RESEARCH COMMUNICATION

Glutathione S-Transferase P1 Genotypes, Genetic Susceptibility and Outcome of Therapy in Thai Childhood Acute Lymphoblastic Leukemia

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Abstract

Glutathione S-transferases (GSTs) are enzymes that involved in bio- transformation by conjugation of electrophillic compounds to glutathione. Polymorphisms within genes that encode GSTs may affect the function of the enzymes. Polymorphisms of GSTP1 at codon 105 residue forms *GSTP1* active site for binding of hydrophobic electrophiles, and the Ile-Val substitution affect substrate specific catalytic activity of this enzyme and may associate with susceptibility to malignant human disease, especially acute lymphoblastic leukemia (ALL), which is the most common leukemia in children younger than 15 years old.Genetic polymorphisms within the *GSTP1* gene of childhood ALL patients were studied. In addition, the association of genetic polymorphism of *GSTP1* and genetic susceptibility of acute lymphoblastic leukemia (ALL) was also determined using Chi-square and Odds ratio. PCR-RFLP was used to study genetic polymorphism of *GSTP1* in 100 ALL patients and 100 healthy individuals.The results show that there is no statistically significant association between each genotypes and genetic susceptibility of acute lymphoblastic leukemia (ALL) (OR=0.92, P –value=0.886). Moreover, there is no statistically significant association between each genotypes and demographic data of acute lymphoblastic leukemia (ALL). However, there are 2 cases of ALL with BM relapse show the polymorphic genotypes of *GSTP1*. It may suggest that *GSTP1*V105* may be involved in relapse of ALL.

Keywords: Acute lymphoblastic leukemia (ALL) - glutathione S-transferase (GST) - GSTP1

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Introduction

Glutathione S-transferases (GSTs) are major phase II detoxifying enzymes and are able to perform a wide variety of functions. Phase II enzymes catalyse conjugation of activated xenobiotics to an endogeneous water soluble substrate, such as glutathione (GSH), UDP-glucuronic acid or glycine (Miller et al., 1999).

The GSTs comprises a complex and widespread enzyme superfamily that has been subdivided into eight classes (Strange et al., 2001). *GSTP1* codes for enzyme glutathione S-transferase pi and is located on chromosome 11q13. It is also subject to polymorphic variation (Hayes and Pulford, 1995). Codon 105 residues from part of the GSTP1 active site for binding of hydrophobic electrophiles and the Ile-Val substitution affect substrate-specific catalytic activity and thermal stability of the encoded protein (Fritz, 2005). Human GSTP1 has been shown to catalyse the isomerization of 13-cisretioic acid to all-trans-retioic acid (Fukai et al., 1992). With this reason, GSTP1 is mostly studied for the susceptibility of acute myeloid leukemia (Allan et al., 2001). Previous studies that examined the relationship between GSTP1 genotype and etiology and outcome of therapy in childhood acute lymphoblastic leukemia (ALL) are still controversial (Davies et al., 2002).

There are still no reports of *GSTP1* genotypes in Thai childhood ALL. Therefore, our study of 100 Thai children with ALL, was to provide an additional and more definitive data to be a preliminary report of *GSTP1* genotypes in Thai childhood ALL. Furthermore, an association study between the polymorphism of *GSTP1* and clinical outcome was also examined.

Materials and Methods

Specimens

One hundred cases of children (43 females and 57 males) with a median age of 5 years (range, 10 months to 14 years 9 months) were diagnosed with ALL at the Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University. Patients were treated on risk-adapted chemotherapy regimens modified total XII protocol (Smith et al., 1996). This was

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Table1. The Sequences of GSTP1 primers

Primers	Sequences
FGSTP1	TCCCCAGTGACTGTGTGTTG
RGSTP1	GAAGCCCCTTTCTTTGTTCA

retrospective case-control study. Normal controls were 100 cases of Thai normal individuals who were healthy (45 females and 55 males) and complete blood counts were normal.

DNA isolation

Genomic DNA was extracted form peripheral blood obtained from subjects using salting out methods with some modification (Miler et al, 1998). Briefly, the pellet of WBC was incubated in lysis buffer (10 mM Tris HCl, pH 8.0, 400 mM NaCl, 2 mM EDTA), 200 µl of 10% Sodium Dodecyl Sulfate (SDS) and 10 mg/ml Proteinase K at 60º C for 3 hours. The solution mixture was mixed with 6M NaCl, shaken and centrifuged at 10,000g at 4ºC for 10 minutes. DNA was precipitated by adding absolute ethanol and then washed 3 times with of 70% ethanol. DNA pellet was then dissolved in TE buffer.

PCR Protocols

PCR was performed from the DNA isolated from 100 ALL patient blood and 100 normal control blood with GSTP1 primers as shown in Table 1. Amplification was carried out in 50 µl reaction mixture containing PCR buffer(10mM Tris-HCl, pH 9.0, 50mM KCl,1.5 mM MgCL2), 200 µM of each dNTP, 1.5 mM $MgCL_2$, 1 Unit of *Taq* DNA polymerase (Pharmacia Biotech, USA), 20 µM of each primers and 100 ng of genomic DNA.. DNA amplification was performed in Gene Amp PCR system 9700 for 40 cycles. Each cycle consists of denaturation at 95ºC for 1 min, annealing at 60ºC for 1 min and extension at 72ºC for 1 min. PCR products were electrophoresized in 2% agarose gel.

Genotyping of GSTP1 gene polymorphism

The A to G polymorphism in codon 105 of GSTP1 gene (AAT to GAT) created *Bsm*AI recognition cleavage site. Genotyping of *GSTP1* gene polymorphism was performed by PCR-RFLP. The PCR product from exon 4 was digested for 18 hrs with 10U of *Bsm*AI (Biolabs,France) and visualized in 2% agarose gel stained with ethidium bromide. The digested products reveal the presence of 3 different patterns; the A/A wide type homozygote, demonstrated a 224 bp fragment, the G/G polymorphic homozygote presented the expected 146 bp fragment and 78 bp fragment, whereas A/G heterozygote exhibited 224, 146 and 78 bp fragments, respectively.

Statistical analysis

The differences in genotypes and allele frequencies between cases and controls were determined by using Chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) were also included. The association between demographic data of ALL patients and distribution of *GSTP1* genotypes were determined by Pearson Chi-square statistic or Fisher exact test. (SPSS version 12.0 (SPSS, Inc., Chicago, IL.) was used for all statistical methods.

Results

Genotype distribution

GSTP1 distribution was assessed in100 ALL childhood and 100 controls. The allele frequency of *GSTP1* polymorphism was 0.23 and 0.20 in ALL patients and controls, respectively. The genotype distribution was in Hardy-Weinberg equilibrium. There was no significant differences in genotype distribution of *GSTP1* between ALL patients and controls (c2=0.256, P=0.886) (Table 2).

GSTP1 and the association of risk classification

With the modern chemotherapy, ALL patients can be classified according to the high risk (white blood cell count ≥50,000/ml) and low risk or standard risk (white blood cell count <50,000/ml) (Smith et al, 1996).It was found that, there was no association between *GSTP1*V105* and the high risk group in ALL patients. (c2=0.11, P=0.918) (Table 3).

GSTP1 and the association of the age at diagnosis

There was no statistically significant difference between genotypes of GSTP1 and (c2 =0.21, P = 0.380) (Table 3).

Discussion

Human GSTP1 has been shown to catalyse the isomerization of 13 cis retinoic acid which is associated with the pathogenesis of AML. In ALL, enhanced GSTP1 expression was described in blast from childhood acute lymphoblastic leukemia (Den Boer et al, 1999). Therefore, it is interesting to study the association between GSTP1*V105 polymorphism and risk of ALL. However, in this study, the results showed that there was no statistically significant association of *GSTP1*V105* polymorphism and ALL susceptibility. This was in accordance with meta-analysis of *GSTP1* in ALL, which found no association between *GSTP1*V105* polymorphism and ALL in most studies (Zheng and Honglin, 2005). From the result of demographic data, there was no statistically significant association between

Table 2. GSTP1 Allele Frequency in Childhood ALL Patients and Normal Controls

	n	Genotype, n (%)			Gene frequency		OR, 95%CI	*p*
GSTP1		I105/I105	I105/V105	V105/V105	I105	V105		
ALL	100	59	36	5	0.77	0.23	0.92 (0.52-1.62)	0.886
Control	100	61	37	2	0.80	0.20		

Table 3. Demographic Data of ALL Patients and Distribution of GSTP1 Genotypes

Characteristic	Total patient n=100, (%)	GSTP1 I105	GSTP1 V105	P value
Sex:				
Male	58	39	20	0.536
Female	42	22	19	
Age (yrs):				
<2	17	10	7	0.380
3-4	27	15	12	
5-8	23	18	5	
>8	24	15	9	
Risk group:				
standard	62	43	19	0.918
high	38	23	15	
Immunophenotype:				
Early-pre B	21	14	7	0.768
Pre-B	55	33	22	
B-cell	1	-	1	
T-cell	13	9	4	

GSTP1*V105 polymorphism and demographic data,including age at diagnosis, immunophenotype and risk classification. However, GSTP1*V105 polymorphism was found in all 2 cases of ALL with BM relapse. It may suggest that GSTP1*V105 may be involved in relapse of ALL. This is in accordance with the previous report that GSTP1 polymorphism may be involved in relapse of ALL (Martin et al, 2000). Because of polymorphic site at codon 105 is the active site for catalytic property of enzyme GSTP1 (Fritz, 2005), therefore, it may alter the expression of GSTP1 which may involve in xenobiotic metabolism of chemotherapeutic agents. This is in accordance with the previous report which suggest that polymorphism in GSTP1 is associated with susceptibility to chemotherapy- induced leukemia (Davies 2002).

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