Analysis of a Novel Functional Polymorphism Within the Promoter Region of the Serotonin Transporter Gene (5-HTT) in Brazilian Patients Affected by Bipolar Disorder and Schizophrenia

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It has been suggested that the serotonin transporter (5-hydroxytryptamine-transporter or 5-HTT) may be involved in the pathogenesis of affective disorders. Recently, Collier et al. (1996) found that the frequency of the low-activity short variant (s) of the 5-HTT-linked polymorphic region (5-HTTLPR) was higher among patients with affective disorders than in normal controls. However, since the observed level of significance was not high, they suggest that these findings should be replicated in independent samples.

We have analyzed 86 unrelated patients (47 with bipolar disorder and 39 with schizophrenia) and 98 normal controls from the Brazilian population for the 5-HTTLPR. Statistical analysis revealed that the genotypes (LL, Ls, ss) as well as the estimated allele frequencies (L,s) did not differ significantly among the three studied groups or between bipolar and normal controls. In addition, although not statistically significant, the genotype ss in our sample was less frequent among our bipolar patients than in our normal controls (12.8% versus 16.3%) which is the opposite of what was found by Collier et al. (24% versus 18%) in the European study.

Although it will be important to extend the present analysis in a larger sample, our preliminary results suggest that the 5-HTTLPR does not seem to play a major role in the genetics of bipolar and schizophrenic disorders at least in this group of Brazilian psychiatric patients. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 81:225–227, 1998.

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Serotonin (5-hydroxytryptamine or 5-HT) is a key neurotransmitter in the central and peripheral nervous system which is implicated in the control of mood, sleep, appetite and a variety of traits and behaviors. Its potential role in psychiatric conditions such as depression, obsessive-compulsive disorders and schizophrenia has been the subject of several publications (Meltzer et al., 1981).

On the other hand, the 5-HT transporter (5-HTT), by regulating the magnitude and duration of serotonergic responses, is central to the fine-tuning of brain serotonergic neurotransmission and of the peripheral actions of 5-HT (Risch and Nemeroff, 1992). According to some investigators 5-HTT may be involved in the pathogenesis of schizophrenia and affective disorders while according to others, dopamine and its receptor may play a more important role in schizophrenia (Seeman et al., 1976; Stahl et al., 1983; Joyce et al., 1993; Lesch et al., 1995; Collier et al., 1996; Okubo et al., 1997).

The 5-HTT is encoded by a gene at 17q11.1-q12, with 14 exons which span –35kb (Lesch et al., 1993; 1994; Ramamoorthy et al., 1993; Gelernter et al., 1995). Recently, two common polymorphisms in the 5-HTT gene were reported: a VNTR (variable number of tandem repeat) in exon 2 and a deletion/insertion polymorphism in the transcriptional control region –1kb upstream of the gene's transcription initiation site (Heils et al., 1995, 1996; Ogilvie et al., 1996).

Two recent studies did not find any evidence that alterations in the primary structure of the 5-HTT gene
are involved in the pathogenesis of uni or bipolar depression (Lesch et al. 1995) as well as in obsessive-compulsive disorders (Altemus et al., 1996). However, an association between allele 12 of the VNTR in intron 2 of the 5-HTT gene and bipolar affective disorder has been found in English and Welsh populations (Collier et al., 1996; Craddock et al., 1996).

Recently, a polymorphism in the transcriptional control region upstream of the 5-HTT coding sequence has been reported. It has been demonstrated that the long (L) and short (s) variants of this 5-HTT gene-linked polymorphic region (5-HTTLPR) have different transcriptional activities. That is, the short variant of the polymorphism reduces the transcriptional efficiency of the 5-HTT gene promoter resulting in decreased 5-HTT expression and 5-HT uptake in lymphocytes (Heils et al., 1995, 1996).

Interestingly, an association between the 5-HTTLPR and anxiety-related traits has been reported by Lesch et al. (1996). According to these authors, this polymorphism accounts for 3 to 4% of total variation and 7 to 9% of inherited variance in anxiety-related personality traits in individuals as well as sibships. Furthermore, in a recent study, Collier et al. (1996) found that the frequency of the low activity allele was higher in patients with affective disorders than in normal controls. However, since the obtained level of significance was not high, these authors suggested that these findings should be replicated in independent samples.

We are not aware of any similar study in the Brazilian population, which is characterized by a high racial admixture. In order to evaluate if there is an association between the 5-HTTLPR with bipolar depression as well as schizophrenia in a different ethnic group, we have analyzed patients affected with these two psychiatric disorders and compared them to a sample of normal controls.

SUBJECTS AND METHODS

A total of 86 unrelated patients (47 with bipolar disorder and 39 with schizophrenia) and 98 normal unrelated controls were included in the present study. Among the patients, 69 or about 80% were caucasoid (of European descent) and 17 (about 20%) were African/Brazilian. Therefore, in order to compare individuals from the same ethnic background, 78 caucasoids and 20 African/Brazilian were selected among normal control subjects.

Patients were diagnosed with schizophrenia and bipolar disorder according to DSM-IV (APA) criteria. These subjects have been followed for many years in two psychiatric clinics, from the Institute of Psychiatry (Universidade de São Paulo) specialized in bipolar disorder and schizophrenia. Normal controls were volunteers who worked at the University or normal relatives of Duchenne muscular dystrophy patients who had no history of major psychiatry or neurological illness.

DNA was extracted from blood, after informed consent, and the 5-HTTLPR was analyzed by polymerase chain reaction (PCR) according to the method reported by Lesch et al., (1996). According to this method two fragments are generated: the short variant (s) with 484-bp and the long one (L) with 528-bp.

RESULTS AND DISCUSSION

The results on genotype frequencies in our sample of normal controls, schizophrenic and bipolar patients are summarized in Table I. Results on the estimated allele frequencies, their respective binomial standard error (s.e.) and results of chi-squared tests for Hardy-Weinberg equilibrium are summarized in Table II.

Statistical analysis revealed that the genotype frequencies did not differ significantly among the three studied groups \( (2(4 \text{ d.f.}) = 2.271; P = 0.686) \). The same result was obtained when allelic frequencies were compared \((2(2 \text{ d.f.}) = 0.411; P = 0.814)\). In addition, no significant difference at the 5% critical level was observed when only bipolar and normal controls were compared \((2(1 \text{ d.f.}) = 0.050 ; P = 0.822)\). On the contrary, the chi-squared value indicates an almost perfect match between allele frequencies in the two groups.

Therefore, in spite of the relatively small size of the sample here reported, our data do not seem to confirm the findings of Collier et al. (1996) who reported a significantly higher frequency \((2(1 \text{ d.f.}) = 6.690 ; P = 0.01)\) of the low activity allele in their combined sample of patients with bipolar and unipolar depression than in control subjects.

It is also important to point out that, although not statistically significant, the genotype ss in our sample was less frequent among our bipolar patients than in normal controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Schizophrenics</th>
<th>Bipolars</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL</td>
<td>39/98 = 0.398</td>
<td>12/39 = 0.308</td>
<td>15/47 = 0.319</td>
</tr>
<tr>
<td>Ls</td>
<td>43/98 = 0.439</td>
<td>21/39 = 0.538</td>
<td>26/47 = 0.553</td>
</tr>
<tr>
<td>ss</td>
<td>16/98 = 0.163</td>
<td>6/39 = 0.154</td>
<td>4/47 = 0.128</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele frequencies</th>
<th>S.E.</th>
<th>( \chi^2 ) (1 d.f.)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P(L) = 121/196 = 0.617</td>
<td>0.035</td>
<td>0.498</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>P(s) = 75/196 = 0.383</td>
<td>0.035</td>
<td>0.498</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P(L) = 45/78 = 0.577</td>
<td>0.056</td>
<td>0.414</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>P(s) = 33/78 = 0.423</td>
<td>0.056</td>
<td>0.414</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Bipolars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(L) = 57/94 = 0.596</td>
<td>0.051</td>
<td>1.037</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>P(s) = 37/94 = 0.404</td>
<td>0.051</td>
<td>1.037</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
our normal controls (12.8% versus 16.3%) in opposition to what was found by Collier et al. (24% versus 18%) in the European study.

In summary, although it will be important to extend the present analysis in a larger sample, these preliminary results suggest that this 5-HTT functional variant does not seem to play a major role in the genetics of these disorders at least in this group of Brazilian psychiatric patients. They also confirm the importance of comparing different populations since as recently pointed out by Gurwitz (1997), the ethnic background seems to influence the degree of penetrance of susceptibility genes.

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REFERENCES


