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RESEARCH COMMUNICATION

Genetic Polymorphisms of CYP2E1 and GSTM1 in a Thai Population

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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 CYP2E1 gene is reported to have genetic and radical variations that are caused by PstI, RsaI, DraI and TaqI polymorphisms (D'Errico 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 (Maezawa 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.

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Table 1. CYP2E1 PstI and RsaI Polymorphisms in Different Ethnic Groups

Country	N	c1/c1 (%)	c1/c2 (%)	c2/c2 (%)	Reference
USA (PstI, RsaI)	454	74.4	22.5	3.1	Le Marchand et al., 1998
UK (PstI, RsaI)	375	93.0	7.0	0.0	Wong et al., 1999
Finland (RsaI)	121	97.5	2.5	0.0	Hirvonen et al., 1993
France (RsaI)	206	95.4	4.2	0.4	Lucas et al., 1996
Sweden (RsaI)	148	89.9	9.4	0.7	Persson et al., 1993
Japan (RsaI)	612	63.9	32.0	4.1	Oyama et al., 1997
Taiwan (RsaI)	320	61.9	35.3	2.8	Hildesheim et al., 1997
Korean (PstI)	333	55.3	39.6	5.1	Wong et al., 2002
China (RsaI)	150	44.0	51.4	4.6	Tan et al., 2000
India (PstI, RsaI)	223	98.0	2.0	0.0	Sikdar et al., 2003
India (PstI, RsaI)	50	100	0.0	0.0	Mittal et al., 2005
Thailand (RsaI)	297	63.7	34.6	1.7	Kongrattanchok et al., 2001
This study (PstI, RsaI)	485	73.2	25.6	1.2	

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 polymorphism 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 µl 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' CCA GTC GAG TCT ACA TTG TCA 3' and 5' TTC ATT CTG TCT TCT AAC TGG 3' for PstI and RsaI polymorphisms (Anwar et

al., 1996), 5' AGT CGA CAT GTG ATG GAT CCA 3' and 5' GAC AGG GTT TCA TCA TGT TGG 3' for DraI polymorphism (Hirvonen et al., 1993). The PCR products including the polymorphic site were digested with PstI, RsaI or DraI 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 TAT ACG GTG G 3' and co-amplified with another pair of primer pair 5' CAA CTT CAT 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 RsaI 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 RsaI, 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

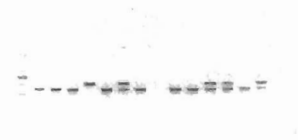


Figure 1. RFLP of PCR-amplified Fragments of CYP2E1 Obtained with RsaI

Table 2. The CYP2E1 DraI Polymorphism in Different Ethnic Groups

Country	N	DD	CD	CC	Reference
USA	452	67.7	26.8	5.5	Le Marchand et al., 1998
UK	375	81.0	18.0	1.0	Wong et al., 2000
Finland	121	79.4	19.8	0.8	Hirvonen et al., 1993
France	206	84.9	14.3	0.8	Lucas et al., 1996
Sweden	152	80.8	18.0	1.2	Persson et al., 1993
Japan	76	56.6	28.9	14.5	Uematsu et al., 1993
Taiwan	320	57.3	38.4	4.3	Hildesheim et al., 1997
India	227	65.0	32.0	3.0	Sikdar et al., 2003
This study	485	59.6	40.0	0.4	

Table 3. The GSTM1 Genotype in Different Ethnic Groups

Country	N	Null (%)	Reference
USA			
Caucasian	465	52.5	London et al., 1995
Mexican-American	146	40.4	Kelsey et al., 1997
African-American	251	27.1	London et al., 1995
UK	577	54.7	Deakin et al., 1996
Norway	342	47.7	Ryberg et al., 1997
Spain	312	49.7	To-Figueras et al., 1997
Japan	447	48.6	Kihara et al., 1995
Taiwan	88	53.4	Hsieh et al., 1996
Korea	1,037	53.8	Cho et al., 2005
China	350	54.6	Zhong et al., 2006
India	370	33.0	Mishra et al., 2004
Singapore	119	63.6	Zhao et al., 2001
Thailand	53	30.2	Kietthubthew et al., 2001
Thailand	145	51.0	Tiwawech et al., 2005
Thailand	485	62.7	This study

(c1/c2) was 25.6% and rare allele (c2/c2) was 1.2%. These two polymorphisms were completely linked with each other (Table 1).

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 (Oyama et al., 1997). In addition, Hayashi et al. (1991) showed P450 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 DraI polymorphism in relation to cancer susceptibility. Hirvonen et al. (1993) provided evidence of no role in susceptibility to lung cancer in Finnish 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 than 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 (Becghly 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.

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