ASSOCIATION OF THE LONG ALLELE OF THE 5-HTTLPR POLYMORPHISM WITH
COMPULSIVE CRAVING IN ALCOHOL DEPENDENCE

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Abstract — Aims: Various studies have reported a role of the serotonin transporter-linked polymorphic region (5-HTTLPR) in alcoholism. Method: The present study investigated an association of this polymorphism with obsessive-compulsive alcohol craving in 124 male patients admitted for alcohol detoxification treatment. Results: We found significantly higher compulsive craving in patients with the long allele of the 5-HTTLPR polymorphism [at admission: analysis of variance (ANOVA): F = 3.48, P = 0.034, general linear model: F = 3.92, P = 0.023; after 7 days: ANOVA: F = 3.12, P = 0.049]. Conclusions: Our results suggest that the long variant of the 5-HTTLPR polymorphism is associated with higher compulsive alcohol craving at the beginning of alcohol withdrawal.

INTRODUCTION

There is growing evidence for involvement of the serotonergic system in alcohol dependence (LeMarquand et al., 1994a; Heinz et al., 1998). Deficits in the serotonergic neurotransmission have been associated with higher alcohol intake (LeMarquand et al., 1994b; Mosner et al., 1997) and with impulsive-aggressive behaviour (Johnson et al., 2000). A polymorphism of the serotonin transporter gene (5-HTT) may play a crucial role in the genesis and maintenance of alcohol dependence (Lichtermann et al., 2000). The short allele of the 5-HTTLPR polymorphism leads to reduced serotonin re-uptake in comparison to the long allele (Collier et al., 1996). Feinn et al. found evidence for an association of the short allele of the 5-HTTLPR with alcohol dependence (Feinn et al., 2005). However, in other than western populations studies have described an association of alcohol dependence with the long allele of the 5-HTTLPR (Kweon et al., 2005). Also, the serotonin transporter polymorphism has been associated with suicidal behaviour in alcohol-dependent subjects (Preuss et al., 2001; Angelova et al., 2003; Limosin et al., 2005).

Craving is an important phenomenon that contributes to relapse in alcohol dependence. While different definitions of craving exist, obsession and compulsion seem to be the most common characteristics (Modell et al., 1992; Lesch et al., 1997). The described findings regarding an involvement of the 5-HTTLPR polymorphism in alcohol dependence, and in suicidal behaviour, lead to the hypothesis that the 5-HTTLPR polymorphism may play a role in obsessive and/or compulsive alcohol craving. Therefore, the aim of the present study was to investigate a possible association of the 5-HTTLPR polymorphism and obsessive-compulsive alcohol craving during withdrawal.

METHODS

The present prospective case control study was part of the FARS (Franconian Alcoholism Research Studies) and was performed as described recently (Bleich et al., 2005; Hillemacher et al., 2004, 2006). All subjects were of Caucasian ancestry and were included in the study after written, informed consent, and after approval by the local ethics committee. We included 124 male patients (age: mean ± SD = 43.3 years, SD = 9.2) suffering from alcohol dependence according to ICD-10 and DSM-IV who were admitted to a closed detoxification unit. Patients with other psychiatric disorders, apart from nicotine dependence, were excluded from the study. Female patients (N = 31) were excluded because of the small number of patients available for statistical analysis, and because of previously described gender differences regarding the neurobiology of alcohol craving (Willner et al., 1998; Kraus et al., 2004; Hillemacher et al., 2005) and the 5-HTTLPR polymorphism (Limosin et al., 2005; Mizuno et al., 2006).

At admission, blood samples were taken and stored at −80°C immediately after collection. DNA extraction was performed using Qiagen DNA blood mini kit (Qiagen GmbH, Hilden, Germany). Amplification of 5-HTTLPR was done with Polymerase Chain Reaction (PCR; primers HTTLPR-F: GAGGGACTGAGCTGGACAACCAC; HTTLPR-R: GAGGGACTGAGCTGGACAACCAC; annealing temperature of 62.5°C; containing 10 ng DNA, 1× PCR buffer [50 mM KCl, 10 mM Tris–HCl, pH 9.0], 1.5 mM MgCl2, 0.2 mM each of dNTP, 0.5 mM of each primer, and 0.5 units of Taq DNA polymerase [Genecraft Supratherm]) on a thermocycler (BioRad). The PCR products were separated with electrophoresis (2% agarose gel) and visualized using 1 μg/ml of ethidium bromide (molecular imager). We measured the extent of withdrawal craving directly at admission for detoxification treatment (day 0) and after one week of treatment (day 7) using the Obsessive Compulsive Drinking Scale (OCDS), differentiating the obsessive and the compulsive subscale (Anton et al., 1995). The severity of withdrawal was assessed by the withdrawal syndrome scale for alcohol and related psychoactive drugs (WSA) (Kristensen et al.,...
1986). Status of intoxication at admission was determined by measurement of the blood alcohol concentration. The Fagerström Test for Nicotine Dependence (FTND) was used to assess the severity of nicotine dependence (Fagerström et al., 1990; Heatherton et al., 1991; Pomerleau et al., 1994).

Demographic data, like current smoking status, age, the daily intake of alcohol (DI: mean = 238.9 g/day, SD = 155.6) or the duration of drinking (YD: mean = 19.8 years, SD = 10.6) were taken in a structured interview by a trained observer (K.B.).

STATISTICAL ANALYSIS

All variables were normally distributed according to the Kolmogorov–Smirnov test. We used parametric methods using one-way ANOVA followed by Tukey’s test for multiple comparisons (post-hoc analysis). General linear models were used to confirm the results. Patients were divided into subjects with 2 long alleles (L/L), 1 short and 1 long allele (L/S) or 2 short alleles (S/S) of the 5-HTTLPR polymorphism. Deviation from Hardy–Weinberg equilibrium was assessed for 5-HTTLPR polymorphism. Analysis done with Tukey’s test for multiple comparison revealed significant differences between S/S and L/L patients (P = 0.031; Fig. 1) and a trend for the differences between S/S and L/S (P = 0.053). No significant differences were found for compulsive craving between L/S and L/L patients.

For the obsessive subscale (day 0) we found no differences regarding the different polymorphisms (data not shown).

RESULTS

Demographic characteristics of the study population are shown in Table 1. In our study sample, we found no deviation from Hardy–Weinberg equilibrium (F = −0.079, Pearson’s P = 0.47). Patients with the S/S variant of the 5-HTTLPR polymorphism showed lower compulsive alcohol craving at day 0 (mean = 9.5; SD = 4.1; N = 15) than patients with L/S (mean = 11.9, SD = 3.6; N = 71) or L/L variant (mean = 12.3, SD = 3.9; N = 38). Groups differed significantly in the extent of craving (ANOVA: F = 3.48, P = 0.034, see Table 1). The post-hoc analysis with Tukey’s test for multiple comparison revealed significant differences between S/S and L/L patients (P = 0.031; Fig. 1) and a trend for the differences between S/S and L/S (P = 0.053). No significant differences were found for compulsive craving between L/S and L/L patients.

From Table 1. Demographic characteristics of the study population

<table>
<thead>
<tr>
<th>5-HTTLPR-PM</th>
<th>L/L (N = 38)</th>
<th>L/S (N = 71)</th>
<th>S/S (N = 15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (years)</td>
<td>41.8 ± 7.4</td>
<td>43.4 ± 9.4</td>
<td>46.8 ± 11.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>daily intake (grams)</td>
<td>225.6 ± 101.3</td>
<td>238.5 ± 161.1</td>
<td>2747 ± 232.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>years of drinking</td>
<td>19.0 ± 10.6</td>
<td>20.1 ± 10.1</td>
<td>20.1 ± 12.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>age of onset</td>
<td>22.7 ± 7.7</td>
<td>23.4 ± 8.8</td>
<td>26.7 ± 11.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>smoking (yes/no)</td>
<td>32/6</td>
<td>55/16</td>
<td>13/2</td>
<td>n.s.</td>
</tr>
<tr>
<td>FTND day 0</td>
<td>5.9 ± 2.5</td>
<td>5.5 ± 2.4</td>
<td>4.4 ± 2.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>WSA day 0</td>
<td>1.49 ± 1.03</td>
<td>1.53 ± 1.25</td>
<td>1.67 ± 1.16</td>
<td>n.s.</td>
</tr>
<tr>
<td>OCDS day 0 total</td>
<td>19.9 ± 7.3</td>
<td>20.7 ± 7.9</td>
<td>17.1 ± 8.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>OCDS day 0 comp.</td>
<td>12.3 ± 3.9</td>
<td>11.9 ± 3.6</td>
<td>9.3 ± 4.1</td>
<td>0.034</td>
</tr>
<tr>
<td>OCDS day 0 obs.</td>
<td>7.6 ± 4.8</td>
<td>8.8 ± 4.9</td>
<td>7.8 ± 4.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>OCDS day 7 total</td>
<td>13.4 ± 6.6</td>
<td>11.1 ± 6.1</td>
<td>11.3 ± 7.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>OCDS day 7 comp.</td>
<td>9.6 ± 3.7</td>
<td>7.6 ± 3.4</td>
<td>7.3 ± 4.0</td>
<td>0.049</td>
</tr>
<tr>
<td>OCDS day 7 obs.</td>
<td>3.8 ± 3.8</td>
<td>3.5 ± 3.4</td>
<td>4.0 ± 3.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>intoxicated admission (yes/no)</td>
<td>28/10</td>
<td>5/17</td>
<td>10/5</td>
<td>n.s.</td>
</tr>
<tr>
<td>BAC day 0 (mg/dl)</td>
<td>106.0 ± 89.9</td>
<td>131.4 ± 119.8</td>
<td>111.1 ± 103.4</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation. OCDS: Obsessive Compulsive Drinking Scale, differentiating the total score, the obsessive subscale (obs.) and the compulsive subscale (comp.); 5-HTTLPR-PM: Serotonin transporter polymorphism with long (L) or short (S) variants; BAC: Blood alcohol concentration at admission; FTND: Fagerström Test for Nicotine Dependence; WSA: withdrawal syndrome score for alcohol and related psychoactive drugs. Day 0: admission for in-patients treatment; day 7: after 7 days of treatment (for variables referring to day 7 please note reduced number of subjects: L/L = 27; L/S = 54; S/S = 10). P: statistical differences on a significant level, using one-way analysis of variance (ANOVA) or Chi-Square test; n.s.: not significant.
for a difference between L/L and L/S patients (Kweon et al., 2005). As described by this study group, the long allele should be associated with higher serotonin transporter function, and therefore be associated with lower serotonin levels due to increased re-uptake. This would be in line with recent studies describing a positive effect on craving and relapse for serotonin re-uptake inhibitors like citalopram and fluvoxamine (Naranjo et al., 1992; Anguelova et al., 1998). Furthermore, the long allele has been associated with obsessive-compulsive disorder (Bengel et al., 1999), which shares various psychological features with alcohol craving. Of course, many other neurotransmission systems are involved in alcohol craving GABA or glutamate (Johnson, 2005). In face of the heterogeneity of the disease ‘alcohol dependence’, future search for endophenotypes and subtypes appears to be helpful.

Lately, a novel variant of the L-allele of the 5-HTTLPR has been described that has some functional relevance (Parsey et al., 2006). This variant has not been analysed in our study in order not to diminish the power of our analysis. Further studies should look at the relevance of this variant for alcohol dependence and craving.

In conclusion, our results suggest an association of compulsive alcohol craving with serotonergic neurotransmission, and the long allele of the serotonin transporter polymorphism. Further studies on the association of the 5-HTTLPR polymorphism and craving in alcohol dependence are needed to verify these preliminary results.

REFERENCES


Using general linear models (dependent variable: compulsive subscale day 0, covariates: age, DI, YD, fixed factor: 5-HTTLPR polymorphism), the results could be confirmed. We found significant results for DI ($F = 10.58$, $P = 0.002$) and for the 5-HTTLPR polymorphism ($F = 3.92$, $P = 0.023$).

We repeated the assessment of the OCDS after seven days of treatment. As shown in Table 1, L/L-patients showed highest scores for compulsive alcohol craving compared to L/S- and S/S-patients (ANOVA: $F = 3.12$, $P = 0.049$; $N = 91$). The post-hoc analysis with Tukey’s test showed a trend for a difference between L/L and L/S patients ($P = 0.054$, Fig. 2). We found no significant differences for the OCDS total score or obsessive subscale at day 7.

DISCUSSION

The findings of the present study show an association of higher compulsive but not obsessive alcohol craving at the beginning of alcohol withdrawal in patients with the long allele of the 5-HTTLPR polymorphism. The association between a ‘static’ variable like the 5-HTTLPR polymorphism and a ‘dynamic’ feature like craving always depends on various possible influencing factors like time of assessment and environmental factors. In our study population, we found no significant differences between the genotype subgroups regarding possible influencing factors like smoking, age of onset, daily ethanol intake or severity of the alcohol withdrawal syndrome. The lack of significant differences using Tukey’s post-hoc test at day 7 may be a result of the smaller number of patients at this second assessment. These results are in contrast to most studies showing that the short allele may be associated with alcohol dependence, including a meta-analysis of 17 studies (Lichtermann et al., 2000; Feinn et al., 2005). However, in a recent study in a Korean population, an association with the long allele was found (Kweon et al., 2005). Kweon et al. explained their results with ethnic differences of the serotonin transporter gene and with uncertainties regarding the 5-HTTLPR polymorphism and its interaction with the central serotonergic activity (Kweon et al., 2005). As described by this study group, the long allele should be associated with higher serotonin transporter function, and therefore be associated with lower serotonin levels due to increased re-uptake. This would be in line with recent studies describing a positive effect on craving and relapse for serotonin re-uptake inhibitors like citalopram and fluvoxamine (Naranjo et al., 1992; Anguelova et al., 1998). Furthermore, the long allele has been associated with obsessive-compulsive disorder (Bengel et al., 1999), which shares various psychological features with alcohol craving. Of course, many other neurotransmission systems are involved in alcohol craving GABA or glutamate (Johnson, 2005). In face of the heterogeneity of the disease ‘alcohol dependence’, future search for endophenotypes and subtypes appears to be helpful.

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In conclusion, our results suggest an association of compulsive alcohol craving with serotonergic neurotransmission, and the long allele of the serotonin transporter polymorphism. Further studies on the association of the 5-HTTLPR polymorphism and craving in alcohol dependence are needed to verify these preliminary results.

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