ASSOCIATION OF THE DOPAMINE TRANSPORTER GENE WITH ALCOHOLISM

MICHAEL D. KÖHNKE*, ANIL BATRA, WERNER KOLB1, ANNETTE M. KÖHNKE, ULRICH LUTZ, SANDRA SCHICK and INES GAERTNER

University Hospital of Psychiatry and Psychotherapy, Tübingen University Hospital, 72076 Tübingen, Germany and Wilhelmshospital, 71570 Oppenweiler, Germany

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Abstract — Aims: It was investigated whether the allele A9 of the dopamine transporter gene (DAT1; SLC6A3) is associated with alcoholism, delirium tremens (DT), alcohol withdrawal seizures (AWS), or the daily alcohol intake. Methods: A group of 102 healthy subjects and 216 alcoholics, including 97 patients with a history of mild withdrawal symptoms, 65 with a history of AWS and 83 with a history of DT were genotyped and personal data were achieved for statistical evaluation in a case–control design. Results: The frequency of individuals carrying the allele A9 [f(A9+) = 0.01] in the group of alcoholics [f(A9+) = 0.48] compared with healthy controls [f(A9+) = 0.32]. There was no significant association of the allele A9 with severe withdrawal symptoms or the daily amount of alcohol consumed. Conclusions: Our results reveal that the allele A9 is strongly associated with alcoholism but not with withdrawal symptoms or daily alcohol intake.

INTRODUCTION

Family studies and animal models give evidence for heritability in the aetiology of alcohol dependence (Merikangas, 1990; Goldman, 1993). The mesolimbic dopamine system plays a critical role in reinforcing alcoholism and in the rewarding effects of alcohol (Fujimoto et al., 1983; Nestler et al., 1993; Mc Bride et al., 1995; Zhou et al., 1995). In comparison to healthy individuals, alterations in dopamine metabolites in the plasma of abstinent alcoholics have been detected (Fulton et al., 1995; Köhnke et al., 2003). Dysfunction in dopaminergic transmission has been associated with craving for ethanol (Wise, 1988) and seems to influence withdrawal symptoms (Harris and Aston-Jones, 1994; Heinz et al., 1996).

Thus, many studies on the genetic background of alcoholism have analysed genes of the dopamine system especially by focussing on homogenous subgroups of alcoholics which can be defined by the onset of delirium tremens (DT) or alcohol withdrawal seizures (AWS). These candidate gene approaches have so far presented inconsistent results (Gelernter, 1993; Batra and Köhnke, 2002).

As the dopamine transporter (DAT) is of major importance for the regulation of dopaminergic neurotransmission (Giros et al., 1996), the DAT gene (DAT1; SLC6A3) can be considered as a candidate gene for alcoholism and severe alcohol withdrawal symptoms. DAT1 is located on chromosome 5q15.3 and includes variable numbers of tandem repeats (VNTR) in the 3’-untranslated region of the gene (Vandenbergh et al., 1992) which can be used as a polymorphic marker of the DAT1 locus and thus is of major interest for research on addiction.

Three independent association studies on different ethnic populations have failed to show that the DAT gene plays a significant role in the vulnerability to alcoholism (Franke et al., 1999; Chen et al., 2001; Foley et al., 2004). Muramatsu and Higuchi (1995) could show in a Japanese sample that the frequency of the 7-repeat allele of the DAT1 VNTR was significantly higher in alcoholics with a point mutation in the aldehyde dehydrogenase-2 gene (ALDH2*2) than in control subjects. In contrast to the results of Franke et al. (1999), two other independent studies on German individuals were able to detect a significant association of the allele A9 with severe withdrawal symptoms (Sander et al., 1997; Schmidt et al., 1998). Gorwood et al. (2003) found an increased number of allele A9 carriers in 34 alcoholics with either AWS or DT compared with 65 healthy controls. Wernicke et al. (2002) could show a significantly increased number of the allele A of DAT G2319A in homozygous carriers of the allele A10 of DAT1 with AWS and DT. Limosin et al. (2004) found that the allele A9 increases the risk of visual hallucinations during alcohol withdrawal in alcohol-dependent women, thus postulating a sex-specific involvement in vulnerability to alcohol withdrawal complications.

All former studies had compared alcoholic patients with AWS or DT with healthy controls. In contrast, we compared for the first time a group of alcoholics with DT and a group of alcoholics with AWS or DT with healthy controls. In contrast, we compared for the first time a group of alcoholics with DT and a group of alcoholics with AWS or DT with healthy controls. In contrast, we compared for the first time a group of alcoholics with DT and a group of alcoholics with AWS or DT with healthy controls.

METHODS

Subjects

The study protocol had been approved by the Ethics Committee of the University Hospital of Tübingen. Written informed consent was obtained from all participants. All participating individuals were unrelated and of German descent. The diagnostic assessment of all participating individuals was carried out without knowledge of the genotype data by an interview based on the Structured Clinical
Interview for the Diagnostic and Statistical Manual of Mental Disorders DSM IV (American Psychiatric Association, 1994), SKID (Wittchen et al., 1997). Persons with primary major psychiatric disorders, severe somatic problems, substance dependence other than alcohol or nicotine, or individuals receiving psychotropic drugs were excluded.

A total of 102 healthy German controls (36 females, 66 males; mean age 37.4 years, SD ± 10.7 years) were recruited in southwest Germany by advertisements. No money was paid for participation. A reason for excluding controls was a positive family history for addiction (other than nicotine) and other psychiatric disorders or an age younger than 24 years to ensure a correct diagnosis. All included controls revealed moderate alcohol consumption in accordance with social drinking.

Inpatients (n = 216) fulfilling the criteria for alcohol dependence according to DSM IV were recruited from the University Hospital Tübingen of Psychiatry and Psychotherapy and from the Hospital for Addictive Disorders at Wilhelmsheim (40 females, 176 males; mean age 43.6 years, SD ± 8.5 years).

A total of 83 patients (10 females, 73 males; mean age 45.8 years, SD ± 8.89 years) were assigned to the group of alcoholics with a history of DT according to DSM IV. A group of 25 patients with DT were recruited at Wilhelmsheim Hospital and 58 patients at the University Hospital Tübingen of Psychiatry and Psychotherapy. The group of alcoholics with a history of AWS included 65 persons (8 females, 57 males; mean age 44.1 years, SD ± 8.5 years). A group of 29 patients were assigned to both groups as they had suffered from AWS and DT.

Alcoholic patients (n = 97) of the University Hospital Tübingen of Psychiatry and Psychotherapy who had experienced only mild withdrawal symptoms as defined by a score <12 in the Clinical Institute Withdrawal Assessment for Alcohol Scale, CIWA-Ar, (Sullivan et al., 1989) within the first 24 h of withdrawal (mean score 1.02, SD ± 2.35), whose withdrawal symptoms did not worsen in the course of withdrawal, and had no history of severe withdrawal were included in a separate group (26 females, 71 males; mean age 41.67 years, SD ± 7.9 years). Most of the individuals of this group took no medication. A minimal dosage of clomethiazol was administered to 21 patients to relieve mild symptoms (high blood pressure, tachycardia) of withdrawal. The mean dosage of clomethiazol for these 21 patients was 1.05 g (SD ± 0.61 g) during the first 24 h of application and was reduced afterwards.

The number of years patients with mild withdrawal symptoms had been diagnosed as alcohol addicted (mean 9 years, SD ± 7 years) did not differ significantly (P = 0.5) from the period of time between the onset of alcoholism and the first DT or AWS in alcoholics with severe withdrawal symptoms (10 years, SD ± 8 years).

The individual average daily alcohol consumption of all included alcoholics was asked for and documented.

**DAT1 genotyping**

Genomic DNA was extracted from all participants using standard procedures (Miller et al., 1988). PCR primers for the DAT1 polymorphism were used as previously described (Sano et al., 1993). PCR reactions were run in a total volume of 30 µl and contained 1.5 mM of MgCl₂, 0.25 mM of each dNTP, 1.1 U of *Taq* DNA polymerase (Gibco-BRL), a 1X PCR-buffer as supplied by the manufacturer, 0.3 µM of each primer and 25 ng of genomic DNA. PCR assays were performed with 35 cycles with denaturation at 93°C for 30 s, annealing at 63°C for 60 s and extension at 72°C for 60 s. The PCR products were separated on 3% agarose gels with direct visualization with ethidium bromide under UV light. A 100 bp ladder (Gibco-BRL) was used for estimation of fragment sizes.

**Statistical analysis**

The frequencies of A9-allele carriers of the DAT1 polymorphism were compared between groups using a 2 X 2 contingency table and Fisher’s exact test. A significance level of 5% was chosen for a type 1 error. Deviations from Hardy–Weinberg equilibrium (HWE) were assessed using the HWSIM program (http://info.med.yale.edu/genetics/kkidd/programs.html) (Cubells et al., 1997) and the GENEPOP program (http://wbiomed.curtin.edu.au/genepop/helpinput.html) (Raymond and Rousset, 1995).

The daily amount of consumed alcohol and its relation to genotype was analysed using the Kruskal–Wallis test.

### RESULTS

Of the 318 individuals who were genotyped for the DAT1 polymorphism, 135 were carriers of the A9 allele (f = 0.42); 17 individuals carried the homozygous A9 genotype (f = 0.05). The allele A10 was the most frequent allele (f = 0.74) followed by the allele A9 (f = 0.24). All other alleles were only found with very low frequencies (f < 0.01).

For all analysed groups genotype frequencies did not deviate significantly from HWE (P > 0.21 in all cases). The frequency of individuals carrying the allele A9 was significantly (P = 0.01) higher in the group of alcoholics [f(A9+) = 0.48] compared with healthy controls [f(A9+) = 0.32] (Table 1). Furthermore, owing to a disproportional distribution of gender in the group of alcoholics and healthy controls, each gender was analysed separately. In male alcoholics (n = 176), the frequency of the allele A9 remained significantly elevated (P = 0.02) compared with 66 male healthy controls. In females, the frequency of the allele A9 was elevated [f(A9+) = 0.55] in alcoholics (n = 40) compared with the frequency in female healthy controls [f(A9+) = 0.32; n = 36], but did not reach statistical significance (P = 0.18).

By comparing the frequencies of allele A9 carriers of the subgroup of alcoholics with a history of severe withdrawal symptoms (DT or AWS) with the frequencies of allele A9 carriers of alcoholics with a history of only mild withdrawal symptoms no significant differences were found (Table 2).

All alcoholic patients (n = 216) were asked to give information on their average daily amount of alcohol consumed in

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>f(A9+)</th>
<th>P(f(A9+))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholics</td>
<td>216</td>
<td>0.48</td>
<td>0.01</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>102</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

f(A9+), frequencies of allele A9 carriers; P(f(A9+)), significance level for 2 × 2 statistic (2 degrees of freedom) are given for comparison of allele A9 carrier frequencies between the two groups.

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the months prior to admission. Kruskal–Wallis test was applied to analyse whether the allele A9 carrier status has an influence on daily alcohol consumption. Of these, 103 alcoholics (f = 0.48) carried the allele A9 and 113 alcoholic patients (f = 0.52) presented another genotype. The daily consumption of alcohol did not differ significantly (χ² = 0.48, P = 0.49) between the group of alcoholics with the allele A9 (median 200 g/day, range 60–1600 g/day, mean 264 g/day, SD 193 g/day) and other genotypes (median 240 g/day, range 60–1600 g/day, mean 264 g/day, SD 204 g/day).

**DISCUSSION**

The major finding of this study is that the DAT1 allele A9 carrier status is significantly associated with the diagnosis of alcoholism in the examined western European sample. Whereas only 32% of the healthy control subjects carried the allele A9, 48% of all alcoholics presented the allele A9 carrier status. When each gender was analysed separately, the results remained significant for male subjects. However, in female alcoholics, an elevation of the allele A9 was also found not reaching statistical significance, most likely because of the small number of female subjects included.

With the results of our study the question arises, how the examined VNTR polymorphism, that finds its location in the 3′-untranslated region of DAT1, could have an effect on the aetiology of alcoholism. Linkage disequilibrium with an influential mutation affecting the functions of the DAT protein is one possibility. However, only two rare missense substitutions and three silent mutations in the neighbouring regions have been detected so far and seem to have no significant influence on the DAT protein (Grunhage et al., 2000). Michelhaugh et al. (2001) could show that the DAT1 VNTR itself may enhance transcription. This enhancing activity suggests that the DAT1 VNTR plays an important role in regulation of DAT gene expression, a finding that was confirmed by another independent study (Miller and Madras, 2002). Heinz et al. (2000) found that alcoholics with the status of an allele A9 carrier had a reduced DAT protein density in the putamen by a mean of 22% compared with other DAT genotypes most probably caused by decreased expression rates. Tiithonen et al. (1995) were able to show that the striatal DAT density was markedly lower in non-violent alcoholics than in healthy controls (P < 0.001). These findings suggest that the DAT1 VNTR is most probably a functional polymorphism and that the DAT1 VNTR might influence the aetiology of alcoholism by lowering the expression rate of DAT. The hypothesis that the allele A9 carrier status of DAT1 is associated with severe alcohol withdrawal symptoms has not been confirmed by this study. In contrast to all former studies, we applied for the first time a case–control design with a clearly defined and exactly examined control group of alcoholics with only mild withdrawal symptoms. Prior studies had examined only very small samples or as in the study of Sander et al. (1997) the individuals with severe withdrawal symptoms were compared with healthy controls and not with persons who had revealed mild withdrawal symptoms. Therefore, it can be concluded that the allele A9 is more likely a marker for alcoholism than for severe withdrawal symptoms.

Instead of a genetic background it could be hypothesized that DT and AWS result from the toxic influence of alcohol on cerebral structures. A post-mortem study revealed destructive influences of alcohol on cerebral structures (Arango et al., 1994). Schuckit et al. (1995) were the first to show that the severity of withdrawal symptoms is associated with the amount of daily alcohol consumption. As previously published, we were able to give further evidence in favour of this hypothesis (Koehnke et al., 2002). Interestingly, the number of years patients with mild withdrawal symptoms had been diagnosed as alcohol addicted did not differ significantly from the period of time between the onset of alcoholism and the first DT or AWS in alcoholics with severe withdrawal symptoms. In the present study, we showed that the allele A9 carrier status of DAT1 did not influence the daily consumption of alcohol. It can be criticized that we relied on retrospective information of patients lacking an objective control as far as the daily consumption of alcohol is concerned.

To summarize the results of this study, strongly suggest an influence of the DAT1 VNTR on the aetiology of alcoholism but not on withdrawal symptoms or daily amount of alcohol consumed. Further studies with large samples should try to replicate our findings.

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