GENETICS AND CELL BIOLOGY

The Short Allele of the Serotonin Transporter Promoter Polymorphism Influences Relapse in Alcohol Dependence

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Abstract — Aims: The short (S) allele of the serotonin transporter gene promoter polymorphism (5-HTTLPR) contributes to the risk of alcohol dependence and co-occurring clinical features. We studied the putative link between this allele and relapse. Methods: 48 alcohol-dependent male patients were recruited and genotyped for the 5-HTTLPR. Relapse to alcohol drinking was monitored during 3 months after standardized withdrawal. Results: The S allele was significantly associated with relapse ($p = 0.008$) while no other factor that was measured played a significant role. Conclusions: The S allele of the 5-HTTLPR polymorphism may influence the risk of relapse in abstinent alcohol-dependent patients, possibly through intermediate phenotypes.

INTRODUCTION

Alcohol dependence (AD) is a common, complex disorder that, on a lifetime basis, affects up to 20% of the adult population in the United States (NIAAA, 2000) and in most developed countries. Family, twin, and adoption studies have demonstrated that genes play a major role in the development of AD (Heath, 1995). Heritability estimates range from 50 to 60% for both men and women (Prescott et al., 1999). Moreover, it has been demonstrated that vulnerability to AD as well as variability in its course among individuals may result from interactions between genetic and environmental factors (Crabbe, 2002). In addition to genes involved in alcohol metabolism, and those encoding γ-aminobutyric acid (GABA) and dopaminergic receptors (see Dick and Bierut, 2006 for a review), genetic modulation of serotonergic (5-HT) neurotransmission has been extensively studied as a potential contributor to vulnerability to alcohol-related disorders.

Among several candidate genes, attention has focused on a 44-basepair insertion/deletion polymorphism in the promoter region of the gene encoding the serotonin transporter (5-HTT), which regulates 5-HT levels in the synaptic cleft (Gorwood et al., 2004). This putatively functional polymorphism (5-HTT-linked polymorphic region: 5-HTTLPR) may affect both transcriptional activity and transporter density. Its short (S) allele exhibits a 2- to 2.5-fold reduced basal transcription rate compared to the long allele (L) and reduces 5-HTT activity in the same proportions, which may affect brain 5-HT activity (Heils et al., 1996).

Since Sander’s first observation of a significant association between severe AD and the S allele of the 5-HTTLPR (Sander et al., 1997), a meta-analysis confirmed that allelic variation at the 5-HTTLPR contributed to risk for AD (Feinn et al., 2005), especially in severe, early-onset alcoholics. Furthermore, many studies have linked the excess of the S allele of the 5-HTTLPR to antisocial, violent, and impulsive type 2 (Hallikainen et al., 1999; Ishiguro et al., 1999) or suicidal alcoholic subjects (Gorwood et al., 2000; Preuss et al., 2000). As impulse control in AD seems to be partially influenced by this allele, we examined its possible impact on relapse in abstinent alcohol-dependent patients.

METHODS AND MATERIALS

Forty-eight male patients of Caucasian descent, who were at least 18 years old, and who met DSM IV criteria for AD participated in the study. Patients were excluded if they met criteria for other substance abuse or dependence except for nicotine, or for any other current or past psychiatric disorder. These individuals were hospitalized for 1 month for alcohol treatment, during which they were given a 1-week standardized benzodiazepine withdrawal treatment (Pinto and Ansseau, 2000) followed by abstinence-targeted individual and group interventions. They donated 3 ml of blood for genotyping. The Ethical Committee of the University of Liège Medical School approved the protocol and all participants provided written informed consent prior to participation in the study.

Participants were reevaluated 1 and 2 months after discharge, but no standardized psychotherapeutic intervention or anticraving medication was provided. Abstinence was evaluated through blood measures of gamma-glutamyl transferase (GGT) and carbohydrate-deficient transferin (CDT) which, when combined, seem to be the most reliable markers of alcohol misuse (Chen et al., 2003). In parallel to self-reported consumption, relapse to alcohol drinking was diagnosed whenever either test score was above cutoff after normalization during hospitalization or when patients failed to show up at scheduled appointments during the 2-month follow-up. Depressive symptoms (measured using the 52-item Carroll Depression Scale; max. score = 52; moderate to severe depression when score is above 19 (Carroll et al., 1981; Charles et al., 1986)) and anxiety symptoms (measured using the State-Trait Anxiety Inventory; min. score = 20; max. score = 80 (Spielberger et al., 1970; Spielberger, 1983)) were evaluated at day 1 of hospitalization. The daily number of drinks and number of previous alcohol...
detoxifications served as estimates of AD severity and were evaluated as possible factors influencing relapse.

Genomic DNA was extracted from peripheral leukocytes using the QIAmp DNA Mini Kit (QIAGEN Inc., Valencia, CA). The evaluated locus was genotyped by previously described PCR methodologies using published primer sequences (Gelernter et al., 1999). Amplification of the 5-HTTLPR gene was performed in 20 µl using 2 µl of genomic DNA, 0.16 µl of 25 mM dNTP, 0.8 µl of 50 mM MgCl2, 0.5 µl of Taq Eurobio Polymerase, and 0.05 µl of each primer 5′-ATG CCA GCA CCT AAC CCC TAA-3′ and 5′-GG ACC GCA AGG TGG GCG GGA-3′. The amplification cycles were as follows: 80°C for 20 min, 95°C for 5 min, followed by 95°C for 30 s, 65°C for 30 s, 72°C for 30 s (35 cycles), and terminated at 72°C for 8 min and 25°C for 10 min. Amplified fragments (L allele: 419 bp and S allele: 375 bp) were then separated by electrophoresis through 3% agarose gels with appropriate size standards and visualized by staining with ethidium bromide.

Fishers’s exact test was used to compare the presence or the absence of the S allele between relapsing and abstinent subjects throughout the 3 months of observation. This approach has been validated through many studies that grouped SS and SL genotypes (Feinn et al., 2005). Analyses of variance estimated the impact of depression, anxiety, and daily number of drinks on relapse. Finally, considering the number of potentially confounding variables, a forward stepwise logistic regression analysis was used to reveal parameters that may be important to distinguish the presence versus absence of relapse. Default P-values were used for stepwise entry (P = 0.10) and removal (P = 0.15) of predictors into the logistic model.

**RESULTS**

The mean age of the sample was 44 ± 8.8 years. Participants had drunk for an average of 15.0 ± 9.8 years. They had undergone an average of 1.9 ± 2.5 alcohol detoxifications prior to this hospitalization and took 19.1 ± 9.2 drinks per day at the time of the study. Their mean depression score was 17.2 ± 8.7 at day 1 while anxiety scores at the same time were 36.0 ± 12.67 for state anxiety and 45.59 ± 13.74 for trait anxiety.

Genotype frequencies in this sample demonstrated Hardy–Weinberg equilibrium. After 3 months, relapse had occurred in 30 patients (62.5%). Nineteen patients (63.4%) self-reported relapse. The mean age of the sample was 44 ± 8.8 years. Participants had undergone an average of 1.9 ± 2.5 alcohol detoxifications prior to this hospitalization and took 19.1 ± 9.2 drinks per day at the time of the study. Their mean depression score was 17.2 ± 8.7 at day 1 while anxiety scores at the same time were 36.0 ± 12.67 for state anxiety and 45.59 ± 13.74 for trait anxiety.

Relapse was neither influenced by depression score (p = .843) nor by anxiety (p = 0.327 for state anxiety and p = 0.878 for trait anxiety) at day 1. Furthermore, daily number of drinks (p = 0.087) and number of previous detoxifications (p = 0.992) did not influence relapse in our sample. None of these parameters was shown to influence type of relapse.

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>Wald Sig.</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>S allele</td>
<td>1.967</td>
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<td>0.010</td>
</tr>
<tr>
<td>Number of drinks</td>
<td>0.067</td>
<td>2.077</td>
<td>0.149</td>
</tr>
<tr>
<td>Carroll Depression Scale score</td>
<td>0.004</td>
<td>0.007</td>
<td>0.937</td>
</tr>
<tr>
<td>STAI—State Anxiety Scale score</td>
<td>−0.011</td>
<td>0.074</td>
<td>0.758</td>
</tr>
<tr>
<td>STAI—Trait Anxiety Scale score</td>
<td>−0.003</td>
<td>0.007</td>
<td>0.933</td>
</tr>
<tr>
<td>Constant</td>
<td>−1.56</td>
<td>0.984</td>
<td>0.321</td>
</tr>
</tbody>
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No other parameter than the S allele of the 5-HTTLPR influences relapse.

However, the higher relapse rate in the S-allele group did not seem to be influenced by the type of relapse (p = 0.068): lost to follow-up individuals did not significantly differ from proven relapsing patients in terms of genotype (Table 2).

A logistic regression confirmed that carrying the S allele of the 5-HTTLPR was the only variable that influenced relapse risk (Table 3). Finally, depression (p = 0.872) symptoms, anxiety symptoms (p = 0.370 for state anxiety and p = 0.635 for trait anxiety), daily number of drinks (p = 0.944), and number of previous detoxifications (p = 0.230) were not influenced by the presence of the 5-HTTLPR S allele.

**DISCUSSION**

There was a highly significant association between the presence of the S allele of the 5-HTTLPR and relapse occurrence during the 12-week observation period of our alcohol-dependent patients. Most of the subjects who showed objective evidence of resuming alcohol drinking or were considered relapers because of their absence at scheduled appointments carried the short variant of this polymorphism. However, even though no significant difference in genotype was observed between proven relapsing patients and those lost to follow-up, it cannot be formally ruled out that the observed difference between S carriers and noncarriers in terms of relapse is not partially influenced by differential dropout in the two groups.

5-HT is known to play a key role in the individual capacity to refrain from immediate achievement of a rewarding
action (Hollander and Rosen, 2000). Furthermore, 5-HT is also implicated in anxiety and depression and may also influence temperament (Deakin, 1998; Hollander and Rosen, 2000). Responsible for a deficit in 5-HT neurotransmission, the S allele of the 5-HTTLPR has been associated with a whole range of disorders characterized by depressive features and anxiety, as well as impulsivity and lack of behavioral inhibition (Gorwood et al., 2004; Feinn et al., 2005). Carriers of the S allele of the serotonin transporter polymorphism may therefore use a short-term, immediately rewarding strategy (alcohol drinking shortly after withdrawal), versus a long-term, apparently less rewarding one: abstinence in spite of craving. However, an A→G SNP within the L allele of the 5-HTTLPR has recently been described as a potential modulator of 5-HTT function (Hu et al., 2006), with the L_G allele being associated with reduced 5-HTT expression making it nearly equivalent to the S allele. Unrecognized L_G alleles in SL and LL phenotypes may therefore create the appearance of S allele dominance as far as 5-HTT expression is concerned. Due to limited availability of DNA for genotyping, we were unable to examine A→G SNP within the L allele in our subjects, so this will have to be taken into account in future studies.

Of course, the small size of our sample is a clear limitation of these results, which should be replicated in larger populations. Moreover, although the observed relapse rates may appear important, they are comparable to those reported in other studies (Willinger et al., 2002). Our results are nevertheless in accordance with many observations of a link between this allele and specific AD phenotypes characterized by high levels of impulsivity, such as early-onset alcoholism or antisocial personality disorder. Therefore, it is possible that the 5-HTTLPR S allele does not directly influence relapse, but rather exerts its effects through intermediate phenotypes, such as high levels of impulsivity or poor behavioral control, which are partly determined by its presence and the resulting 5-HTT hypoactivity. A clear evaluation of these parameters in future studies may contribute to get a clearer picture of the potential role of the 5-HTTLPR S allele in increasing the risk of relapse to alcohol drinking. However, depression and anxiety had no impact on alcohol consumption after withdrawal in our sample and were not influenced by the S allele of the 5-HTTLPR, consistent with a central role of the polymorphism in determining relapse risk.

Altogether, our results emphasize the necessity of using precisely defined phenotypes when studying complex behavioral phenomena such as AD from a genetic point of view. If replicated in larger samples, they may be useful in evaluating and developing novel pharmacogenetic approaches to treat AD and prevent relapse to alcohol drinking.

REFERENCES


