Serotonin Transporter Gene Polymorphisms, Alcoholism, and Suicidal Behavior

Philip Gorwood, Philippe Batel, Jean Adès, Michel Hamon, and Claudette Boni

Background: Dysfunction of serotoninergic transmission could predispose to excessive alcohol consumption and dependence. The functional polymorphism of the serotonin transporter gene (5-HTTLPR) has been associated with different disorders, including alcoholism. Considering the likelihood of heterogeneity in the “alcohol dependence” phenotype, 5-HTTLPR may be more specifically implicated in subsamples of patients or in related traits of alcoholism, such as impulsivity.

Methods: We analyzed the role of this functional polymorphism in the risk for suicide attempt in a population of male alcohol-dependent subjects. One hundred ten male alcohol-dependent patients (DSM-III-R criteria), French for at least two generations, were personally interviewed with the Diagnostic Interview for Genetic Studies and compared with 61 unaffected blood donors.

Results: The “short” (S) allele of the 5-HTTLPR appeared to be unrelated to alcohol dependence and comorbid depression in our sample, but was found associated with an increased risk for suicide attempts. This association was predominantly observed in severe and repetitive suicide attempts, with a significant dose effect of the S allele (0, 1, or 2) on the number and the severity of suicide attempts.

Conclusions: Mood disorders and alcohol dependence may interact with a genetic (relative) deficiency in serotonin reuptake, thereby increasing the risk for aggressive/impulsive behaviors such as suicide attempts. Biol Psychiatry 2000;48:259–264 © 2000 Society of Biological Psychiatry

Key Words: Suicide, serotonin, genetics, behavior, impulsivity, addiction, dependence

Introduction

Evidence has accumulated for the last years that serotonergic neurotransmission is involved in alcoholism (McBride et al 1993; Sellers et al 1992). Thus alcohol-drinking behavior seems to be associated with low activity levels of central serotonin (5-HT) systems (Ferreira and Soares-Da-Silva 1991; LeMarquand et al 1994b; Zhou et al 1994). Alterations in 5-HT uptake have also been described in alcohol-dependent patients (Ernouf et al 1993; Heinz et al 1998) that may sustain such alcohol–5-HT relationships because selective 5-HT reuptake inhibitors, such as fluoxetine, sertraline, and citalopram, regularly produce a decrease in alcohol craving and drinking in alcoholic patients (Balldin et al 1994a, 1994b; Higley et al 1998; Lejoyeux 1996; Lu et al 1994; Naranjo et al 1994).

Alcoholic subjects are dramatically exposed to suicidal behavior (Kessler et al 1994) and suicide mortality (Rossov and Amundsen 1995), which may also be causally related to altered 5-HT neurotransmission. Indeed, low levels of 5-HT and/or its metabolite 5-hydroxyindoleacetic acid (5-HIAA) have regularly been found in blood and cerebrospinal fluid (CSF) of subjects who attempted suicide (Pihl and LeMarquand 1998). Interestingly, alterations in 5-HT uptake have also been reported in these subjects (Ellis and Salmond 1994; Meltzer et al 1981). The interaction between alcoholism and 5-HT uptake may thus be relevantly tested to explain the increased risk for suicidal behavior in alcohol-dependent patients.

Polymorphism of the gene encoding the 5-HT transporter (5-HTT) notably concerns its promoter (Heils et al 1996). The region between base pair (bp) 1376 and bp 1027 contains 16 tandem repeats of a 20- to 23-bp G+C rich sequence. Two common forms of this region were found in a white population: a 528-bp allele with 16 repeats (“long” [L] allele) and a 448-bp allele (“short” [S] allele) with a deletion of 44 bp extending from bp 1255 to bp 1212. The 528-bp long allele of the 5-HTT promoter is two- to threefold more active in driving transcription than the 484-bp allele in transfection experiments (Heils et al 1996). In addition, 5-HT uptake is twice as high in lymphoblastoid cells from individuals homozygous for the 528-bp allele as in cells from individuals of the other genotypes (Lesch et al 1996). The potential role that this functional polymorphism in the 5-HTT promoter may play in personality traits and psychiatric diseases has already been the matter of numerous studies; however, to date, rather controversial data have been reported regarding a possible association of either the short or the long allele with sometimes mood disorders and sometimes anxiety-

Similarly, conflicting data have been reported about the possible association of polymorphism of the 5-HTT gene promoter with alcoholism. Thus, Sander et al (1998) and Schmidt et al (1997) found that the frequency of the short allele is significantly increased in alcoholic patients with severe dependence as compared with nonalcoholic control subjects. On the other hand, Türker et al (1998) noted the existence of a significant association between the short allele of the 5-HTT gene promoter and high ethanol tolerance in young adults. In contrast, Edenberg et al (1998) and Jorn et al (1998) could not find any association between polymorphism of the 5-HTT gene promoter and alcohol misuse and dependence.

Because heterogeneity in the population of alcoholic patients might have contributed to these discrepancies, further investigations on a possible association between this polymorphism and various subgroups of alcoholics have been performed. Distinction of subgroups based on the presence and type of suicidal behavior allowed the discovery of a significant association between the presence of the short allele of the 5-HTT gene promoter and severe suicide attempts in alcoholic-dependent patients.

Methods and Materials

Seventy male control subjects, recruited from a blood transfusion center, were at least 35 years old and French for at least two generations, without any substance dependence (DSM-III-R). The age of 35 was considered as a cutoff to reduce the risk that control subjects may later develop alcohol dependence. Using the same clinical instruments as for the patients, we eliminated four subjects who fulfilled the DSM-III-R alcohol dependence or abuse criteria, and three others because they made a suicide attempt at least once in their life. In addition, two subjects refused to participate. Finally, 61 control subjects were included in this study.

We recruited 110 male alcoholics from two general hospitals that specialize in the treatment of alcoholism, in Paris and its suburbs. Inclusion criteria consisted of DSM-III-R criteria of alcohol dependence, French origin for at least two generations, and no comorbid dementia. Schizophrenia and bipolar mania–depressive disorder were exclusion criteria. The lifetime psychiatric and addictive diagnoses were made by face-to-face interview using the Diagnostic Interview for Genetic Studies (DIGS; Nurnberger et al 1994). The alcoholic patients were seen at least once in their life. In addition, two subjects refused to participate. Finally, 61 control subjects were included in this study.

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Table 1. Genotype Counts of Polymorphism within the Serotonin Transporter (5-HTT) Promoter in Alcoholic Patients (with or without Suicide Attempts) and Unaffected Matched Control Subjects

<table>
<thead>
<tr>
<th>5-HTTLPR genotypes</th>
<th>Control subjects</th>
<th>Without suicide attempt</th>
<th>With suicide attempt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>LL</td>
<td>24 39.3%</td>
<td>21 38.2%</td>
<td>12 21.8%</td>
</tr>
<tr>
<td>LS</td>
<td>26 42.6%</td>
<td>26 47.3%</td>
<td>30 54.5%</td>
</tr>
<tr>
<td>SS</td>
<td>11 18.0%</td>
<td>8 14.5%</td>
<td>13 23.6%</td>
</tr>
</tbody>
</table>

Genotype counts are not significantly different in alcohol-dependent patients and control subjects ($\chi^2(2) = 1.61, p = .45$). Genotypes with the “short” (s) allele (LS and SS) are more frequent in alcoholic-dependent patients with suicide attempt (78.1%) than in alcohol-dependent patients without suicide attempt [61.8%; $\chi^2(1) = 3.51, p = .06$] and control subjects [60.6%; $\chi^2(1) = 4.15, p = .04$].

subjects (without suicidal behavior), 61.8% in alcoholics without suicidal behavior, and 78.2% in alcoholics who tried at least once to commit suicide (Table 1). Thus, alcoholics with suicidal behavior have different genotypes than normal control subjects and a tendency for different genotypes than alcoholics with no history of suicidal behavior (Table 1).

Number and lethality of suicide attempts were then compared between alcoholic patients according to the presence of the short variant of the 5-HTT gene promoter. We noted a regular increase in the frequency of the S allele with the number of suicide attempts for each subject: 61.8% for patients with no suicide attempt, 74.2% for patients who committed a suicide attempt once or twice, 82.3% for those who tried three or four times, and 83.3% for patients with more than four suicide attempts. The linear trend for increased S allele frequency according to the number of suicide attempts (none, once or twice, three or four times, and more than four) was significant [$\chi^2(1) = 3.89, p = .05$, Armitage test]. Furthermore, a “dose effect” of the S allele was observed, with a significant positive correlation between the number of suicide attempts and the number of the S allele (0, 1, or 2; $\rho = .185, p = .05$).

We also observed an increase in the presence and the frequency of the S allele in alcoholic-dependent patients according to the lethality of suicide attempts. Sixty-two percent of patients with no suicide attempt ($N = 55$) had the S allele; this percentage increased to 78% for patients with moderate lethality suicide attempts ($N = 40$) and to 80% for those with more severe suicide attempts ($N = 15$). In this last group, the two patients with very severe suicide attempts both had the S allele. In this view, we found a significant increase in the S allele frequency according to the lethality of suicide attempts [i.e., no suicide attempt, at least a suicide attempt, at least one severe suicide attempt, and at least one very severe attempt; $\chi^2(1) = 4.41, p = .04$, Armitage test]. A dose effect of the S allele was also observed, as there is a correlation between the degree of lethality of suicide attempts and the number of the S allele (0, 1, or 2; $\rho = .185, p = .05$).

Comorbid depression might explain increased suicide attempts in samples of alcohol-dependent subjects. In our sample, the two variables “existence of at least one major depression episode” and “at least one suicide attempt” were significantly interacting with the number of the S allele (Table 2). Taking into account this interaction, we found a significant impact of 5-HTTLPR polymorphisms on suicide attempts alone, but not on major depression alone. For example, in this sample 76.2% of alcohol-dependent patients with the S allele and with lifetime comorbid major depressive disorder ($N = 21$) made at least one suicide attempt. On the other hand, only 44.4% of patients with no S allele and no comorbid lifetime major depression disorder ($N = 27$) made a suicide attempt [$\chi^2(1) = 5.46, p = .02$].

Discussion

For the sample studied, no association could be found with 5-HTTPR polymorphisms and alcohol dependence per se, in agreement with most recent studies (Edenberg et al 1998; Jorm et al 1998). Accordingly, it can be inferred that the changes in 5-HT reuptake capacity in alcoholic patients that were noted in previous studies (Ernouf et al 1993; Heinz et al 1998) do not probably reflect changes in expression of the 5-HTT–encoding gene that would depend on the presence of the S or L allele promoter. Indeed, opposite changes in 5-HT reuptake—that is, an increase in platelets (Ernouf et al 1993) versus a decrease in brain (Heinz et al 1998)—have been reported in alcoholic patients, strongly suggesting that these changes are independent of the 5-HTT gene promoter but are related to local environmental conditions, affecting 5-HTT functioning differently in the periphery versus in the central nervous system. Furthermore, postmortem studies showed that the density of 5-HTT binding sites in the midbrain of alcoholic subjects was significantly higher than that in con-
control subjects with the same SS or SL genotypes, in line with the hypothesis that alcohol consumption per se can affect the 5-HTT at posttranscriptional level(s) (Little et al 1998).

Interestingly, further phenotypic definition of alcoholic patients led to the distinction of alcohol-dependent subjects with dissocial personality disorder for whom a significant association was found between the presence of the S allele of the 5-HTT gene promoter and a temperamental profile of high novelty seeking and low harm avoidance (Edenberg et al 1998). In the present sample, analysis of various phenotypic parameters revealed that other subgroups could be identified within the alcoholic population, depending on both a history of suicide attempts and the presence of mood disorders.

First, we found that in the recruited alcohol-dependent patients the risk for suicidal behavior and, particularly, for repeated suicide attempts and attempts with high lethality is partly dependent on 5-HTTLPR polymorphisms. In our sample, the S allele is qualitatively distinguishing patients with or without suicide attempts and is quantitatively correlated to number and severity of suicide attempts.

The second result of our study is that comorbid mood disorder is significantly interacting with 5-HTTLPR polymorphisms to "explain" suicide attempts in alcohol-dependent patients. It is the interaction that is involved and not mood disorder per se, as 5-HTTLPR polymorphisms are not associated with depression in our sample. These results are consistent with those of Bellivier et al (2000) showing no direct impact of 5-HTTLPR on recurrent mood disorder, but a significant impact of the S allele on suicide attempts, especially for those with high lethality.

Long-term, prospective studies of psychiatric patients have shown that affective disorder and alcoholism are most frequently found in suicidal cases. Thus, Robins (1981) reported that of 134 subjects who committed suicide, 47% were suffering from depression and 25% from alcoholism. Similarly, in their study Barrclough et al (1974) noted that most patients among 100 suicide cases were suffering from either depression (70%) or alcoholism (15%). Furthermore, the most prevalent psychopathology among alcoholics who commit suicide is affective disorder (Berglund 1984; Cornelius et al 1995; Murphy et al 1979; Whitters et al 1985).

Acute alcohol intake transiently increases central 5-HT functioning, whereas chronic intake decreases it (LeMarquand et al 1994a). Chronic alcohol intake may lead to a state of lowered central 5-HT functioning characterized by a propensity toward disinhibited behavior, thus increasing the potential for aggressive behavior (Pihl and LeMarquand 1998). When expressed towards the self (suicide), aggressive behavior has been associated with low CSF levels of the 5-HT metabolite 5-HIAA regardless of diagnosis (Traskman-Bendz et al 1986; Tuinier et al 1995; van Praag 1983), this relationship being particularly significant for violent suicide (Asberg et al 1976). It is the severity and impulsivity of suicide that may thus be related to low 5-HT functioning. On the other hand, depression per se is also associated with reduced 5-HT neurotransmission, as evidenced by low CSF 5-HIAA levels (Meltzer and Lowy 1987) and plasma free tryptophan levels (Coppen and Wood 1978; DeMeyer et al 1981) in depressed patients. The existence of a causal relationship between depression and reduced 5-HT neurotransmission is strongly supported by the observation that tryptophan depletion (which efficiently decreases central 5-HT synthesis [Nishizawa et al 1997]) can produce a lowering of mood both in normal subjects and in recovered depressed patients (Benkelfat et al 1994; Salomon et al 1993; Smith et al 1997).

In our sample, the S allele, leading to lower 5-HT transport capacity than the L allele (Heils et al 1996), is associated with an increased risk of suicide attempts, this association being stronger for numerous suicide attempts with high lethality. Variation of the S allele frequency according to number and severity of suicide attempts could be interpreted in two ways. First, the impact of the S allele could be associated with the aggressive/impulsive dimension, which is probably (quantitatively) increased in severe suicide attempts. Another explanation is that the (qualitative) presence of nonviolent, nonimpulsive suicide attempts (e.g., pill ingestion) is probably reduced in more severe suicide attempts.

The impact of the S allele is also especially significant when lifetime comorbid depression is present, and when suicide attempts are characterized by frequent antecedents and severe lethality. Mood disorders and alcohol dependence may interact with a genetic (relative) deficiency in 5-HT reuptake, thereby increasing the risk for aggressive/impulsive behaviors such as suicide attempts.

Some limitations have to be raised in this study, however, notably regarding the limited size of our sample. The main analyses are based on the comparison of two groups of 55 subjects each, but some subsamples, such as those with severe suicide attempts (N = 15), are of limited size, especially as interaction between different variables has been tested. Although very similar results have been found in Bellivier and colleagues’ study (2000)—namely, the S allele is not increasing the risk for any specific psychiatric disorder, but instead is associated with suicide behavior—other replications have to be made on larger samples of alcohol-dependent subjects.

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