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Association of a polymorphism of the serotonin 1B receptor gene and alcohol dependence with inactive aldehyde dehydrogenase-2

Y. Hasegawa[1], **S. Higuchi**[2], **S. Matsushita**[2], and **H. Miyaoka**[1]

[1] Department of Psychiatry, Kitasato University, School of Medicine, Sagamihara, Kanagawa, and
[2] Institute of Clinical Research, National Alcoholism Center, Kurihama Hospital, Yokosuka, Kanagawa, Japan

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Summary. The use of persons who become alcoholic despite having a well-defined negative risk for alcoholism (inactive aldehyde dehydrogenase-2 or ALDH2) is advantageous in genetic research because of this population's reduced heterogeneity and possible genetic factors conferring susceptibility to alcohol dependence. This investigation of central serotonin neurotransmission, specifically the serotonin 1B (5HT1B) receptor gene and its role in both regulating alcohol consumption and developing alcohol dependence revealed overrepresentation of the C allele of the 861G>C polymorphism of 5HT1B in alcoholics with inactive ALDH2, compared with its frequency in nonalcoholic controls. No significant differences in 5HT1B genotype and allele distributions were observed between alcoholics with active ALDH2 and controls, however. Taken together with recent observations, these results suggest that genetic variability of the 5HT1B receptor is involved in the development of some type of alcohol dependence.

Keywords: 5HT1B receptor gene, polymorphism, alcohol dependence, inactive ALDH2.

Introduction

Genetic factors' contribution to the development of alcohol dependence has been consistently supported by both animal studies (Crabbe et al., 1999) and clinical observations (Goodwin et al., 1973; Cloninger et al., 1981; Hrubec and Omenn, 1981; Kendler et al., 1992). Researchers have estimated the heritability of alcohol dependence to be 0.4–0.6 in both males and females (Pickens et al., 1991; Enoch and Goldman, 1999). The most likely model of inherited alcohol dependence involves the interaction of multiple genes that have minor effects and environmental factors, an interaction that leads to the development of the disease in genetically susceptible individuals. One of the major

obstacles in identifying genes that produce minor effects is the heterogeneity of the disease to be examined. Such difficulties hinder pinpointing genes responsible for alcohol dependence in both association and linkage approaches (Reich et al., 1998; Long et al., 1998).

Aldehyde dehydrogenase-2 (ALDH2) and alcohol dehydrogenase (ADH) are two major ethanol-oxidizing enzymes known to alter genetic susceptibility to alcohol dependence. The gene encoding ALDH2 is located on chromosome 12q24, wherein the single nucleotide difference in exon 12 produces a catalytically inactive isozyme (inactive ALDH2) (Yoshida et al., 1991). Due to delayed oxidation in individuals with the ALDH2*2 allele, a high blood acetaldehyde concentration after drinking causes adverse reactions. These reactions are severe enough to deter further drinking, and together constitute a negative risk for alcohol dependence (Harada et al., 1982; Higuchi et al., 1996). Like ALDH2, naturally occurring nucleotide substitutions at ADH2 and ADH3 loci are capable of altering ethanol metabolism (Yoshida et al., 1991). Superactive ADH encoded by the ADH2*2 allele has also been reported to reduce the risk for alcohol dependence (Thomasson et al., 1991; Higuchi et al., 1996).

Despite having inactive ALDH2, some individuals develop alcohol dependence. Alcoholics with inactive ALDH2 are considered advantageous for studies of the genetics of alcohol dependence because these individuals must overcome the discomforts of drinking to become alcoholic. The heterogeneity of the disease is likely to be relatively restricted in this genetically defined subgroup of alcoholics, compared with that in an entire alcoholic population. An additional benefit of studying individuals who have become alcoholics despite having inactive ALDH2 is that they probably have one or more factors that increase their susceptibility to alcohol dependence.

Using the 861G>C polymorphism as a marker, we examined a possible association between the serotonin 1B (5HT1B) receptor gene and alcohol dependence. There are good reasons for selecting this gene as a promising candidate for an association analysis. First, both animal and human studies have shown that the function of the serotonergic system is closely related to alterations in food and ethanol intake (Sellers et al., 1992; LeMarquand et al., 1994a,b). Second, a quantitative trait locus for the alcohol preference drinking phenotype has been provisionally linked in mice to the middle of chromosome 9, where the 5HT1B receptor gene is located (Crabbe et al., 1994, 1999). Third, knockout mice that lack the 5HT1B receptor have shown increased spontaneous alcohol consumption, reduced sensitivity to ethanol-induced ataxia, and slower development of tolerance than wild-type mice (Crabbe et al., 1996). Fourth, a Finnish study has shown that the 861G>C polymorphism of the 5HT1B receptor gene – the same marker as the one examined in this study – is associated with antisocial alcoholism but not with non-antisocial alcoholism (Lappalainen et al., 1998).

In this study, we screened nearly 1,400 Japanese alcoholics and identified a substantial number with inactive ALDH2, for whom we compared genotype and allele distributions of 5HT1B 861G>C polymorphism with those of non-alcoholic controls. We also examined the relationships between this marker

and phenotype variations, including the presence of antisocial personality disorder (ASPD) and the severity of alcohol dependence. This study excluded female alcoholics for the following reason: Because clinical characteristics of female alcoholics differ in many aspects from those of male alcoholics (Hesselbrock et al., 1985; Gomberg et al., 1993), analyses involving the 5HT1B receptor gene have to be carried out separately by gender. However, the limited number of female alcoholics in Japanese clinical samples (approximately 10% of all alcoholics), especially those with inactive ALDH2 (approximately 1% of the total number of alcoholics) (Higuchi et al., unpublished data), would hinder reliable statistical analysis.

Material and methods

Subjects

The Ethics Committee of the National Alcoholism Center, Kurihama Hospital, approved this study and written informed consent was obtained from all of the subjects. The study population consisted of 1,385 Japanese male alcoholics who were consecutively hospitalized at Kurihama Hospital (June 1992 to December 1993, and January 1996 to June 1997). Although each had been diagnosed as having either DSM-III-R (American Psychiatric Association, 1987) alcohol dependence or alcohol abuse, based on the Structured Clinical Interview for DSM-III-R (SCID) assessment (Spitzer et al., 1990), the actual frequency of patients with alcohol abuse was negligible. Information on patients who were unable to respond adequately to the SCID interview for various reasons, including dementia, was obtained from their collaterals.

The 200 control subjects – unrelated nonalcoholic Japanese males age-matched with the patients (mean ± SD, 50.5 ± 10.0 years) – were Institute employees, their acquaintances, or persons living near the Institute. We examined their drinking patterns, using a previously described questionnaire (Higuchi et al., 1992), and assessed their drinking problems with the most widely used screening test for alcohol dependence in Japan: the Kurihama Alcoholism Screening Test or KAST (Saito and Ikegami, 1978). On the basis of KAST results, we excluded control subjects suspected of being alcohol dependent from the study.

Genotyping ALDH2, 5HT1B, and ADH2

ALDH2 genotyping was done by a previously described polymerase chain reaction (PCR)-restriction fragment length polymorphism method (Harada and Zhang, 1993), using DNA extracted from the subjects' peripheral leucocytes. Among the 1,385 alcoholics, we identified 151 (10.9%; ages 50.8 ± 9.8 years) with inactive ALDH2. All of these alcoholics with inactive ALDH2 and 356 alcoholics (ages 50.8 ± 9.8 years) with active ALDH2 – a total of 507 – were subjected to association analysis.

The 861G>C polymorphism of the 5HT1B gene was determined by the method of Lappalainen et al. (1998). Briefly, for amplification of a 548-base pair (bp) DNA fragment by PCR, we used two primers (5′-GAAACAGACGCCCAACAGGAC-3′ and 5′-CCAGAAACCGCGAAAGAAGAT-3′), denaturing at 94°C for 20 sec, annealing at 58°C for 60 sec, and extension at 72°C for 30 sec, with 30 cycles in a GeneAmp PCR system 9600 thermocycler (PerkinElmer Cetus, Norwalk, CT). Digestion of the PCR product with HincII and electrophoresis on 1% agarose gel yielded two fragments (452 and 96 bps) when the polymorphic site was G, and three fragments (142, 310, and 96 bps) when the site was C.

Determination of ADH2*1 and ADH2*2 alleles was carried out as previously described (Xu et al., 1988).

Clinical characteristics

To examine the possible influences of 5HT1B 861G>C polymorphism on the alcohol dependence phenotype, we obtained information on the subjects' clinical characteristics. Antisocial personality disorder was determined by SCID evaluation (Spitzer et al., 1990). Age at onset of alcohol dependence was defined as the age when an individual first met DSM-III-R criteria for alcohol dependence. We used the Family History Research Diagnostic Criteria (Andreasen et al., 1977) to assess alcohol dependence among first-degree relatives. Careful examination of patients' histories was used to determine whether they had experienced delirium tremens.

Statistics

For statistical analysis of the differences in the allele and genotype frequencies of the 5HT1B receptor gene between the alcoholic and control groups and between alcoholic subgroups, we used the chi-square test. Probability differences of $P < 0.05$ were considered statistically significant. For multiple pair-wise comparisons among the three groups (i.e., comparing 5HT1B allele frequencies in alcoholics with inactive ALDH2 vs. controls, in alcoholics with active ALDH2 vs. controls, and in alcoholics with inactive ALDH2 vs. alcoholics with active ALDH2), we set the significance level at 0.017 to reduce type I errors [$1 - (1 - P)^3 = 0.05$, $P = 0.017$]. Odds ratios with 95% confidence intervals were calculated to compare allele frequencies between the groups.

Results

Consistent with previous reports (Higuchi et al., 1994, 1996), all alcoholics with inactive ALDH2 (n = 151) had the heterozygous ALDH2*1/*2 genotype; we detected no cases of homozygous ALDH2*2/*2 (Table 1). As expected, the frequency of the ALDH2*2 allele was significantly lower in the alcoholic group than in the control group.

Table 2 compares the genotype and allele distributions of the 861G>C polymorphism in alcoholics, subgrouped by ALDH2 genotype, and controls. All genotype distributions were in Hardy-Weinberg's equilibrium. The C allele was overrepresented in alcoholics with inactive ALDH2, compared with its frequency in the controls. In contrast, there were no significant differences between the 861G>C genotype and allele distributions in alcoholics with

Table 1. ALDH2 genotype and allele frequency distributions in Japanese alcoholics and controls

	Genotype			Allele	
	2*1/2*1 N (%)	2*1/2*2 N (%)	2*2/2*2 N (%)	2*1 N (%)	2*2 N (%)
Alcoholics (n = 1,385)	1,234 (89.1%)	151 (10.9%)	0 (0.0%)	2,619 (94.5%)	151 (5.5%)
Controls (n = 200)	117 (58.5%)	73 (36.5%)	10 (5.0%)	307 (76.8%)	93 (23.3%)

Note: Percentages may not total 100, because of rounding. * Alcoholics vs. controls: Genotype, $\chi^2 = 169.47$, df = 2, $P < 0.001$; allele, $\chi^2 = 155.85$, df = 1, $P < 0.001$

Table 2. 5HT1B receptor genotype and allele distribution in a sample of 507 alcoholics with active or inactive ALDH2 and 200 nonalcoholic controls

	Alcoholics		Controls (n = 200) N (%)
	Inactive ALDH2* (n = 151) N (%)	Active ALDH2* (n = 356) N (%)	
Genotype			
G/G	34 (22.5%)	104 (29.2%)	62 (31.0%)
G/C	72 (47.7%)	186 (52.3%)	104 (52.0%)
C/C	45 (29.8%)	66 (18.5%)	34 (17.0%)
Allele			
G	140 (46.4%)	394 (55.3%)	228 (57.0%)
C	162 (53.6%)	318 (44.7%)	172 (43.0%)
Odds ratio** (95% CI)	1.53 (1.14–2.07)	1.07 (0.84–1.37)	

* Alcoholics with inactive ALDH2 vs. controls: Genotype, $\chi^2 = 9.152$, df = 2, P = 0.010; allele, $\chi^2 = 7.814$, df = 1, P = 0.004. Alcoholics with active ALDH2 vs. controls: Genotype, $\chi^2 = 0.307$, df = 2, P = 0.858; allele, $\chi^2 = 0.287$, df = 1, P = 0.592. Alcoholics with inactive ALDH2 vs. alcoholics with active ALDH2: Genotype, $\chi^2 = 8.584$, df = 2, P = 0.014; allele,$\chi^2 = 7.372$, df = 1, P = 0.007. ** Ratio of odds (C/G) for each alcoholic group compared to that of controls and its 95% confidence interval

active ALDH2 and the controls. Within the alcoholic samples, the frequency of the C allele was significantly higher in alcoholics with inactive ALDH2 than in those with active ALDH2.

To identify possible associations of the 5HT1B 861G>C polymorphism with other alcoholic subpopulations, we examined several pertinent genetic and clinical characteristics (detailed data not shown). The frequency of the C allele tended to be higher in alcoholics with ASPD, in those who became alcoholic prior to the age of 30 years, and in those with a history of alcoholism in first-degree relatives. The C allele was present in 50.0% of the 21 alcoholics with ASPD and 47.3% of the 486 without ASPD, in 50.8% of the 63 early-onset patients and 47.0% of the 444 with late-onset alcohol dependence, and in 52.4% of the 62 with positive family history compared with 46.7% of the 445 who had no family history of alcoholism. In none of these comparisons did the difference in C allele frequency reach statistical significance. Likewise, the presence or absence of delirium tremens had little to do with 861G>C genotype and allele distributions.

ADH2 is another well-defined negative risk factor for alcohol dependence, but stratification of our sample of 507 alcoholics by the presence of the superactive ADH2*2 allele did not yield differences in 861G>C genotype and allele distributions. Finally, we separately examined the relationships between 5HT1B 861G>C and these characteristics in alcoholics with inactive ALDH2 and those with active ALDH2; however, in none of the comparisons of genotype and allele distributions could we detect significant differences.

Discussion

In this study, we demonstrated that the 861G>C polymorphism of the 5HT1B receptor gene is associated with a particular type of alcohol dependence, that is, alcohol dependence with inactive ALDH2. The C allele of the 861G>C marker was overrepresented in this alcoholic subpopulation, compared with its frequency in nonalcoholic controls, whereas we observed no difference in C allele frequency between alcoholics with active ALDH2 and controls. We did not look for an association between 5HT1B 861G>C and the total of 1,385 subjects considered representative of clinical alcoholism, inasmuch as the age-matched samples of alcoholics with inactive ALDH2 and active ALDH2 examined in this study were selected from these subjects. However, because the proportion of alcoholics with active ALDH2 is overwhelming, the positive association with 5HT1B observed in alcoholics with inactive ALDH2 would most likely have disappeared within the total of 1,385 alcoholics.

What are the clinical features of alcoholics with inactive ALDH2? Our previous work has shown that this type of alcoholic does not differ from alcoholics with active ALDH2 in either sociofamilial background or comorbid psychopathology, including ASPD. However, the onset of alcohol dependence is relatively delayed in alcoholics with inactive ALDH2, who tend to experience each stage or event in the history of drinking and to become dependent on alcohol 1–5 years later in life than alcoholics with active ALDH2 (Murayama et al., 1998). Examination of alcoholics' personality traits has revealed that those with inactive ALDH2 have higher novelty-seeking and lower harm-avoidance tendencies than those with active ALDH2 (Higuchi et al., unpublished data). These characteristics suggest that alcoholics with inactive ALDH2 clinically resemble Cloninger's type 1 alcoholics (Cloninger, 1987), although they appear to have personality traits similar to those of type 2 alcoholics and alcoholics with ASPD.

To the authors' knowledge, three studies examining the genetic association between the 5HT1B gene and alcohol dependence have been published. The original finding was reported by Lappalainen and colleagues (1998), who showed a higher frequency of the C allele of the 861G>C polymorphism in alcoholics with ASPD than in unaffected controls. They did not find a significant association in alcoholics without ASPD. Subsequently, Fehr et al. (2000) examined the same marker and alcoholism, finding that the frequency of the G allele, but not the C allele, was higher in alcoholics than in controls. The difference having been observed only in an allele comparison, and the significance being modest ($P = 0.04$), the authors themselves suggested that the difference might be due to type I error. Using multiple markers of the 5HT1B gene, to compare the frequencies of these markers in individuals with alcohol abuse or dependence and controls, Cigler et al. (2001) failed to find any significant differences. However, in that study the sample sizes appeared to be too small (alcohol abuse, $n = 20$; alcohol dependence, $n = 10$) for drawing firm conclusions.

Our results seem to support the original findings of Lappalainen et al. (1998). Both their study and ours demonstrated that 5HT1B 861G>C is

associated with some type of alcohol dependence, possibly alcohol dependence with antisocial personality traits. Our study actually examined 861G>C in our sample of 507 alcoholics by the presence or absence of ASPD, finding a slight but not significant increase in the frequency of the C allele in alcoholics with ASPD. It is possible that the small sample size (n = 21) of alcoholics with ASPD in our study might have produced a type II error. Additional studies are warranted to examine these relationships, using multiple markers of the 5HT1B gene (including 861G>C) in an increased number of antisocial alcoholics and focusing on personality traits such as novelty seeking and harm avoidance.

There are three potential explanations for the association we found. First, because 861G>C does not cause a structural change in the 5HT1B receptor, this polymorphism may be in linkage disequilibrium with other polymorphisms elsewhere in the gene, demonstrating biologically relevant variability. This appears unlikely, inasmuch as an extensive search has failed to identify polymorphisms accompanying amino acid replacement in this intronless gene (Nothen et al., 1994; Lappalainen et al., 1995; Ohara et al., 1996; Huang et al., 1999; Cigler et al., 2001). Second, 861G>C may be linked to a functional polymorphism located in the regulatory region of the 5HT1B gene. For example, the polymorphism could alter the expression of the 5HT1B receptor and, subsequently, central serotonin neurotransmission. Finally, there exists the possibility that 861G>C is in linkage disequilibrium with a genetic variation of another gene located near the 5HT1B receptor gene. If this possibility proves true, elucidation of the structure of human genome now in progress will facilitate identifying the gene that may confer susceptibility to alcohol dependence with antisocial personality traits.

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Authors' address: S. Higuchi, M.D., Ph.D., Institute of Clinical Research, National Alcoholism Center, Kurihama Hospital, 5-3-1 Nobi Yokosuka, Kanagawa 239-0841, Japan, e-mail: h-susumu@db3.so-net.ne.jp