Population-Based Case-Control Study of DRD2 Gene Polymorphisms and Alcoholism

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Available online: 05 Oct 2010
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ABSTRACT. Several independent lines of evidence for genetic contributions to vulnerability to alcoholism exist. Dopamine is thought to play a major role in the mechanism of reward and reinforcement in response to alcohol. D2 dopamine receptor (DRD2) gene has been among the stronger candidate genes implicated in alcoholism. In this study, alcohol use was assessed in 196 randomly selected Kota individuals of Nilgiri Hills, South India. Six DRD2 SNPs were assessed in 81 individuals with alcoholism and 151 controls to evaluate the association between single nucleotide polymorphisms (SNPs) and alcoholism. Of the three models (dominant, recessive, and additive) tested for association between alcoholism and DRD2 SNPs, only the additive model shows association for three loci (rs1116313, TaqID, and rs2734835). Of six studied polymorphisms, five are in strong linkage disequilibrium forming a single haplotype block. Though the global haplotype analysis with these five SNPs was not significant, haplotype analysis using all six SNPs yielded a global P value of .033, even after adjusting for age. These findings support the importance of dopamine receptor gene polymorphisms in alcoholism. Further studies to replicate these findings in different populations are needed to confirm these results.

KEYWORDS. DRD2, SNP, alcoholism, India

INTRODUCTION

Alcoholism is a complex and multifactorial disorder arising from interactions between genetic and environmental risk factors. Several independent lines of evidence for genetic contributions to vulnerability to alcoholism exist. Evidence from twin, adoption, and family studies show that alcoholism is genetically influenced, with a heritability of 50% to 60%. Although the prevalence of alcoholism differs significantly between cultures, as well as between sexes within the same culture, the vulnerability to alcoholism corresponds in part with...
interindividual differences in pharmacokinetics and the pharmacodynamic responses to alcohol. Our knowledge about alcohol’s action in the brain is very little, unlike other drugs such as cocaine, marijuana, and heroin that interact with specific proteins; a more complex drug such as alcohol has the potential to interact with many proteins in the brain. The genes encoding these proteins are a possible source of variation in susceptibility to alcoholism.

The central catecholamines, especially dopamine, are thought to play a major role in the central reward process. Animal and human studies of addiction indicate that the D2 dopamine receptor (DRD2) plays a critical role in the mechanism of reward and reinforcement behavior. Any genetic polymorphism that affects the expression of DRD2s may be a possible candidate for susceptibility to alcoholism. The DRD2 gene, which encodes one receptor for dopamine, is localized on human chromosome 11 at q22–q23, extends over 270 kb, and has 8 exons. This transmembrane receptor is a member of the rhodopsin family. To date, the DRD2 gene has been among the stronger candidate genes implicated in alcoholism and other substance use disorders.

TaqIA restriction fragment length polymorphism (rs1800497) has been reported to be located in the 3’ flanking region of the DRD2 gene, which was subsequently recognized as a functional coding polymorphism of the adjacent functionally unrelated gene ANKK1. The A1 allele of Taq1A has been associated with alcoholism, and with other substance use disorders, including cocaine, nicotine, opiate dependence, polysubstance abuse, and obesity. Several more recent studies have reported an increase of A1 frequency in alcoholics with more severe forms of alcoholism. Eriksen et al. reported that individuals with alcoholism with the A1 allele have low platelet monoamine oxidase-B activity compared with those with the A2 allele. The A1 allele is associated with high dopamine transporter density in detoxified alcoholics. In this study, we examined the association between alcoholism and 6 SNPs in the DRD2 gene using a population case-control sample.

**MATERIALS AND METHODS**

**Participants**

We have recruited 196 participants from the Kota population of Nilgiri Hills, Tamil Nadu. Among the study population, 81 participants were defined as alcoholism cases and 115 were defined as controls based on the Michigan alcoholism screening test. A score of 0 to 2 was considered a controls, more than 6 was considered an alcoholics, and 3 to 5 were excluded from the analysis because they may develop alcoholism in future. In the control sample, present and past alcohol-related problems were ruled out using the Alcohol Use Disorders Identification Test. All samples were collected with written informed consent from the participants; procedures for protection of human participants in this study were approved by the Institutional Ethical Review Committee of CCMB, Hyderabad.

**DRD2 Genotyping**

Genomic DNA was extracted from peripheral blood leukocytes using the detailed protocol described in Thangaraj et al. The details of the selected SNPs are described elsewhere. Genotyping was carried out by using direct sequencing (Applied Biosystems, Foster City, CA). Briefly, PCR reactions were performed in a Gene Amp 9600 Thermal cycler (Perkin Elmer, Pomona, CA) in a 10 µL total volume and using 1.0 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). PCR amplicons (70 ng) were directly sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) in an ABI3730 automated DNA Analyser (Applied Biosystems, Foster City, CA).

**Statistical Analyses**

Allele frequencies were determined by direct gene counting. The genotype distribution for each site in each sample was evaluated for departure from Hardy-Weinberg equilibrium using the HWSIM program. We examined the effect of the common allele in three genetic models.
TABLE 1. Six DRD2 Gene SNPs and Their Associations with Alcoholism Under Different Genetic Models

<table>
<thead>
<tr>
<th>SNP</th>
<th>Alcoholism</th>
<th>Genotype (n)</th>
<th>MAF</th>
<th>HWE P</th>
<th>Overall HWE P</th>
<th>OR (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dominant</td>
<td>Recessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqIB</td>
<td>Case</td>
<td>AA (9)</td>
<td>AG (25)</td>
<td>GG (47)</td>
<td>0.265 0.061</td>
<td>0.091 0.846 (0.568) 1.313 (0.574) 0.958 (0.852)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>AA (10)</td>
<td>AG (43)</td>
<td>GG (62)</td>
<td>0.274 0.52</td>
<td>0.274 0.52</td>
</tr>
<tr>
<td>BcII</td>
<td>Case</td>
<td>CC (9)</td>
<td>CT (25)</td>
<td>TT (47)</td>
<td>0.265 0.061</td>
<td>0.091 0.846 (0.568) 1.313 (0.574) 0.958 (0.852)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>CC (10)</td>
<td>CT (43)</td>
<td>TT (62)</td>
<td>0.274 0.52</td>
<td>0.274 0.52</td>
</tr>
<tr>
<td>rs1116313</td>
<td>Case</td>
<td>CC (4)</td>
<td>CT (32)</td>
<td>TT (45)</td>
<td>0.247 0.575</td>
<td>0.348 0.655</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>CC (15)</td>
<td>CT (50)</td>
<td>TT (50)</td>
<td>0.247 0.575</td>
<td>0.348 0.655</td>
</tr>
<tr>
<td>TaqID</td>
<td>Case</td>
<td>TT (5)</td>
<td>TC (31)</td>
<td>CC (45)</td>
<td>0.253 0.912</td>
<td>0.357 0.876</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>TT (15)</td>
<td>TC (52)</td>
<td>CC (48)</td>
<td>0.253 0.912</td>
<td>0.357 0.876</td>
</tr>
<tr>
<td>rs2734835</td>
<td>Case</td>
<td>CC (5)</td>
<td>CA (31)</td>
<td>AA (45)</td>
<td>0.253 0.912</td>
<td>0.357 0.876</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>CC (15)</td>
<td>CA (52)</td>
<td>AA (48)</td>
<td>0.253 0.912</td>
<td>0.357 0.876</td>
</tr>
<tr>
<td>TaqIA</td>
<td>Case</td>
<td>TT (20)</td>
<td>TC (35)</td>
<td>CC (26)</td>
<td>0.463 0.238</td>
<td>0.463 0.238</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>TT (29)</td>
<td>TC (51)</td>
<td>CC (35)</td>
<td>0.463 0.238</td>
<td>0.463 0.238</td>
</tr>
</tbody>
</table>

*Significant at P < 0.05.
SNP = Single-nucleotide polymorphism; HWE = Hardy Weinberg Equilibrium; MAF = Minor allele frequency; OR = Odds ratio.

(additive, dominant, and recessive) using the $\chi^2$ test. Linkage disequilibrium (LD) values of $D'$ and $r^2$ were estimated using HaploView 3.12.22 We also used the Hap-Clustering program23 to perform haplotype analysis for each haplotype block.

RESULTS

Summary statistics, by locus and sample, are provided in Table 1. Genotype frequencies were tested for deviation from Hardy Weinberg equilibrium both separately among case and control groups and combined. All SNPs were in Hardy Weinberg equilibrium (Table 1). Of the three models tested for association between alcoholism and DRD2 SNPs, only the additive model shows association for three loci (rs1116313, TaqID, and rs2734835), with P values and odds ratios (OR) of .033 (0.615), .030 (0.612), and .030 (0.612), respectively. No other significant association was observed in the analyses. The pairwise LD values ($D'$ and $r^2$) among studied SNPs are provided in Table 2. Of the 6 studied polymorphisms, 5 are in strong LD and form 1 haplotype block, suggesting that haplotype analysis might be informative. The minimum $D'$ value between the markers present in the LD block is 0.861 (between rs1116313 vs. TaqID and rs1116313 vs. rs2734835). The SNP located outside the block (TaqIA) is not in LD with 3 of the 5 other SNPs (rs1116313, TaqID, and rs2734835). Haplotype analysis using all 6 SNPs and only 5 SNPs that are located in the LD block are provided in Table 3. Haplotype analysis using all 6 SNPs yielded a global $P$

TABLE 2. Linkage Disequilibrium Indices in the Current Study

<table>
<thead>
<tr>
<th>SNP</th>
<th>TaqIB</th>
<th>BcII</th>
<th>rs1116313</th>
<th>TaqID</th>
<th>rs2734835</th>
<th>TaqIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>0.952</td>
<td>0.952</td>
<td>1</td>
</tr>
<tr>
<td>BcII</td>
<td></td>
<td>–</td>
<td>1</td>
<td>0.952</td>
<td>0.952</td>
<td>1</td>
</tr>
<tr>
<td>rs1116313</td>
<td>0.164</td>
<td>0.164</td>
<td>–</td>
<td>0.861</td>
<td>0.861</td>
<td>0.165</td>
</tr>
<tr>
<td>TaqID</td>
<td>0.154</td>
<td>0.154</td>
<td>0.716</td>
<td>–</td>
<td>1</td>
<td>0.041</td>
</tr>
<tr>
<td>rs2734835</td>
<td>0.154</td>
<td>0.154</td>
<td>0.716</td>
<td>1</td>
<td>–</td>
<td>0.041</td>
</tr>
<tr>
<td>TaqIA</td>
<td>0.419</td>
<td>0.419</td>
<td>0.014</td>
<td>0.001</td>
<td>0.001</td>
<td>–</td>
</tr>
</tbody>
</table>

a$D'$ and $r^2$, above and below diagonal, respectively.
value of .033 with adjustment for age. A global $P$ value of .105 was found for the haplotype block comprised of only the 5 SNPs in LD (Table 3). Two of the haplotypes containing the TaqIA site are associated with alcoholism, whereas none of the haplotypes lacking the TaqIA site are associated with alcoholism.

**DISCUSSION**

The role of the dopaminergic system and its genetic determinants in the etiology of alcoholism has long been suspected.\(^5,24\) Previous animal and human in vivo positron emission tomographic and single-photon emission tomographic studies have provided much evidence for an association between the DRD2 gene and alcoholism.\(^25\) Beyond association studies, pharmacologic studies have shown reduced brain D₂ dopamine receptor numbers in A₁⁺ allele carriers (A₁A₁ and A₁A₂ genotypes) compared to A₁⁻ allele carriers (A₂A₂ genotype). We examined the association between the occurrence of alcoholism and 6 SNPs, including 1 well-studied TaqIA polymorphism. The genotype and allele frequencies for all variants were similar to those found in previous studies.\(^20,26,27\) We present strong evidence for an association between alcoholism and various polymorphisms within the DRD2 gene. In analyzing each SNP independently, we found a significant association between alcoholism and 3 of the 6 DRD2 SNPs (rs1116313, TaqID, and rs2734835) under the additive model. However, the Taq1A SNP was not significantly associated with alcoholism. This finding is contradictory to the previous studies where they report higher A₁ allele frequency among alcoholics.\(^8,28\) Hallikainen et al.\(^29\) reported 30% lower alcohol consumption in A₁/A₂ and 40% lower in A₂/A₂ group than in A₁/A₁ group. The association between DRD2 TaqIA variation and alcoholism remains controversial.\(^30\) Neville et al.\(^31\) determined that the DRD2 TaqIA is actually located not within DRD2, but rather within a protein-coding region, exon 8, of the adjacent ANKK1 gene. ANKK1 is located downstream of DRD2 and is involved in signal transduction pathways.\(^31\) Even though TaqIA is not associated with alcoholism at the genotype level, TaqA₁ allele-containing haplotypes are associated with alcoholism, indicating its potential role in the development of alcoholism.

In the current study, we did not find any significant association between alcoholism and TaqIB. TaqIB polymorphism lies much closer to the dopamine-coding region and 5' regulatory
regions. This positive association between TaqIB and alcoholism was observed in 2 independent studies on White American men, where B1 allele is found to be more than other populations. The current study reports association between alcoholism and 3 intronic polymorphisms that have not been previously studied. The first significant association is with rs1116313 SNP, which is located in intron 1, 179 bp down-stream of the TaqIB site, and is in 100% LD with TaqID allele. The second significant association is with the rs2734835 SNP, which is located in the second intron of the DRD2 gene and is very close to DRD2 dual repressor element (D2-DRE; position 12432–12461, GenBankTM AF050737), which negatively regulates its expression. The third polymorphism associated with alcoholism in the current study is TaqID, which is located intron 2. The TaqID is in strong linkage disequilibrium with rs1116313. Further studies of these variations using cellular experiments could potentially provide an in-depth view of the mechanism of our observations. Out of 6 studied polymorphisms, 5 are in strong LD and form 1 haplotype block. TaqIA is not in LD with 3 SNPs, which are associated with alcoholism (rs1116313, TaqID, and rs2734835) and is located outside the LD block. The distribution of the 6 site haplotypes in both cases and controls is significant even after adjusting for age.

Our study design has strengths and limitations. We recruited only men because women in this population never drink alcohol due to social restrictions. The study population was also perfectly homogenous in terms of ethnicity and geographic location: all participants came from the same Kota population of Nilgiri Hills, Tamilnadu. Thus, confounding from variation in these factors was avoided. At the same time, the limited samples size decreases the possibility of observing moderate effects. Because of these limitations, replication of these results using larger samples is required.

REFERENCES


