Association between alcoholism and the dopamine D4 receptor gene

Taro Muramatsu, Susumu Higuchi, Masanobu Murayama, Sachio Matsushita, Motoi Hayashida

Abstract
A point mutation in the aldehyde dehydrogenase 2 gene (ALDH2) allele is considered to be a genetic deterrent for alcoholism; however, 80 of 655 Japanese alcoholics had the mutant allele. Genotype factors that might increase susceptibility by overriding the deterrent showed a higher frequency of a five repeat allele of the dopamine D4 receptor 48 bp repeat polymorphism in alcoholics with ALDH2 than in 100 other alcoholics and 144 controls. Alcoholics with the five repeat allele also abused other drugs more often. These data suggest the involvement of the dopamine system in the development of alcoholism and other addictive behaviour.

Key words: alcoholism; dopamine D4 receptor.

Genetic factors certainly exist in alcoholism, as twin, family, and adoption studies consistently show. However, substantial research efforts to find the “alcoholism gene” have yielded little information on these factors. One of the biggest obstacles encountered in genetic studies of alcoholism is its heterogeneity. The most likely model of alcoholism is one in which multiple genetic loci interact with complex environmental factors; therefore, determining a single susceptibility locus is extremely difficult.

In a bid to overcome this problem, some researchers have focused on “severe” alcoholism and investigated the dopamine D2 receptor polymorphism; their results have been highly controversial.1,2

At present, only one defined genetic factor is known to affect the risk of developing alcoholism: a deficiency of low Km aldehyde dehydrogenase (ALDH2), the enzyme regarded as responsible for the oxidation of most of the acetaldehyde generated in alcohol metabolism. The gene for this homotetrameric enzyme is located on chromosome 12q24, where the single base pair difference in exon 12 produces a catalytically inactive isozyme.3 The mutant allele, ALDH2, is prevalent in Orientals and encodes an inactive subunit with a single point mutation corresponding to an amino acid substitution.4 People with at least one ALDH2 allele have little or no ALDH2 activity and after one or two alcoholic drinks exhibit the “Oriental flushing response”. Besides facial flushing, this response includes other symptoms, such as tachycardia, headache, and nausea, owing to a high concentration of blood acetaldehyde.5 These symptoms are unpleasant enough to prevent people from heavy drinking and the development of alcoholism.6

Significantly, there are alcoholic patients who have the ALDH2 allele, although they are few. In Japan and China, approximately 10% of alcoholics have this mutant allele, compared with approximately 50% of control subjects.7,8 These patients probably have some factor(s) that increase their susceptibility to the development of alcoholism. Whatever their nature, these factors must be strong enough to overcome the adverse effects of acetaldehydemia. Therefore, it is plausible that these factors reside in the brain’s so-called “reward system,” in which dopamine plays a crucial role.9

Among the structural polymorphisms in the dopamine system, the 48 bp tandem repeat sequence polymorphism of the dopamine D4 receptor (DRD4) is the only one that has been reported to produce altered receptor function. This polymorphism is located in the putative third cytoplasmic loop of the receptor, which is believed to be a binding site for G proteins.10 Van Tol et al11 have suggested the receptor function covaries with variation of the repeat sequence. The polymorphic repeat also serves as a genetic marker for detecting other unknown susceptibility mutations within or close to the DRD4 gene, and has been localised to chromosome 11q15,12 To explore the possibility of an association between the DRD4 structural mutation and alcoholism in Japanese with the ALDH2 allele, we examined the prevalence of the 48 bp repeat alleles in alcoholics with and without the ALDH2 allele and in control subjects. We further compared the clinical characteristics of the alcoholic subjects.

Subjects and methods
In this study, approved by the Ethics Committee of the National Institute on Alcoholism, the study population included 655 alcoholic inpatients (all Japanese, 597 males and 58 females, mean age 50 (SD 1·1) years), all of whom met DSM-III-R criteria for alcohol dependence. Information on alcohol consumption and medical history was obtained by one of the authors in face to face semistructured interviews.13

The control group, consisting of 144 unrelated Japanese (70 males and 74 females, mean age 38·2 (SD 11·9) years), mainly hospital employees or persons connected with them. To evaluate drinking problems, we administered the questionnaire type Kurihama
Alcoholism Screening Test (KAST)\(^6\) to this group and found all were normal drinkers.

After obtaining informed consent, we extracted DNA from lymphocytes and determined the ALDH2 allele of each subject by dot blot analysis, as previously described.\(^8\) DRD4 genotyping was performed for all controls, all 80 alcoholic subjects with the ALDH2\(^2\) allele (12% of patients, 73 males and 7 females, mean age 50.6 (SD 10.5) years), and 100 alcoholic subjects randomly selected from the remaining alcoholics without ALDH2\(^2\) (90 males and 10 females, mean age 49.0 (SD 9.6) years).

The 48 bp repeat polymorphism was examined by the method of Nanko et al.\(^7\) slightly modified. Briefly, 100 ng of genomic DNA was mixed with 5 pmol of each primer \(5'-\)AGGTGGCACGTCGCGCCAAGCTGCA-\(3'\) sense; \(5'-\)TCTGCGGTGGAGTCTGGGGGTGGGAG-\(3'\) antisense) in a total volume of 25 \(\mu\)l containing 100 \(\mu\)mol/l dCTP, 100 \(\mu\)mol/l dTTP, 100 \(\mu\)mol/l dATP, 100 \(\mu\)mol/l 7-deaza-guanosine, 1.5 \(\mu\)mol/l MgCl\(_2\), 10% (v/v) DMSO, and 1 U of \(Taq\) DNA polymerase (Promega, Madison, WI). Thirty cycles of PCR (denaturation, 15 seconds at 94°C; annealing, 30 seconds at 65°C; polymerisation, 15 seconds at 72°C) were performed in a Perkin-Elmer GeneAmp PCR System 9600. Whole PCR reactions were loaded on a 2% agarose gel, electrophoresed, stained with ethidium bromide, and visualised using an FMBIO-100 image analyser (Takara, Japan). For some samples, rhodamine labelled primer was used for PCR and the products were electrophoresed on a 7% denatured polyacrylamide gel, where exact sizes were determined compared with M13 mp18 single strand DNA sequencing ladder. Differences in allele frequencies were tested for significance by using Fisher's exact probability test. Computations were performed using the statistical analysis system (SAS).\(^9\)

### Results

Dot blot analyses showed that all 80 alcoholics with the ALDH2\(^2\) were ALDH2\(^2/ALDH2^2\) heterozygotes. There were no significant differences between the ages (50.6 \(v\) 49.0 years) or sex distribution (8.8% \(v\) 10.0% female) of the alcoholics with and without the ALDH2\(^2\) allele.

Table 1 shows the distribution of DRD4 allele variants in the three groups. This distribution in the controls was in general agreement with a previously reported Japanese

<table>
<thead>
<tr>
<th>Allele</th>
<th>No of chromosomes (%)</th>
<th>Alcohols With ALDH2(^2)</th>
<th>Without ALDH2(^2)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>13 (8.1)</td>
<td>20 (10.0)</td>
<td>22 (7.6)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0 (0.0)</td>
<td>3 (1.5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>125 (78.1)</td>
<td>172 (86.3)</td>
<td>254 (88.1)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18 (11.3)</td>
<td>5 (2.5)</td>
<td>10 (3.5)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0 (0.0)</td>
<td>0 (0)</td>
<td>2 (0.7)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4 (2.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>160 200</td>
<td>288</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Dopamine D4 receptor genotype distribution in alcoholics with and without the ALDH2\(^2\) allele

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No of subjects (%)</th>
<th>Alcohols With ALDH2(^2)</th>
<th>Without ALDH2(^2)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/2</td>
<td>0 (0)</td>
<td>2 (2.0)</td>
<td>1 (0.7)</td>
<td></td>
</tr>
<tr>
<td>2/3</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.7)</td>
<td></td>
</tr>
<tr>
<td>2/4</td>
<td>1 (1.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>2/5</td>
<td>12 (15.0)</td>
<td>16 (16.0)</td>
<td>19 (13.1)</td>
<td></td>
</tr>
<tr>
<td>2/6</td>
<td>0 (0)</td>
<td>3 (3.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>3/3</td>
<td>46 (57.5)</td>
<td>74 (74.0)</td>
<td>112 (77.8)</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>18 (22.5)</td>
<td>5 (5.0)</td>
<td>9 (6.3)</td>
<td></td>
</tr>
<tr>
<td>4/4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (1.4)</td>
<td></td>
</tr>
<tr>
<td>4/5</td>
<td>3 (3.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>80 100</td>
<td>144</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The DRD4 genotype frequency in alcoholics without the ALDH2\(^2\) allele did not differ significantly from that in controls. In alcoholics with ALDH2\(^2\), however, the difference was significant: the five repeat sequence allele occurred three to four times more often than in the other alcoholics and controls (\(p<0.001\)). When those with five repeat or longer alleles ("longer\(^\ast\) alleles") were combined, they occurred more often in alcoholics with the ALDH2\(^2\) allele than other alcoholics and controls (\(p<0.001\)).

The genotype distribution of DRD4 is shown in table 2. Most subjects with the five repeat allele were four repeat/five repeat heterozygotes; the genotype occurred approximately four times more often in the alcoholics with the ALDH2\(^2\) allele than in other alcoholics and controls (\(p<0.001\)).

In control subjects, 64 (44.4%) had the ALDH2\(^2\) allele and six of them were homozygous for the allele. There was no significant difference in distribution of the DRD4 five repeat allele between those with and without the ALDH2\(^2\) allele (3.8% \(v\) 3.1%).

Comparison of the clinical characteristics of the alcoholics with and without the five repeat allele showed that the comorbidity of drug abuse was significantly higher in the patients with the five repeat allele, six of 23 (26.1%) versus 14 of 157 (8.9%) of other patients (\(p<0.03\)). In contrast, neither their drinking histories and clinical features of alcoholism nor the prevalence of other DSM-III-R Axis I and Axis II disorders differed significantly (data not shown).

### Discussion

In psychiatric research, the dopamine system has been of special interest because of its possible involvement in schizophrenia and other disorders, including alcoholism. Consequently, the DNA polymorphisms in this system have been subjected to many association studies. Although this approach is attractive, attempts to replicate initial positive results have often failed. Accumulated false positive findings in genetic research show substantial heterogeneity in various psychiatric disorders and demand more refined strategy.

In this association study of alcoholism, we focused on patients with the ALDH2\(^2\) allele, predicting that they would exhibit some al-
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Our study supports the idea that a single repeat allele, or the "longer" allele, affects the linkage disequilibrium with some unknown locus within or near the allele. Further investigations are needed to clarify these problems.

A recent study of the association of the DRD4 polymorphism with Finnish alcoholics showed no overall differences between cases and controls but indicated a non-significant trend towards greater prevalence of the five repeat allele among alcoholics. This result supports our assumption that the heterogeneity of alcoholism could mask an association between the disease and certain structural polymorphisms. Our results were consistent with the Finnish study, in that there was no overall difference in the allele and genotype distribution when all alcoholics were considered as a single group. However, by focusing on those with the mutant ALDH2 allele, we found the significant association between the DRD4 five repeat allele and this relatively homogeneous subset of alcoholics. The results of another association study from Canada are hard to interpret, because it lacked racial and ethnically matched controls. This study, made possible by the presence of the ALDH2 allele characteristic of Orientals, cannot be extended directly to other racial populations. However, the data obtained can serve as a key to help open the black box of the genetics of alcoholism, and possibly other substance abuse disorders.

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