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DOPAMINE TRANSPORTER GENE POLYMORPHISM AND ALCOHOLISM

Taro Muramatsu and Susumu Higuchi*

National Institute on Alcoholism, Kurihama National Hospital

5-3-1 Nobi Yokosuka, Kanagawa 239, Japan

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In this search for a possible association between the dopamine transporter gene (DAT1) polymorphism and alcoholism, 655 Japanese alcoholics were grouped according to their aldehyde dehydrogenase-2 (ALDH2) genotypes. Because inactive ALDH2 is an established negative risk factor for alcoholism, alcoholics with the mutant allele, ALDH2*2, were considered a relatively homogeneous group. The frequency of the 7-repeat allele of the DAT1 variable number of tandem repeat was significantly higher in alcoholics with ALDH2*2 than in control subjects. These results are consistent with the hypothesis that alteration in the dopaminergic system plays some role in the development of alcoholism.

Among several genes that may contribute to an individual's susceptibility to alcoholism, those in the mesolimbic/mesocortical dopamine system are of special interest because addictive substances share the ability to activate this particular system. The first report of positive association of alcoholism and gene polymorphism in this system came from Blum et al., who demonstrated that the risk for becoming alcoholic is higher in persons who have the A1 allele of the *TaqI* polymorphism in the 3'-flanking region of the dopmine D2 receptor gene (DRD2) (1). Although substantial effort has been devoted to replicating that study, the results have been equivocal (2-7).

The association of a recently identified structural polymorphism of the DRD2 gene and alcoholism has also been reported (8-10). Like the putative association of the *TaqI* polymorphism and alcoholism, this DRD2 association with alcoholism is controversial. However, the hypothesized involvement of genetically defective dopaminergic neurotransmission in alcoholism is

*To whom all correspondence should be sent. Fax:81-468-49-7743.

Abbreviations: DAT1, dopamine transporter; ALDH, aldehyde dehydrogenase; VNTR, variable number of tandem repeat; PCR, polymerase chain reaction; dNTP, dideoxynucleoside-triphosphate.

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supported by a large body of evidence indicating that this system mediates reward mechanisms as well as alcohol-seeking behavior (11). Therefore, it seems reasonable to consider other candidate genes within the dopamine system.

The dopamine transporter gene (DAT1) is one such candidate. After the synaptic release of catecholamine, the protein encoded by the DAT1 gene uptakes catecholamine and is the direct target of cocaine action (12). Human DAT1 has a variable number of tandem repeat (VNTR) at its 3' noncoding region (40-bp repeat),which enables study of a possible allelic association of this marker and alcoholism (13).

In this association study we divided Japanese alcoholic subjects into two groups: those with and those without inactive aldehyde dehydrogenase-2 (ALDH2), a negative risk factor for the development of alcoholism (14). The inactive form of this enzyme is attributable to a point mutation in the ALDH2 gene, designated ALDH2*2 (15). The rationale for investigating the two groups separately was that alcoholics with the ALDH2*2 allele are relatively homogeneous, i.e., they have overcome its genetic protection, hence they should share some yet-unknown susceptibility factor(s) for becoming alcoholic.

MATERIALS AND METHODS

Subjects: The study was approved by the Ethics Committee of the National Institute on Alcoholism, Kurihama National Hospital, and all subjects participated gave informed consent. The sample included 655 alcoholic inpatients (597 males and 58 females, mean age 50 ± 1.1 years; all met DSM-III-R (16) criteria for alcohol dependence), of whom 80 (12%) has been identified as having inactive ALDH2 by dot-blot analysis (17). The control group consisted of 235 unrelated Japanese (111 males and 124 females, mean age 39.8 ± 12.7 years), mainly hospital employees or persons connected with them. The DAT1 VNTR genotyping was performed for all controls, all 80 alcoholic subjects with the ALDH2*2 allele, and 132 alcoholic subjects randomly selected from the remaining alcoholics without ALDH2*2.

DAT1 Genotyping: DNA was extracted from peripheral leukocytes by a standard method and DAT1 genotyping was performed by a slight modification of the method of Sano et al (18). Briefly, 100-200 ng of genomic DNA was mixed with 5 pmol of each primer (5'-TGTGGTGTAGGGAACGGCCTGAGA-3' sense; 5'-TGTTGGTCTGCAGGCT GCCTGCAT-3' antisense) in a total volume of 25 μl containing 50 μM of each dNTP, 1.5 mM $MgCl_2$, 10% (v/v) dimethyl sulfoxide, and 1 U of *Taq* DNA polymerase (Promega, Madison, WI). Thirty cycles of PCR (denaturation, 94 ℃, 20 sec; annealing, 63 ℃, 30 sec; polymerization, 72 ℃, 15 sec) were performed in a Perkin-Elmer GeneAmp PCR System 9600. Five μl of the each PCR reaction were loaded on 2% agarose gel, electrophoresed, stained with ethidium bromide and visualized. Differences in allele frequencies were tested for significance by using the Chi-square test.

RESULTS AND DISCUSSION

Table 1 shows the distribution of the DAT1 VNTR alleles across the three groups. As in previous studies (18, 19), the 10-repeat allele was the

Table 1 Allele frequencies of DAT1 VNTR (%)[a]

Allele (no. of repeat)	6	7	8	9	10	11
Controls (N[b]=470)	0	2.1	0.2	6.2	90.2	1.3
Alcoholics without ALDH2*2 (N=264)	0	1.9	0	5.3	92.0	0.7
Alcoholics with ALDH2*2 (N=160)	1.9	5.6[c]	0	6.3	85.6	0.6

a Because of rounding, percentages may not add to 100.
b Number of chromosomes.
c $p<0.05$ vs control (χ^2 test).

most prevalent form throughout the three groups, occurring in 90% of the controls and 92% and 86% of the two alcoholic groups, respectively; other variants were found at much lower frequencies. The DAT1 VNTR allele frequency in alcoholics without the ALDH2*2 allele did not differ significantly from that in controls. However, in alcoholics with ALDH2*2, the difference was significant: The 7-repeat allele occurred 2 to 3 times more often than in controls ($\chi^2=3.867$, $p<0.05$).

The search for specific genes that cause susceptibility to alcoholism is complicated by the heterogeneity of the disease. Focusing on genetically or phenotypically defined subpopulations to reduce heterogeneity should help elucidate the complex developmental pathway that must exist between alcoholic's genotype and environment and his or her alcohol-related behavior. Therefore, we focused on these alcoholics with the mutant ALDH2*2 allele, the only defined genetic factor known to affect the risk of developing alcoholism. ALDH2 is responsible for the oxidation of most of the acetaldehyde generated in alcohol metabolism (20). A point mutation in the ALDH2 gene, prevalent in Orientals, produces a deficiency in the enzyme activity (21). The mutant allele is dominant; persons with at least one ALDH2*2 allele have little or no ALDH2 activity and exhibit the "Oriental flushing response" after one or two alcoholic drinks (15). Besides facial flushing, this response includes symptoms, such as tachycardia, headache and nausea. These symptoms, like the adverse reaction to alcohol ingestion in patients treated with the ALDH inhibitor disulfiram, are associated with a high concentration of acetaldehyde in blood (22). Thus persons with the ALDH2*2 are genetically protected from

heavy drinking and the development of alcoholism (14). In Japan and China, approximately 10% of alcoholics have the mutant allele, compared with approximately 50% of control subjects (14, 17, 23). Because such individuals have overcome their genetic protection from alcoholism, we assumed for this study that they share some unknown factor(s) and represent a less heterogeneous subgroup of alcoholics.

The recent report that cocaine binds to the dopamine transporter and the interaction is correlated with the reinforcing properties of the drug highlighted the possibility of this protein as a key to human addictive behavior (12). Moreover, the DAT1 gene's VNTR marker provided a means of studying the possible association of this gene and addictive diseases.

In this study, we demonstrated that the frequency of the 7-repeat allele of the DAT1 VNTR in alcoholics who have the ALDH2*2 allele is significantly higher than in controls. This finding suggests that the VNTR is in linkage disequilibrium with an unknown structural polymorphism, which in turn contributes to the susceptibility of alcoholism. Thus, the candidate region we identify here warrants further investigation. Furthermore, in the search for the association between other dopaminergic system gene polymorphisms and alcoholism, study of alcoholics with the ALDH2*2 allele promises to be fruitful.

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