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Brief Research Communication

No Evidence of Association Between Structural Polymorphism at the Dopamine D3 Receptor Locus and Alcoholism in the Japanese

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Dopaminergic systems mediate reward mechanisms and are involved in reinforcing self-administration of dependence-forming substances, including alcohol. Studies have reported that polymorphisms of the dopamine D2 receptor, whose structure and function are similar to those of the dopamine D3 receptor, increase the susceptibility to alcoholism. These observations led to the examination of the possible association between a structural polymorphism of the D3 receptor gene and alcoholism. Genotyping results, employing a PCR-RFLP method, showed no difference in allele and genotype frequencies of the D3 *BalI* polymorphism (Ser^9/Gly^9) between Japanese alcoholics and controls. Moreover, these frequencies were not altered in alcoholics with inactive aldehyde dehydrogenase-2 (ALDH2), a well-defined negative risk factor for alcoholism. These results strongly suggest that the dopamine D3 receptor is not associated with alcoholism.

KEY WORDS: dopamine D3 receptor, polymorphism, alcoholism, association

INTRODUCTION

Dopaminergic systems are involved in reinforcing self-administration of dependence-forming substances such as alcohol, opiates, and cocaine [Ritz et al., 1987; Wise and Rompre, 1989]. Pharmacological or structural lesions blocking these dopamine systems attenuate the self-administration of several kinds of abused substances [Uhl et al., 1993]. These findings have led many researchers to speculate that polymorphisms of the genes constituting dopaminergic systems may alter susceptibility to alcoholism.

Blum et al. [1990] first reported an overrepresentation of the A1 allele of the Taq I polymorphism located in the 3′ flanking region of the dopamine D2 receptor (DRD2) gene in patients with alcoholism. However, evidence against the association of the A1 allele with alcoholism [Bolos et al., 1990] has generated considerable controversy.

Recently, with the identification of increasing numbers of gene polymorphisms leading to alterations in amino acid structures, more and more association studies have taken a case-control approach, employing sequence variations affecting protein structure or expression (VAPSE). Using this approach to examine associations between the DRD2 gene and alcoholism, we found an increased frequency of the Cys^{311} allele of the DRD2 gene in Japanese alcoholics [Higuchi et al., 1994b]. Further, we identified an association between the dopamine D4 receptor and alcoholism [Muramatsu et al., 1996]. However, this association was observed only in alcoholics with inactive aldehyde dehydrogenase-2 (ALDH2), which will be described below.

In 1990, the gene encoding the dopamine D3 receptor (DRD3) was cloned [Sokoloff et al., 1990; Giros et al., 1990]. Like the DRD2 gene, the DRD3 gene contains introns [Lannfelt et al., 1992]. Characterization of the D3 gene revealed the receptor's potential to mediate some of the effects of antipsychotic drugs and drugs used against Parkinson's disease, drugs previously thought to interact only with D2 receptors [Sokoloff et al., 1990].

The dopamine D3 receptor is an important constituent of the dopamine systems that mediate reward mechanisms as well as alcohol-seeking behavior [Wise and Rompre, 1989]. These actions and the resemblance of the structure and function of the D3 receptor to those of the dopamine D2 receptor led us to speculate that genetic variation of the D3 receptor may lead to an alteration in the susceptibility to alcoholism. The identification of a structural polymorphism (*BalI* polymorphism, allele 1/allele 2 or Ser^9/Gly^9) in the first exon of the DRD3 gene [Lannfelt et al., 1992] enabled us to un-

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dertake the following VAPSE-based case-control study of the DRD3 gene's possible allelic association with alcoholism.

MATERIALS AND METHODS

Our study was approved by the Ethics Committee of the National Institute on Alcoholism, Japan, and written informed consent was obtained from all subjects and controls after we carefully explained the details of the study.

The subjects were 230 alcoholics (208 males and 22 females; mean age ± SD, 49.7 ± 10.6 years) who were hospitalized at the Institute. Because in Japan heavy alcohol consumption occurs much less often among females than among males (National Institute on Alcohol Abuse & Alcoholism and National Institute on Alcoholism, 1991), we included the proportionate one-ninth as many female alcoholics as male alcoholics. All met DSM-III-R (the Diagnostic and Statistical Manual of Mental Disorders, Third Revised Edition) diagnostic criteria for alcohol dependence [American Psychiatric Association, 1987]. The control group, consisting of 200 unrelated Japanese, age 18 years or older (86 males, 114 females; mean age, 39.4 ± 12.3), were mainly hospital employees or persons connected with them.

Peripheral blood was collected from the patients and controls, and leukocyte DNA was purified for genotyping by a standard method [Sambrook et al., 1989]. The method used to genotype aldehyde dehydrogenase-2 (ALDH2) has been described elsewhere [Higuchi, 1996].

We developed a new method to determine the D3 *Bal*I polymorphism, using the endonuclease *Hae*III. For amplification of a 112-base pair (bp) DNA fragment by PCR, we used two primers (downstream, 5′-ATGGCATCTCTGAGTCAGCTGAG-3′; upstream, 5′-GGATGAGCGCGCAGTAGGAGA-3′), denaturing at 94°C for 20 sec, annealing at 58°C for 60 sec, and extension at 72°C for 15 sec, with 30 cycles in a GeneAmp PCR System 9600 thermocycler (Perkin-Elmer Cetus, Norwalk, CT). After ethanol precipitation, the PCR product was digested with *HaeIII* (GIBCO BRL, Gaithersburg, MD) and electrophoresed on 20% polyacrylamide gels. An amplified DNA fragment for allele 1 was cleaved into 76-bp and 45-bp fragments, and the 76-bp DNA fragment for allele 2 was further cleaved into 50-bp and 26-bp fragments. There was a constant band at 45 bp for every subject. When we duplicated a part of the D3 receptor genotype data by the method of Lannfelt et al. [1992] using the endonuclease *Bal*I, the data resulting from these two independent methods matched perfectly (Fig. 1).

For statistical analysis of the differences in the allele and genotype frequencies of the dopamine D3 receptor gene among Japanese alcoholics with inactive ALDH2, those with active ALDH2, and controls, we used the chi-square test. $P < 0.05$ was considered statistically significant. Computations were performed with the Statistical Analysis System [SAS Institute, 1985].

RESULTS AND DISCUSSION

The distribution of DRD3 genotype and allele frequencies in the control group was uniform, indicating that 1) age- and gender-matching with the alcoholic group was unnecessary, and 2) aggregating the data for each gender was appropriate. Because the DRD3 genotype frequencies did not differ by gender in the alcoholic population, we examined aggregate data for both genders.

As shown in Table I, we detected no difference in the allele and genotype frequencies of the dopamine D3 (Ser^9/Gly^9) polymorphism between alcoholics and controls. Examination of the 80 alcoholics with inactive ALDH2 revealed all of them to be heterozygous ALDH2*1/2*2. Although we separately examined D3

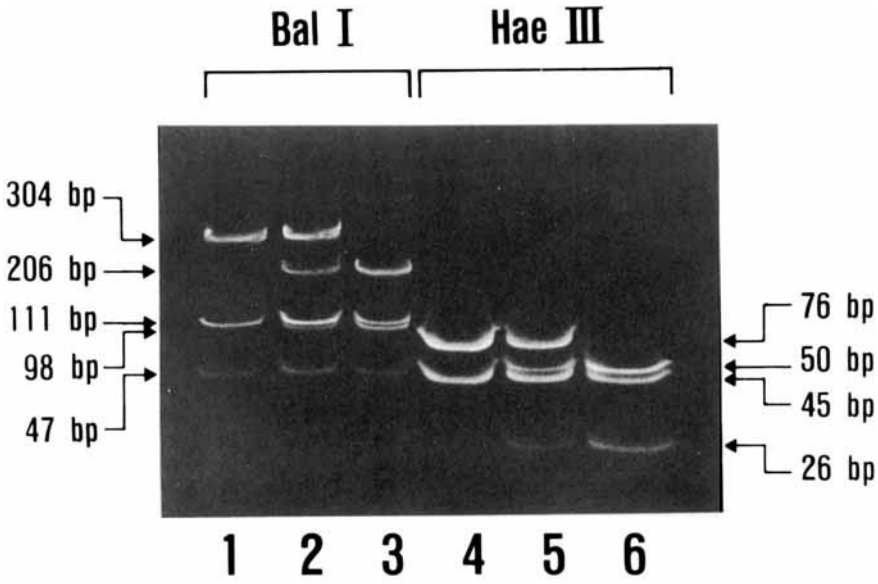

Fig. 1. Comparison of dopamine D3 Ser^9/Gly^9 genotyping results between a method by Lannfelt et al. [1992] using *Bal*I and our *Hae*III method. **Lanes 1** and **4** (Ser^9/Ser^9), **lanes 2** and **5** (Ser^9/Gly^9), and **lanes 3** and **6** (Gly^9/Gly^9) are the same subjects, respectively. Results of the two methods showed perfect agreement.

TABLE I. Genotype and Allele Frequencies of Dopamine D3 Receptor Gene in Japanese Alcoholics With Inactive ALDH2, Alcoholics With Active ALDH2, and Controls

	Alcoholics			
Frequencies[a]	Inactive ALDH2 (n = 80)	Active ALDH2 (n = 150)	Total (n = 230)	Controls (n = 200)
Genotype				
1/1 (Ser^9/Ser^9)	0.438	0.487	0.470	0.475
1/2 (Ser^9/Gly^9)	0.450	0.420	0.430	0.440
2/2 (Gly^9/Gly^9)	0.113	0.093	0.100	0.085
Allele				
1 (Ser^9)	0.663	0.697	0.685	0.695
2 (Gly^9)	0.338	0.303	0.315	0.305

[a] Frequencies may not total 1 because of rounding.

allele and genotype frequencies by the ALDH2 genotype, we found no statistically significant differences between these two groups and controls.

One of the major difficulties in identifying gene(s) of susceptibility to alcoholism is the heterogeneity of the disease. To overcome this problem, a strategy focusing on genetically- or phenotypically-defined subpopulations, in which heterogeneity is reduced, should yield significant implications. One such subpopulation comprises alcoholics with inactive ALDH2, encoded by either heterozygous ALDH2*1/2*2 or homozygous ALDH2*2, a well-defined genetic factor that lowers the risk for alcoholism [Harada et al., 1982; Higuchi et al., 1994a; Thomasson et al., 1994]. In addition, individuals who have become alcoholic despite their inactive ALDH2 probably have one or more factors that increase their susceptibility to alcoholism. If the DRD3 polymorphism were one of these factors, the allele and genotype distributions of the polymorphism in alcoholics with inactive ALDH2 might differ from those in alcoholics with active ALDH2 or in controls.

In this study, however, the allele and genotype frequencies of the DRD3 (Ser^9/Gly^9) polymorphism in alcoholics with inactive ALDH2 did not differ from those in either alcoholics with active ALDH2 or controls. These results further support our findings that the DRD3 gene locus is not associated with alcoholism.

In summary, our VAPSE-based case-control examination of the association between allelic polymorphism of the DRD3 gene (Ser^9/Gly^9) and alcoholism revealed no difference in allele and genotype frequencies of the polymorphism between alcoholics and controls. Even when the alcoholics were subdivided into two groups by ALDH2 genotype, we detected no significant differences among alcoholics with inactive ALDH2, those with active ALDH2, and controls. These results strongly suggest that the dopamine D3 receptor is not associated with alcoholism.

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