depressed patients (Beck et al., 1985, 1990; Fawcett et al., 1990). Moreover, hopelessness may be both a state and trait dimension (Beck et al., 1985, 1990). With respect to the latter, there is evidence that many patients suffer from chronic hopelessness even after other symptoms of depression have remitted, and that these patients are at particularly high risk of engaging in suicidal behavior (Rifai et al., 1994; Young et al., 1996; Szanto et al., 1998). Although our findings seem to be discrepant with the those of Furlong et al. (1998) whose meta-analysis revealed a significant association between the s allele and both unipolar and bipolar depression, it is possible that different genotypes may be associated with hopelessness and suicidal behavior on the one hand, and depressive illness on the other. The findings of Du et al. (1999) are consistent with this view. Alternatively, this discrepancy may be explained by sampling error.

Our results are consistent with the possibility that homozygosity for the l allele is related to heightened suicide risk (Mann et al., 1999b) in a subgroup of patients with the highest hopelessness scores. This study must be viewed as preliminary, given its many limitations including small sample size (132 patients and 132 control subjects would be needed to achieve 80% power, \( \alpha = 0.05, OR = 2.0 \), lack of structured diagnostic assessments of patients and control subjects, and diagnostic heterogeneity in the patient sample. Nevertheless, our findings suggest that future studies exploring the role of the 5-HTT promoter polymorphism in suicide risk and hopelessness are warranted.

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References


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