

Association between alcoholism and the dopamine D4 receptor gene

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Abstract

A point mutation in the aldehyde dehydrogenase 2 gene (ALDH2² 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 ALDH2² 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.

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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, ALDH2², 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 ALDH2² 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 ALDH2² 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 acetaldehyde. 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 al*¹¹ 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 11q15.5.¹² To explore the possibility of an association between the DRD4 structural mutation and alcoholism in Japanese with the ALDH2² allele, we examined the prevalence of the 48 bp repeat alleles in alcoholics with and without the ALDH2² 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

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Table 1 Dopamine D4 receptor allele distribution in alcoholics with and without the ALDH2² allele

Allele	No of chromosomes (%)		
	Alcoholics		
	With ALDH2 ²	Without ALDH2 ²	Controls
2	13 (8.1)	20 (10.0)	22 (7.6)
3	0 (0)	3 (1.5)	0 (0)
4	125 (78.1)	172 (86.0)	254 (88.1)
5	18 (11.3)	5 (2.5)	10 (3.5)
6	0 (0)	0 (0)	2 (0.7)
7	4 (2.5)	0 (0)	0 (0)
Total	160	200	288

Table 2 Dopamine D4 receptor genotype distribution in alcoholics with and without the ALDH2² allele

Genotype	No of subjects (%)		
	Alcoholics		
	With ALDH2 ²	Without ALDH2 ²	Controls
2/2	0 (0)	2 (2.0)	1 (0.7)
2/5	0 (0)	0 (0)	1 (0.7)
2/7	1 (1.3)	0 (0)	0 (0)
4/2	12 (15.0)	16 (16.0)	19 (13.1)
4/3	0 (0)	3 (3.0)	0 (0)
4/4	46 (57.5)	74 (74.0)	112 (77.8)
4/5	18 (22.5)	5 (5.0)	9 (6.3)
4/6	0 (0)	0 (0)	2 (1.4)
4/7	3 (3.8)	0 (0)	0 (0)
Total	80	100	144

Alcoholism Screening Test (KAST)¹⁴ 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 ALDH2² 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 ALDH2² (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*¹⁵ slightly modified. Briefly, 100 ng of genomic DNA was mixed with 5 pmol of each primer (5'-AGGTGGCACGTCGCGCCAAGCTGCA-3' sense; 5'-TCTGCGGTGGAGTCTGGG-GTGGGAG-3' antisense) in a total volume of 25 µl containing 100 µmol/l dCTP, 100 µmol/l dTTP, 100 µmol/l dATP, 100 µmol/l 7-deazaguanosine, 1.5 mmol/l MgCl₂, 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).¹⁶

Results

Dot blot analyses showed that all 80 alcoholics with the ALDH2² were ALDH2¹/ALDH2² 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 ALDH2² allele.

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

study.¹⁵ The DRD4 genotype frequency in alcoholics without the ALDH2² allele did not differ significantly from that in controls. In alcoholics with ALDH2², 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" alleles) were combined, they occurred more often in alcoholics with the ALDH2² 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 ALDH2² allele than in other alcoholics and controls (*p*<0.001).

In control subjects, 64 (44.4%) had the ALDH2² 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 ALDH2² 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 ALDH2² allele, predicting that they would exhibit some al-

teration in the "reward system" because they must overcome the adverse experience of acetaldehyde to develop alcoholism. Genotyping showed specific allele differences in the DRD4 48 bp repeat polymorphism among different patient groups. The frequency of the five repeat allele was significantly higher in the alcoholics with the ALDH2² allele. Moreover, those with the five repeat allele had high comorbidity of other drug abuse.

The DRD4 is enriched in mesolimbic/mesocortical dopamine pathways, disturbance of which has been suggested in the pathogenesis of alcoholism.⁹ The 48 bp repeat polymorphism we investigated in this study is located at the third cytoplasmic loop of the protein, the site believed to interact with G proteins in this family of receptors.¹⁰ Therefore, it is conceivable that the different size of the cytoplasmic loop affects the conformation of the protein and the receptor function, thereby leading to susceptibility to alcoholism. That the five repeat allele or the "longer" alleles occurred more often in the alcoholics with the ALDH2² allele does not contradict this view. Another possible explanation for the results is that the five repeat allele is in 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.¹⁷ These results support 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 ALDH2² allele, we found the significant association between the DRD4 five repeat allele and this relatively ho-

mogeneous subpopulation of alcoholics. The results of another association study from Canada are hard to interpret, because it lacked racially and ethnically matched controls.¹⁸

This study, made possible by the presence of the ALDH2² 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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