RESEARCH COMMUNICATION

Genetic Polymorphisms of CYP2E1 and GSTM1 in a Thai Population

Suleeporn Sangrajrang, Adisorn Jedpiyawongse, Petcharin Srivatanakul

Abstract

Cytochrome P450 2E1 and GSTM1 play major roles in metabolic activation and detoxification of many carcinogens and polymorphisms in the encoding genes have been reported to be individually associated with increased susceptibility to certain cancer. In the present study, we investigated the RsaI, PstI and DraI polymorphisms of the CYP2E1 gene and the null GSTM1 genotype in a Thai population. DNA samples from 485 individuals were analysed by polymerase chain reaction with restriction fragment length (PCR/RFLP). The frequency of RsaI and PstI predominant homozygous alleles (c1/c1) was 73.2%, heterozygous allele (c1/c2) was 25.6% and rare homozygous allele (c2/c2) was 1.2%. For the DraI polymorphism, the frequency of the predominant allele (DD) was 59.6%, heterozygous (CD) was 40% and rare allele (CC) was 0.4%. The frequency of GSTM1 null genotype was 62.7%. The distribution and frequencies of these alleles showed different pattern from those found in Caucasian and some other Asian populations. With the large population in this study, we believed that our results are reliable estimates of the frequencies of the polymorphic CYP2E1 and GSTM1 alleles in Thai population and should provide a base for further epidemiological studies on their links with cancer development.

Key Words: CYP2E1 - GSTM1 - genetic polymorphisms

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Introduction

It has been estimated that over 80% of human tumors are due to the action of environmental carcinogens (Doll and Peto, 1981). Most chemical carcinogens are not capable of inducing genetic damage by themselves but require metabolic activation to electrophilic proximate carcinogens. The amount of the ultimate carcinogens produced depends on the action of competing activation and detoxification pathways involving cytochrome P450 and glutathione-S-transferase enzymes (Guengerich, 1990; Mannervik et al., 1992). Genetic differences in these pathways are likely to be major sources of interindividual variation in susceptibility to cancer (Idle, 1991) and some other diseases (Wong et al., 2000).

The oxidation by CYP 450 enzymes is primarily regarded as the phase-I activating process in carcinogenesis (Roots et al., 1992). Genetic polymorphism in CYP2E1 might be a contributing cancer risk factor since this enzyme activates procarcinogen, such as N-nitrosamine, benzene, butadiene, vinyl chloride, PAHs, and other low molecular weight chemicals (Wang et al., 1999). The PstI and RsaI polymorphism is the 5′ flanking (promoter) region of the gene which are reported to affect the transcriptional activity of the gene (increase inducibility) and are linked with each other: an allele possessing a positive restriction point for RsaI is designated as a c1 allele and one with a PstI restriction point, a c2 allele (Maizawa et al., 1994). The DraI polymorphism is associated with a mutation in intron 6 of the gene. Although a direct relationship of the RFLP to CYP2E1 expression and activity has not been established, the distribution of the DraI genotype in Japanese was different between lung cancer patients and controls (Uematsu et al., 1991). For the TaqI polymorphism, there is no report of a relationship between polymorphism activity and cancer incidence.

In contrast to phase-I, most phase-II metabolizing enzymes are considered to be predominantly protective enzymes since they detoxify a number of reactive chemical carcinogens. The glutathione S-transferase M1 (GSTM1) gene is responsible for detoxification of certain reactive intermediates of potential human carcinogen, including PAHs by conjugation to glutathione. Many studies indicated that GSTM1 polymorphism is correlated with bladder cancer (Bell et al., 1993) and lung cancer (Brockmoller et al., 1993). Cancer is the leading cause of death in Thailand.
Occupational and environmental exposure to potential carcinogens occurs in recently industrialized countries. In particular, exposure to known and suspected carcinogens might have occurred in a large industrial complex in the Rayong province of Eastern Thailand. Among the chemicals that have been detected in a survey conducted in November 2005 are benzene, vinyl chloride, 1,3-butadiene, dichloromethane, dichloroethane and various polycyclic aromatic hydrocarbons (PAH) (data from Department of Pollution Control, Ministry of Public Health, Thailand). We therefore undertook a study to determine the frequencies of the polymorphic of CYP2E1 and GSTM1 alleles among people in Rayong province. The results will provide a basic database for future clinical and genetic studies concerning variability in the response and/or toxicity to drugs known to be substrates for CYP2E1 and GSTM1.

### Materials and Methods

#### Study population

A total of 485 healthy Thai were recruited in the study. Subjects were resident of Rayong province in Eastern region of Thailand. This people had a mean age of 34.5 ± 8.2 years (range, 21 to 65). A 7 ml blood sample was obtained from each after receiving informed consent. DNA were extracted using the spin column procedure (QIAmp blood kit, Qiagen, Germany).

#### Identification of genetic polymorphisms

The identification of the CYP2E1 genotypes was carried out by the PCR/restriction digest-genotyping methods. The 50 ml reaction mixture will be contained 50 mM KCL, 20 mM Tris–HCl (pH 8.4), 1.5 mM MgCl2, all four deoxynucleoside triphosphates (each at 0.2 mM), 2.5U of Taq DNA polymerase (Qiagen) and 10 pmol of each primer. DNA amplification were carried out using a DNA thermal cycle model 480 (Perkin-Elmer Cetus). CYP2E1 gene was amplified using the following primers 5’ AGT CGA CA T GTG A TG GA T CCA 3´ and 5´ GAC AGG GTT TCA TCA TGT TGG 3´ for Dral polymorphism (Hirvonen et al., 1993). The PCR products including the polymorphic site were digested with PstI, Rsal or Dral restriction enzymes, then analysed by electrophoresis in 2% agarose gels. Deletion status of GSTM1 was determined by a PCR method using the primers 5’ GAA CTC CCT GAA AAG CTA AAG C 3´ and 5´ GTT GGG CTC AAA TA T ACG GTG G 3´ and co-amplified with another pair of primer pair 5’ CAA CTT CA T CCA CGT TCA CC 3’ and 5’ GAA GAG CCA AGG ACA GGT AC 3’ to amplify β-globin, included in the assay as a positive control for target DNA.

#### Results

**CYP2E1 Rsal and PstI polymorphisms**

The predominant homozygous allele, the heterozygous allele, and the rare homozygous allele were named c1/c1, c1/c2 and c2/c2, respectively. On digestion with Rsal, the fragments from c1/c1 gave bands at 360 and 50 bp (base pair); c2/c2 gave a single band at 410 bp; c1/c2 gave all three bands (Figure 1). On PstI digestion of the fragments amplified from c1/c1 DNA, only a single undigested band was observed at 410 bp; the fragment from c2/c2 DNA gave bands of digestion products at 290 and 120 bp; c1/c2 gave three bands at 410, 290, 120 bp. The frequency of predominant allele (c1/c1) was 73.2%, heterozygous allele 67.6% and rare allele (c2/c2) 0.89%.

#### Table 1. CYP2E1 PstI and Rsal Polymorphisms in Different Ethnic Groups

<table>
<thead>
<tr>
<th>Country (PstI, Rsal)</th>
<th>N</th>
<th>c1/c1 (%)</th>
<th>c1/c2 (%)</th>
<th>c2/c2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA (PstI, Rsal)</td>
<td>454</td>
<td>74.4</td>
<td>22.5</td>
<td>3.1</td>
</tr>
<tr>
<td>UK (PstI, Rsal)</td>
<td>375</td>
<td>93.0</td>
<td>7.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Finland (Rsal)</td>
<td>121</td>
<td>97.5</td>
<td>2.5</td>
<td>0.0</td>
</tr>
<tr>
<td>France (Rsal)</td>
<td>206</td>
<td>95.4</td>
<td>4.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Sweden (Rsal)</td>
<td>148</td>
<td>89.9</td>
<td>9.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Japan (Rsal)</td>
<td>612</td>
<td>63.9</td>
<td>32.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Taiwan (Rsal)</td>
<td>320</td>
<td>61.9</td>
<td>35.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Korean (PstI)</td>
<td>333</td>
<td>55.3</td>
<td>39.6</td>
<td>5.1</td>
</tr>
<tr>
<td>China (Rsal)</td>
<td>150</td>
<td>44.0</td>
<td>51.4</td>
<td>4.6</td>
</tr>
<tr>
<td>India (PstI, Rsal)</td>
<td>223</td>
<td>98.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>India (PstI, Rsal)</td>
<td>50</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Thailand (Rsal)</td>
<td>297</td>
<td>63.7</td>
<td>34.6</td>
<td>1.7</td>
</tr>
<tr>
<td>This study (PstI, Rsal)</td>
<td>485</td>
<td>73.2</td>
<td>25.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Reference

Le Marchand et al., 1998
Wong et al., 1999
Hirvonen et al., 1993
Lucas et al., 1996
Persson et al., 1993
Oyama et al., 1997
Hildesheim et al., 1997
Wong et al., 2002
Tan et al., 2000
Sikdar et al., 2003
Mittal et al., 2005
Kongrattanachok et al., 2001

**Figure 1. RFLP of PCR-amplified Fragments of CYP2E1 Obtained with Rsal**
The GSTM1 gene is polymorphic in human in that the product of GSTM1 and β-globin were 215 and 268 bp in length respectively. The homozygous null genotype was 62.7% (Table 2).

**CYP2E1 DraI polymorphism**

In the PCR-based RFLP analysis, the rare homozygous genotype (CC) gave an undigested 373 fragment. In the predominant homozygous genotype (DD), this fragment was digested into 240 and 133 bp fragments. In the heterozygous (CD), all three polymorphic restriction fragments were visible. The frequency of DD genotype was 59.6%, CD genotype was 40%, and CC genotype was 0.4% (Table 2).

**GSTM1 analysis**

The GSTM1 gene is polymorphic in human in that the gene is either present or absent (null genotype). The PCR product of GSTM1 and β-globin were 215 and 268 bp in length respectively. The homozygous null genotype was 62.7% (Table 3).

**Discussion**

The activities of metabolizing drugs and carcinogens are known to be genetically variable in human individuals (Guengerich, 1989). As cytochrome P450 plays an important role in this system, we studied the PstI, RsaI and DraI polymorphisms of CYP2E1 in a Thai population. We found frequencies similar to those reported in a previous study in Thailand (Kongruttanachok et al., 2001) and also in other Asian countries (Oyama et al., 1997; Hildesheim et al., 1997; Wong et al., 2002). However, the results of two studies from India showed the frequency of c2 allele to be very low in an Indian population (Sikdar et al., 2003; Mittal et al., 2005). CYP2E1 c2/c2 and c1/c2 genotypes are more common in Asians than in Caucasians (Table 2). It has been reported that these polymorphisms are correlated with the incidences of bladder (Anwar et al., 1996) and lung cancer s(Oyama et al., 1997). In addition, Hayashi et al. (1991) showed V50 2E1 RsaI polymorphism caused marked differences in its transcriptional activity, the enhancer activity for c2/c2 DNA was about 10 times that of c1/c1 DNA.

At the DraI site, the frequency of DD, CD, and CC was 59.6%, 40%, 0.4%, respectively. The frequency of C allele is more common in Asians than in Caucasians (Table 3). Currently, there is much confusion about the role of CYP2E1 Dra polymorphism in relation to cancer susceptibility. Hirvonen et al. (1993) provided evidence of no role in susceptibility to lung cancer in Finish population, but Uematsu et al. (1994) found a link with lung cancer especially in a population with low smoking exposure (<20 pack-year). There has also been a report of a higher frequency of the DraI RFLP in other alcohol-related disease (Lucas et al., 1996).

The GSTM1 polymorphism is a deletion of the gene and results in a loss of enzymatic activity. We observed 62.7% of the population were homozygous for the GSTM1 deletion. The frequency was higher that reported in a previous study that analysed the GSTM1 polymorphism in Thai population (Kietthubthew et al., 2001; Tiwawech et al., 2005). The percentage of individuals who do not express the GSTM1 enzyme is higher in Caucasian and Asians than Africans (Table 3). Polymorphism of GSTM1 have been shown to be associated with susceptibility to various forms of cancer, particularly those caused by cigarette smoking (Strange and Fryer, 1999), resistance to chemotherapy treatment (Hayes and Pulford, 1995). Deleted GST may be associated with less detoxification of cyclophosphamide, resulting in more available drug compared to the wild-type enzyme (Beeghly et al., 2006).

This study report the preliminary results on metabolic enzyme gene. The frequency of gene polymorphism varies among different ethnic groups. The ability to characterize polymorphic genes involved in metabolism of carcinogens will open up a new approach for human cancer risk and could apply to study environment interactions in pathogenesis of cancer and other disease.

**Acknowledgment**

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References


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