ALDH2 PROMOTER POLYMORPHISM HAS NO EFFECT ON THE RISK FOR ALCOHOLISM

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Abstract — Aim: To test for the possible association between mitochondrial aldehyde dehydrogenase (ALDH2) promoter polymorphism and alcoholism. Methods: Genotyping to identify the polymorphisms in 515 alcoholic patients and 361 normal controls was performed. Results: There were no significant differences between the genotype and allele frequencies of the –357A/G polymorphism in alcoholics and normal controls. Linkage disequilibrium was observed between the promoter and exon 12 polymorphisms. Conclusion: These results suggest the ALDH2 promoter polymorphism does not affect the risk for alcoholism.

INTRODUCTION

The inactive form of mitochondrial aldehyde dehydrogenase (ALDH2) is regarded as a preventive factor against the risk for alcoholism. ALDH2 is an enzyme responsible for the oxidative metabolism of most of the acetaldehyde generated during alcohol elimination (Agarwal and Goedde, 1990). The ALDH2 gene is located at chromosome 12q24 (Raghunathan et al., 1988) and consists of 13 exons and 12 introns (Hsu et al., 1989). This gene has a single point mutation in the exon 12 coding region corresponding to a Glu to Lys amino acid substitution (SNP ID: rs671) (Hsu et al., 1985). This polymorphic site encodes both active (Glu487, ALDH2*1) and inactive (Lys487, ALDH2*2) sub-units of ALDH2, but the isozyme containing the Lys487 subunit plays no role in the elimination of acetaldehyde.

Inactive ALDH2 causes a high acetaldehyde level in the blood after drinking ethanol, resulting in the ‘flushing response’ characterized by facial flushing, palpitations, nausea, headache, and other symptoms. Such discomfort suppressing further drinking in the general population (Muramatsu et al., 1995; Higuchi et al., 1996) and decreases the occurrence of alcoholism (Harada et al., 1982; Higuchi et al., 1994). The inactive Lys487 allele is common in Asian populations, but it is very rare, if any, in Caucasians (Goedde et al., 1992).

Two rare polymorphisms (ALDH2*3 and ALDH2*2Taiwan) have been reported near the Glu487/Lys locus in exon 12 (Novoradovsky et al., 1995). The ALDH2*3 (SNP ID: rs1064933) allele was detected in Pima Indians at a frequency of 4.4%, and ALDH2*2Taiwan was detected in the Chinese of Taiwan, although the frequency of this allele was very low (0.3%). ALDH2*3 does not cause amino acid replacement. ALDH2*2Taiwan accompanies amino acid substitution, but exists only as a subtype of the already inactive Lys487 allele. In spite of many attempts to detect other polymorphisms, no other variant in exons or introns of the ALDH2 gene has been reported to be associated with alcoholism.

In 1999, two groups reported a novel polymorphism in the promoter region of the ALDH2 gene (SNP ID: rs886205) (Chou et al., 1999; Harada et al., 1999). Located at –357 bp from the starting codon, this polymorphism has a G to A substitution and has been detected in Caucasians as well as Asian populations. The distribution of the –357A/G polymorphism differs across ethnic groups; the G allele is more frequent in Asian populations, while the A allele is more common among Caucasians. The exon 12 polymorphism and the –357A/G polymorphism in the promoter region are in linkage disequilibrium (LD).

The ALDH2 promoter has a CCAAT box that binds to nuclear factor Y (NF-Y), which is vital for the expression of ALDH2 (Stewart et al., 1996). The upstream region of the promoter contains direct repeat (DR) elements that bind the steroid nuclear receptor superfamily (Stewart et al., 1998). The region is divided into two subregions, one of which interacts with hepatocyte nuclear factor 4 (HNF-4) (Stewart et al., 1998), the retinoic acid receptor (RAR) (Pinaire et al., 2003), and the retinoid X receptor (RXR) (Pinaire et al., 2000). The other interacts with the apolipoprotein A regulatory protein 1 (ARP-1) and the chicken ovalbumin upstream promoter transcription factor (COUP-TF). These elements are considered to be co-regulators of the expression of ALDH2 (Pinaire et al., 1999; You et al., 2002). The –357A/G polymorphic locus exists far upstream from the CCAAT box but adjacent to the binding site for ARP-1 and COUP-TF.

Chou and colleagues suggested that –357A/G polymorphism is functional (Chou et al., 1999). They carried out a transfection assay study and reported that the promoter constructs encoded by the G allele were more active than those encoded by the A allele in a hepatoma cell line.

Harada and coworkers suggested that the –357A/G polymorphism is associated with alcoholism (Harada et al., 1999). They performed an association study in Glu487– homozygous 185 alcoholics and 206 controls, and reported a significant difference between genotype frequencies in alcoholics and normal controls. However, their results failed to show a significant difference in allelic frequencies; thus, the association between the –357A/G polymorphism and alcoholism remains controversial.

To confirm the association between this ALDH2 promoter polymorphism and alcoholism, we designed an association...
study using larger samples of Japanese alcoholic patients and normal controls, including subjects with the inactive ALDH genotype.

MATERIALS AND METHODS

Samples
This study was approved by the Ethics Committee of the National Hospital Organization, Kurihama Alcoholism Center. All alcoholic subjects were Japanese, hospitalized in the National Hospital Organization’s Kurihama Alcoholism Center. All alcoholic patients met DSM-III-R diagnostic criteria for alcohol dependence (American Psychiatric Association, 1987). The diagnostics was based upon the Alcohol and Drug Use Disorders module of the Structured Clinical Interview for DSM-III-R (SCID). First, genotyping a sample of >1500 patients to detect the Glu487Lys polymorphism in exon 12 identified 159 Glu487Lys heterozygotes. The rest were homozygous for Glu487; none had homozygous Lys487.

For the present study, we selected the 159 patients with inactive ALDH2 (Glu487Lys) and another 356 alcoholics with active ALDH (Glu487Glu) to focus on alcoholics who have inactive ALDH2, because patients who develop alcoholism against the strong negative risk factor inactive ALDH2 might also possess another genetic factor that increases their risk for alcoholism. In all, 515 alcoholic patients (508 males, mean age 50.6 ± SD 9.1 years; 7 females, 45.3 ± 9.2 years) underwent genotyping of the –357A/G polymorphism in the ALDH2 promoter region.

The normal control group consisted of 361 unrelated Japanese volunteers (355 males, 48.4 ± 15.2 years, 6 females, 45.0 ± 8.2 years), mainly hospital employees or persons connected with them. They were selected from our database and matched by age and gender with the alcoholic group. Drinking problems were assessed using the Kurihama Alcoholism Screening Test (KAST) (Saito and Ikegami, 1978), and control subjects who were suspected to be alcohol-dependent on the basis of KAST results were excluded from the study.

Genotyping
Polymorphism in exon 12 was assessed by the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method (Crabb et al., 1989). Briefly, after informed consent, peripheral blood was obtained from each subject and leucocyte DNA was extracted. Portions of exon 12 of the ALDH2 gene were amplified using PCR, digested with MboII, electrophoresed on 12% acrylamide gel, and viewed with the aid of ethidium bromide staining.

Genotyping of the ALDH2 promoter –357A/G polymorphism was also performed by the PCR–RFLP method on DNA extracted from peripheral leucocytes. Two PCR primers were prepared:

Forward primer: 5’-CGCATCGGCTGACCGTCT-3’
Reverse primer: 5’-GGTACTTATAAAAGCGTGG-GCTC-3’

PCR amplification of DNA was accomplished by denaturing at 94°C for 30 seconds, annealing at 63°C for 110 s, extending at 72°C for 30 s, and 35 cycles of a GeneAmp PCR system 9600 thermal cycler (Perkin-Elmer, Norwalk, CT). We amplified a total of 50–400 ng of genomic DNA in 50 µl of PCR mixture containing 80 µM of each dNTP, 1.5 mM magnesium chloride, 50 mM potassium chloride, and 1 U of Taq DNA polymerase. A 10 µl sample of each PCR product was digested with 20 U of HindIII overnight at 37°C, electrophoresed on 13.3% acrylamide gel, and subsequently visualized by staining with ethidium bromide.

Statistical analysis
Differences in the distribution of genotypes and alleles were tested using the χ²-test. To test for LD between the two polymorphic loci, we used the program ASSOCIAT, version 2.35, programmed by Dr J. Ott (http://linkage.rockefeller.edu/ott/linkutil.htm).

RESULTS
The distribution of genotype and allele frequencies of the ALDH2 promoter polymorphism is shown in Table 1. Because of the LD between two polymorphisms, we analysed the genotype and allele frequencies in active ALDH2 (Glu487Glu) and inactive ALDH2 (Glu487Lys) groups. None of the subjects in the alcoholic sample was a Lys487 homozygote.

Among the subjects with active ALDH2, the frequency of the A allele was 25.8% in alcoholics and 30.2% in normal controls; among subjects with inactive ALDH2, it was present in 19.2% of alcoholics and 19.7% of normal controls. These frequencies in the active ALDH2 group were almost the same as those reported in the previous study (Harada et al., 1999), in

Table 1. Distribution of genotype and allele frequencies of the ALDH2 promoter polymorphism in alcoholics and normal controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. subjects (n = 876)</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GG (%)</td>
<td>GA (%)</td>
</tr>
<tr>
<td>Active ALDH2 (Glu487Glu)*</td>
<td>553</td>
<td>195 (54.8)</td>
<td>138 (38.8)</td>
</tr>
<tr>
<td>Alcoholics</td>
<td>356</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>197</td>
<td>93 (47.2)</td>
<td>89 (45.2)</td>
</tr>
<tr>
<td>Inactive ALDH2 (Glu487Lys)**</td>
<td>301</td>
<td>98 (61.6)</td>
<td>61 (38.4)</td>
</tr>
<tr>
<td>Alcoholics</td>
<td>159</td>
<td>87 (61.3)</td>
<td>54 (38.0)</td>
</tr>
<tr>
<td>Controls</td>
<td>142</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The number of subjects is denoted by n (the number of alleles is 2n). Total control group includes 22 with Lys487Lys. Alcoholic group had none with Lys487Lys. Differences in distribution between alcoholics and controls. *Genotype: χ² = 2.91, df = 2, P = 0.23; allele: χ² = 2.42, df = 1, P = 0.12. **Genotype: χ² = 1.12, df = 2, P = 0.57; allele: χ² = 0.28, df = 1, P = 0.87.
Table 2. Distribution of polymorphisms in the ALDH2 promoter region and in exon 12

<table>
<thead>
<tr>
<th>Exon 12 genotype</th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls**</td>
<td>93</td>
<td>89</td>
<td>15</td>
<td>197</td>
</tr>
<tr>
<td>Glu487Glu</td>
<td>87</td>
<td>54</td>
<td>1</td>
<td>142</td>
</tr>
<tr>
<td>Lys487Lys</td>
<td>21</td>
<td>1</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Alcoholics**</td>
<td>195</td>
<td>138</td>
<td>23</td>
<td>356</td>
</tr>
<tr>
<td>Glu487Glu</td>
<td>98</td>
<td>61</td>
<td>0</td>
<td>159</td>
</tr>
</tbody>
</table>

Alcoholic group had none with Lys487Lys. Statistical analysis for LD by ASSOCIAT: *delta value ($D^*$) = 0.056, $\chi^2$ = 33.8, df = 4, $P = 0.000001$. **$\chi^2$ = 17.8, df = 4, $P = 0.013$.

which 24% of alcoholics and 27% of normal control Japanese homozygous for Glu487Glu had the A allele.

There were no significant differences between the ALDH promoter genotype and allele frequencies in alcoholic patients and normal controls in each group with active and inactive ALDH2. In the active ALDH2 group, the frequency of the A allele tended to be slightly lower in the subgroup with subjects who were alcoholic, but there was no significant difference between alcoholics and controls (for the genotype, $\chi^2 = 2.91$, df = 2, $P = 0.23$; for the allele, $\chi^2 = 2.42$, df = 1, $P = 0.12$). Among the subjects with inactive ALDH2, the alcoholic and control groups were approximately equal with regard to genotype and allele frequencies (for the genotype, $\chi^2 = 1.12$, df = 2, $P = 0.57$; for the allele, $\chi^2 = 0.28$, df = 1, $P = 0.87$).

Table 2 shows the distribution of the two alleles within the promoter and exon 12. Among control subjects, statistical analysis revealed very strong LD between Glu487 and the −357/A allele (delta value = 0.056, $\chi^2 = 33.8$, df = 4, $P = 0.000001$). The delta value of alcoholic subjects could not be calculated because the alcoholic samples included only a part of samples with Glu/Glu genotype and the proportion of Glu/Glu samples were arbitrary, but the distribution of alleles among alcoholic subjects confirmed the same LD.

DISCUSSION

In this first replication study of the association between the ALDH2 promoter −357A/G polymorphism and alcoholism, the results suggest lack of an association. The distribution of genotype and allele frequencies revealed no statistical differences between alcoholic and control subjects in either Glu487Glu or Glu487Lys genotype of exon 12 polymorphism.

An earlier investigation revealed a significant difference between the frequencies of the −357A/G genotype and alcoholism (Harada et al., 1999). That study showed a significant difference in the genotype frequencies, but no significant difference in allele frequencies. That study’s sample size (185 alcoholics; 206 controls) was smaller than ours (515 alcoholics; 361 controls). While the allele frequencies were approximately equal in both studies, it is more likely that the significant difference in the previous study was the result of a statistical error due to small sample size, and that the −357A/G promoter polymorphism does not affect the risk for alcoholism.

Our sample comprised homozygotes and heterozygotes with the Lys487 allele as well as subjects homozygous for the Glu487 allele, although the previous study had restricted subjects with the Glu487Glu genotype. Because of LD between the ALDH2 promoter −357A/G polymorphism and the Glu487Lys variant in exon 12, it is possible that the effect of the −357A/G polymorphism on alcoholism is due to linkage to the Glu487Lys polymorphism and is not attributable to changes in promoter activity. Therefore, we investigated the genotype and allele frequencies within groups with active (Glu487Glu) and inactive (Glu487Lys) ALDH2. Owing to linkage of the two polymorphisms, the distributions of the −357A/G genotypes differed between the active and inactive ALDH2 groups; however, the differences between alcoholics and controls were not statistically significant for either group.

If the −357A/G polymorphism alters the expression of ALDH2, hence acetaldehyde elimination, it might affect drinking behaviour and the risk for alcoholism in the same way that the Glu487Lys polymorphism influences drinking behaviour. Chou et al. (1999) reported an ~50% difference between the activity of the A and G alleles in the expression of the gene in a transfection assay using a hepatoma cell line. The function of the −357A/G polymorphism in vivo remains uncertain, however, because that study failed to show the same difference of expression in transfection assays using other cell lines, and there was no difference between the abilities of the transcription factors COUP-TFI and ARP-1 to bind with oligonucleotides representing the A and G alleles. Polymorphism of the ALDH1 promoter region was reported to be associated with alcoholism in spite of its less important role in the elimination of ethanol (Ehlers et al., 2004). If those findings are valid, our results suggest that the −357A/G promoter polymorphism has no effect or much less influence on the metabolism of acetaldehyde.

In our study, all subjects were Japanese. Among Caucasians, the A allele frequency is higher than in Asian populations, and the Lys487 allele is not detectable. The role of the −357A/G polymorphism might vary across different racial groups because of racial differences in allele frequencies. Moreover, our study did not examine the clinical characteristics of the subjects, such as the severity, symptoms, and course of alcoholism. Although a more detailed study might be needed to consider clinical characteristics and to include multiethnic samples, these results strongly suggest that the −357A/G polymorphism has no effect on the incidence of alcoholism.

REFERENCES


