ABSTRACT The human dopamine receptor D2 gene is an important candidate gene for drug addiction, alcoholism and/or for the modification of its severity. In the present study, the distribution of DRD2 polymorphism (Ser311/Cys311) among a random Siberian female population sample was examined and correlated with self-reported alcohol drinking and alcohol-induced flushing response. The Cys311 variant was detected by DNA amplification using PCR followed by digestion with the restriction enzyme Sau96I. The Ser311/Cys311 heterozygous genotype was present in 4% of the samples and the frequency of the Cys311-encoding allele was found to be 0.021. Subjects with Cys311/Cys311 allele consumed alcohol less frequently than subjects with Ser311/Cys311 allele.

INTRODUCTION

The human dopamine 2 receptor gene (DRD2) is one of the candidate genes possibly contributing to the genetic vulnerability for several neuropsychiatric diseases, including schizophrenia, alcoholism and/or drug abuse (Blum et al. 1995; Noble 2000; Finckh 2001; Lu et al. 2001). Restriction of DRD2 by TaqI yields polymorphic fragment lengths identifying alleles A1 and A2 which differ in average amounts of receptor sites. Since the first report by Blum et al. (1990) suggesting an association of D2 dopamine receptor gene and alcoholism, the possible role of DRD2 receptor locus in the etiology of alcoholism has been the focus of considerable attention (Pato et al. 1993; Kidd et al. 1996; Noble 2000). However, no evidence of linkage of A1 allele and increased susceptibility to alcoholism in family-based and association studies has been found (Gelernter and Kranzler 1999; Sander et al. 1999; Blomqvist et al. 2000; Parisian et al. 2000). More recent reports, nevertheless, do hint to a possible linkage of the dopamine receptor gene with medical complications of alcoholism (Lawford et al. 1997). Moreover, polymorphism of the D2 dopamine receptor gene seems to be an important determinant of F300 latency (Nacher 2000). The observed association of the alleles of the human D2 dopamine receptor locus may not possibly be due to linkage with a gene for alcoholism, but could be a cause of the progression of the disease in individuals genetically predisposed to alcoholism.

In addition to the widely distributed TaqI polymorphism (Kidd et al. 1998), the human dopamine D2 receptor gene has three other polymorphic variants that predict the amino acid substitutions Val96→Ala, Pro310→Ser, and Ser311→Cys in the receptor protein. The C→G polymorphism in exon 7 of the D2 receptor gene leads to Ser311/Cys311 mutation (Itokawa et al. 1993). The reported frequencies of the Ser311/Cys311 variant allele vary from 0.019 to 0.035 in Japanese (Itokawa et al. 1993; Arinami et al. 1994; Higuchi et al. 1994; Nanko et al. 1994), and from 0.008 to 0.018 in Caucasian healthy subjects (Gejman et al. 1994; Sobell et al. 1994). An association of the structural Ser311/Cys311 variant with alcoholism was reported in a large Japanese sample (Higuchi et al. 1994). Moreover, an association of the DRD2 Ser311/Cys311 variant with schizophrenia has been reported (Arinami et al. 1994; Kaneshima et al. 1997; Serretti et al. 1998). However, data from other investigators do not support an association of Ser311/Cys311 variant with schizophrenia and/or alcoholism (Chen et al. 1996; Crawford et al. 1996; Finckh et al. 1996; Tanaka et al. 1996; Verga et al. 1997; Goldman et al. 1998; Spurlock et al. 1998).

In the present study we have examined the incidence of Ser311/Cys311 mutation among a Siberian female population sample from Novosibirsk (n=299). The frequency of the variant allele was correlated with the self-reported alcohol consumption and the incidence of alcohol-induced flushing response.
MATERIALS AND METHODS

The study was carried out on blood samples collected under the WHO multinational program MONICA (“Monitoring of Trend and Determinants in Cardiovascular Diseases”). It is a larger project that includes an investigation of cardiovascular disorders, diabetes mellitus, anemias, alcohol consumption and other medical anomalies. A total of 875 women (age = 25-65 yrs) were investigated, using confidential interviews with the help of a questionnaire and medical examinations. Venous blood was obtained for biochemical and genetic analysis from 299 females DNA samples were prepared from clotted blood using conventional methods (treatment with proteinase K, phenol-chloroform extraction and ethanol precipitation).

For DNA amplification the following primers (5’-CCAGCTGACTCTCCCCGACCGGT-3’) and (5’-TTGGGCTATGGCTGGATCTCAAA-3’) were used as forward and reverse primers, respectively. Twenty five microliters of the PCR reaction mixture contained 0.5 µl of 10 mM solution of dNTP; 2.5 µl of PCR buffer; 0.5 µl of 100 pM solution of each primer; 0.8 µl of 50 mM MgCl$_2$; 19 µl H$_2$O; 1 µl DNA and 1 unit of Taq polymerase. Amplification was performed in the following mode: 1 cycle 4 min at 95 °C, 2 min at 65 °C; 35 cycles, 30 sec at 95 °C, 50 sec at 65°C, 10 sec at 72°C; 1 cycle, 10 min at 72°C.

Eight microliters of PCR product was digested with the restriction enzyme Sau969 for overnight. The DNA bands were separated on 8% polyacrylamide gel and visualized by silver staining. Amplification product size was 112 bp for the homozygous Ser$_{311}$/Ser$_{311}$ genotype, 112 and 133 bp for the heterozygous Ser$_{311}$/Cys$_{311}$ genotype and 133 bp for the homozygous Cys$_{311}$/Cys$_{311}$ genotype.

RESULTS AND DISCUSSION

A number of studies have been published concerning alcohol drinking pattern in Russian populations (Bobak et al. 1999; Nemtsov 2000). While in many studies a relationship between alcohol metabolism genes and alcohol consumption has been investigated (Kurilovich et al. 1998; Belkovets et al. 2001), only a few studies have reported on the polymorphism of DRD2 gene in Russian populations (Avksentyuk et al. 1995; Galeeva et al. 2000). The major objective of the present study was to document drinking patterns and flushing response in relation to DRD2 receptor gene polymorphism among the female urban white population living in Novosibirsk.

Genotype and Allele Frequency Distribution

The distribution of the DRD2 genotypes among Novosibirsk sample is presented in Table 1. Cys$_{311}$ variant allele was found to be 0.021, quite similar to that found in other Caucasian populations (0.008-0.018) (Gejman et al. 1994; Sobell et al. 1994; Craddock et al. 1995).

The distribution of DRD2 genotypes in groups with different frequency of alcohol consumption are presented in table 2. While the common Ser$_{311}$/Ser$_{311}$ homozygous genotype was found in all the three alcohol drinking groups, the mutant allele was found only in group 2 and group 3 subjects. However, neither the genotype nor the allele frequency distribution showed a statistically significant difference between any of the three groups.

Table 1: Distribution of DRD2 genotypes and allele frequency in Siberia (n=299)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype frequency (%)</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser$<em>{311}$/Ser$</em>{311}$</td>
<td>95.98</td>
<td>Ser$<em>{311}$ = 0.979 Cys$</em>{311}$ =0.021</td>
</tr>
<tr>
<td>Ser$<em>{311}$/Cys$</em>{311}$</td>
<td>4.01</td>
<td></td>
</tr>
<tr>
<td>Cys$<em>{311}$/Cys$</em>{311}$</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Percentage distribution of DRD2 genotypes in different alcohol drinking groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Alcohol consuming group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>Ser$<em>{311}$/Ser$</em>{311}$</td>
<td>5.3%</td>
</tr>
<tr>
<td>Ser$<em>{311}$/Cys$</em>{311}$</td>
<td>-</td>
</tr>
<tr>
<td>Cys$<em>{311}$/Cys$</em>{311}$</td>
<td>-</td>
</tr>
</tbody>
</table>

Alcohol Drinking Habits vs. Genotypes

The majority of the subjects (84.7%) reported to consume alcohol only episodically (1-2 times a month). Only about 7% of the subjects reported to consume alcohol at least once per week (regular drinkers). The current and past abstainers constituted about 8.3%. The average dose of alcohol consumed per drinking occasion was about 27.2 g pure alcohol.

Alcohol-induced Flushing Response

Out of a total of 726 females questioned, 28.5% reported that they experienced facial flushing; 15.6% experienced flushing “sometimes” and 12.9% experienced flushing “often”. The major-
ity of those females who experienced flushing (83.8%) were able to continue drinking after flushing began. However, 8.1% were not able to continue drinking and 8.1% could continue only sometimes. Many females reported to have adverse manifestations after alcohol ingestion (symptoms which usually accompany flushing). Nausea was reported by 0.3% of the often-flushed and by 2.3% of females who flushed sometimes only; in females reporting often and sometimes flushing, feeling drowsy was reported by 1.1% and 5.9%; feeling dizziness by 0.3% and 3.5%; increased heart beat by 0.6% and 2.2%; sweating by 0.5% and 1.8%; headache by 1.0% and 3.0%, respectively. Many of the flushers noted that not all types of drinks caused flushing but it was more often induced by vodka (42.6% cases) and sparkling wine (champagne) (32.6% cases).

No significant relationship between distribution of the Cys311 variants and alcohol drinking habits was noted. Moreover, no significant relationship between the genotype frequency and flushing response as well quantity of drinking and the prevalence of alcohol-related symptoms was observed. Taken together the present study do not hint to a strong association between Ser311/Cys311 mutation and alcohol drinking habits in Siberian females. Moreover, as the mutant allele (Cys311/Cys311) is present in very low frequency in Siberians and other Europeans, it is difficult to assess its involvement in the etiology of alcohol abuse. Thus, the results of the present study support an earlier report (Goldman et al. 1998) showing that Ser311/Cys311 mutation in DRD2 gene is not linked to alcohol abuse and/or alcoholism.

REFERENCES


Kidd KK, Morar B, Castiglione CM, Zhao H, Pakstis AJ, Speed WC, Bonne-Tamir B, Lu RB, Goldman D,


