Association of Glutatione S-Transferase (GSTM1 and GSTT1) Gene Deletions in Korean Patients with Alcoholism

Zheng Long Tai¹, Yoon Kyung Uhm², Jong-Woo Kim³ and Sung-Vin Yim²,4*

¹Department of Pharmacology, School of Medicine, Brain Science and Engineering Institute, CMRI, Kyungpook National University, Daegu 702-701, Departments of ²Pharmacology, ³Neuropsychiatry, ⁴Clinical Pharmacology, School of Medicine, Kyung Hee University, Seoul 130-701, Korea

ABSTRACT

Alcoholism is caused by a complex interaction between genetic and environmental factors. Findings obtained from several studies indicate that some tissue damage occurring in alcohol abusers is due to the generation of reactive oxygen species during the ethanol metabolism. The objective of this study was to examine the associations between the polymorphisms of glutathione S-transferase (GST) M1 and T1 genes and Korean male patients with alcoholism. We investigated the distribution of deletion of GSTM1 and GSTT1 in Korean male patients diagnosed with alcoholism (n=133) and Korean male control subject without alcoholism (n=91) with polymerase chain reaction (PCR) method. GSTM1 showed significant associations with alcoholism susceptibility (p=0.0002). But GSTT1 showed no significant associations (p=0.0948). In combined analysis, both gene deletion and GSTM1 deletion were associated with alcoholism (p < 0.0001 and p < 0.0150). These results suggest that GSTM1 gene deletion might play an important role in risk for alcoholism.

Key words: alcoholism, genetic polymorphism, glutathione S-transferase, association

INTRODUCTION

Alcoholism is a clinically and etiologically heterogeneous syndrome that is caused by a complex interaction between genetic and environmental factors (Merikangas, 1990; Oroszi and Goldman, 2004). Recently, many studies are focused with genetic polymorphism of alcoholism. Studies about drug-metabolizing enzymes such as, cytochrome P450 enzymes, alcohol dehydrogenase, and aldehyde dehydrogenase showed possible associations with alcoholism susceptibility (Suzuki et al., 2004). Other genes such as, serotonin-related genes and dopamine-related genes were also studied. Also there is considerable evidence implicating reactive oxygen species (ROSs) and their products in the pathology of alcoholic disease (Arteel, 2003). ROSs are generated during alcohol metabolism as a result of the generation of both NADH from the conversion of ethanol to acetaldehyde by alcohol...
dehydrogenase and NADPH from the metabolism by cytochrome P450 2E1 (CYP2E1) (Kunitoh et al., 1997). In addition ROSs are generated by alcohol-related cell damage (Hoek and Pastoria, 2002), which suggests that ROSs are central to alcohol-related organ damage. ROSs are highly reactive and damage cellular macromolecules such as lipids, DNA and proteins (Arteel, 2003).

The glutathione S-transferases are dimeric phase II metabolic enzymes that catalyze the reaction between reduced glutathione and toxic, as well as carcinogenic compounds with electrophilic center such as aliphatic and heterocyclic radicals, epoxides, or aren oxides (Keen and Jakoby, 1978; Hayes and Pulford, 1995; Armstrong, 1997; Hayes and McLellan, 1999). GSTs are a superfamily, in which seven classes have been found, but only class (GSTM1) and class theta (GSTT1) have gene deficiency (null genotype). The GST enzymes are believed to exert a critical role in cellular protection against reactive oxygen species (Hayes and McLellan, 1999). GSTs are associated with protection from pro-oxidant stress (Mari and Cederbaum, 2001). In humans, genetic polymorphisms have been described in GSTM1, GSTT1 and GSTP1 (Hayes and Pulford, 1995). Three different polymorphisms have been described at the GSTM1 locus on chromosome 1p13.3. The most important polymorphism encodes for a partial gene deletion in GSTM1 (GSTM1 null genotype) resulting in complete absence of GSTM1 enzyme activity. The two other polymorphisms do not lead to functional differences (Beckett and Hayes, 1993). The frequency of the GSTM1 null genotype ranges from 23 to 62% in different populations over the world.

In this paper, we studied the possible association between alcoholism and the null polymorphism of GSTM1 and GSTT1 genes in Korean male alcoholics.

**MATERIALS AND METHODS**

**Human subjects and DNA extraction**

The control subjects consisted of 91 healthy, unrelated male Koreans visiting public health centers for health status evaluation. They did not have any psychiatric or physical disease, nor a personal or familial history of psychiatric or neurologic illness, and their mean age was 45 years. Most of the control group members were nondrinkers, and some were occasional light drinkers. One hundred thirty three unrelated male alcoholism cases were recruited from the Kyung Hee Medical Center and diagnosed by trained psychiatrists according to Diagnostic and Statistical Manual of Mental Disorders, 4th. Edition (DSM-IV) criteria. The mean age of the patients was 46.1 years. All control and patients participating in the study signed a written consent form approved by the Institutional Review Board of Kyung Hee Medical Center. Blood samples were obtained from all subjects and collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted by using a DNA isolation Kit for mammalian blood (Boehringer Mannheim, Indianapolis, IN, USA).

**Genetic polymorphism analysis of GSTM1 and GSTT1 gene**

The genetic polymorphism analysis for the GSTM1 and GSTT1 gene was determined using the multiplex polymerase chain reaction (PCR) approach (Abdel-Rahman et al., 1996). The CYP1A1 gene was co-amplified as an internal positive control. The following primers were used in PCR reaction: GSTM1 primer of 5-GAICTCCCTGAAGGCTAAAGC-3, 5-GTTGGCTCAATATACCGTGG-3, GSTT1 primers of 5-TTCCTGACTGGTCCTCACATCTC-3, 5-TGACCTGCAGCAAG-3 and CYP1A1 5-GAACTGCACCTGCTGCT-3, 5-CAGCTGCATTGGAAGTGCTC-3. PCR was carried out in a total volume of 25 μl with 0.5 μg of DNA, 10 pmol of each of GSTM1, GSTT1 and CYP1A1 primer, 0.2 mM dNTP, 2 mM MgCl2, 2.5 μl of 10X-PCR buffer and 2 U Taq DNA polymerase. The amplification conditions were initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 59°C for 50 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. Amplified products (GSTM1: 215 bp; GSTT1: 480 bp; CYP1A1: 312 bp) were then analyzed electrophoretically on an ethidium bromide-stained 2% agarose gel.

**Statistical analyses**

The distribution of the genotypes in the group of patient with alcoholism was compared with that of
the control group by using \( \chi^2 \) test (2×2 continuity table). All data were analyzed using the Statistical Analysis System software (SAS, version 8.2).

**RESULTS**

The PCR products from amplification of GSTM1 (215 bp), GSTT1 (480 bp) and CYP1A1 (312 bp) on 2% agarose gel are shown in Fig. 1.

Table 1 shows the association between the GSTM1 and GSTT1 gene deletion and alcoholism.

The frequencies of GSTM1 null genotype in alcoholism group and control group were 53.15% (76/133) and 31.87% (29/91), respectively. The frequencies of GSTT1 null genotype in alcoholism group and control group were 56.46% (85/133) and 52.75% (48/91), respectively. There was a significant association of null genotype of the GSTM1 with Korean male alcoholism (p=0.0002). But there was no significant association of null genotype of GSTT1 with alcoholism (p=0.0948).

In combination analysis with both genes, both GSTM1 and GSTT1 null genotypes that occurred in alcoholism group and control group were 14.29% (19/133) and 30.77% (28/91), respectively. The significant difference was found between alcoholism and null genotypes (p=0.0001). GSTT1 positive but GSTM1 null genotype cases in alcoholism group and control group were 15.39% (47/133) and 15.39% (14/91), respectively, and the difference was significant (p=0.0150). GSTT1 null but GSTM1 positive genotype cases in alcoholism group and control group were 28.57% (38/133) and 37.36% (34/91) respectively, and the two groups had no significant difference compared with the controls (p=0.1873) (Table 2).

**DISCUSSION**

Genetic factors are known to be associated with alcohol dependence, and many genes are likely to be involved in the susceptibility to alcohol seeking behavior (Pickens et al., 1991; Sander et al., 1997).

---

**Table 1.** Genotype frequencies and ORs for GSTM1 and GSTT1 polymorphisms in Korean male with and without alcoholism

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Alcoholism (%)</th>
<th>Control (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=133)</td>
<td>(n=91)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>76 (53.15%)</td>
<td>29 (31.87%)</td>
<td>2.8506</td>
<td>1.6301~4.9849</td>
<td>0.0002</td>
</tr>
<tr>
<td>Positive</td>
<td>57 (42.86%)</td>
<td>62 (68.13%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>85 (56.46%)</td>
<td>48 (52.75%)</td>
<td>1.5864</td>
<td>0.9219~2.7298</td>
<td>0.0948</td>
</tr>
<tr>
<td>Positive</td>
<td>48 (33.56%)</td>
<td>43 (47.25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Risk of alcoholism according to the number of putative higher risk GST genotypes

<table>
<thead>
<tr>
<th>GST genotype</th>
<th>Cases (n=133)</th>
<th>Control (n=91)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1</td>
<td>GSTT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>19 (14.29%)</td>
<td>28 (30.77%)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>Null</td>
<td>47 (35.34%)</td>
<td>14 (15.39%)</td>
<td>4.9474</td>
<td>2.1488~11.3910</td>
</tr>
<tr>
<td>Null</td>
<td>Positive</td>
<td>29 (21.80%)</td>
<td>15 (16.48%)</td>
<td>2.8491</td>
<td>1.2139~6.6873</td>
</tr>
<tr>
<td>Positive</td>
<td>Null</td>
<td>38 (28.57%)</td>
<td>34 (37.36%)</td>
<td>1.6471</td>
<td>0.7827~3.4658</td>
</tr>
</tbody>
</table>
Therefore, identifying the candidate genes associated with an increased risk of alcoholism might have important implications for classifying high-risk individuals and determining the specific therapeutic approaches.

In this study, we analyzed GSTM1 and GSTT1 as candidate genes for alcoholism. GSTs are known to be associated with various diseases such as, rheumatoid arthritis, vitiligo, and various cancers (Egan et al., 2004; Yun et al., 2005; Gonlugur et al., 2006; Uhm et al., 2007; Shao et al., 2008). Previous studies showed that the deletion of GSTM1 and GSTT1 showed different susceptibility for disease. Study by Uhm et al. (2007) showed that GSTM1 not T1 is associated with vitiligo susceptibility (Uhm et al., 2007). Yun et al. (2005) reported similar results in rheumatoid arthritis. In their study, GSTM1 showed significant association but GSTT1 showed no significant association with rheumatoid arthritis susceptibility (Yun et al., 2005). Our study also showed similar results. GSTM1 polymorphism showed highly significant association with alcoholism but GSTT1 polymorphism showed no significant association.

In combined analysis, both null type showed highly significant association with alcoholism, GSTM1 only deletion showed also significant association with alcoholism. However, other study by Brind et al. showed contrary result. In their study, GSTT1 gene deletion was associated with alcoholic liver disease (Brind et al., 2004).

We found that GSTM1 null genotype were significantly association between alcoholism in Korean patients. When the potential combined effect of GSTM1 null and GSTT1 null genotype was examined, evidence of an interaction between these polymorphism and alcoholism was observed. These results are well consistent with study by Harada et al. Although small case-control studies of alcoholism, they suggested an increased risk of alcoholism in Caucasian patients with GSTM1 null, but not GSTT1 polymorphism (Harada et al., 1987).

In conclusion, our results suggest a significant relation between GSTM1 null and both null genotype and alcoholism in Korean patients. Of course it is impossible to conclude whether this polymorphism determines an individual susceptibility for alcoholism these results will help to provide clues for understanding of genetic and environmental determinants of alcoholism.

ACKNOWLEDGMENTS

This study was supported by Kyung Hee University Research Fund (2004).

REFERENCES


Marti M and Cederbaum AI (2001) Induction of catalase, alpha, and microsomal glutathione S-transferase in CYP2E1 overexpressing HepG2 cells and protection against...