Genetic Polymorphisms of Glutathione-S-Transferase P1, T1 and M1 in Pediatric Patients with Acute Lymphocytic Leukemia in a Philippine Tertiary Hospital

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ABSTRACT

Introduction. Glutathione S-transferases (GSTs) are major detoxifying enzymes that modify susceptibility in cancers including acute lymphocytic leukemia (ALL). This paper determines the frequency of GST polymorphisms (M1, T1, P1) in Filipino ALL patients and control subjects and compares the frequencies between the two groups.

Methods. Pediatric ALL patients at the UP-PGH Medical Center seen from January to June 2007 were enrolled. Age and sex matched subjects without ALL from the UP-PGH Outpatient Department were included as controls. Genomic DNA was extracted from peripheral blood of each subject. GSTM1 and T1 polymorphisms were determined using polymerase chain reaction (PCR) while restriction fragment length polymorphism (RFLP) analysis was employed for the determination of GSTP1 polymorphisms. Matched Odds Ratio was used to compare the genomic frequencies of control and ALL patients.

Results. The presence of GSTT1 and GSTM1 polymorphisms showed a trend towards protection from having ALL, with OR 0.59 (95% CI: 0.24-1.36) and OR 0.86 (95% CI: 0.36-2.00), respectively. Having the GSTP1 polymorphism was shown to be a risk factor [OR 1.7 (95% CI: 0.74-4.15)].

Conclusion. Differences in GST polymorphism frequencies were noted between the control group and ALL patients. GSTT1 and GSTM1 polymorphisms appear protective while having the GSTP1 polymorphism confers increased risk for ALL.

Key Words: Glutathione-S-transferase, Acute lymphocytic leukemia, polymorphic enzymes

Introduction

Research on cancer is now geared towards identifying host factors that modify susceptibility and treatment outcome. Numerous studies have been undertaken in the search for genes that may increase or decrease an individual's risk of susceptibility of having cancer. Much focus is given to genes encoding for detoxifying enzymes that interact with various carcinogens and genes that play a role in the prevention of carcinogenesis.

Glutathione-S-transferases are major detoxifying enzymes that provide critical defense from various chemical and environmental carcinogens. They are a multigene family of phase II detoxification enzymes that catalyze the conjugation of both endogenous and exogenous electrophiles with the non-protein thiol glutathione. The resulting conjugate is more water soluble and in most instances, less toxic. This results in protection against toxicity and chemical carcinogenesis, especially during the initiation phase.[1] Six classes of GSTs have been identified with four (α, μ, σ, π) being well characterized and known to be polymorphic. The polymorphisms that have been most extensively evaluated as biomarkers of cancer risk are GSTM1, GSTT1, and GSTP1. Mutations in subtypes P1, T1, and M1 polymorphisms have been implicated as risk factors in a number of malignancies - colorectal carcinoma,[2,3] gastric carcinoma,[3,4] lung cancer,[5,6,7] bladder carcinoma,[5] hamartomas,[7] gallbladder carcinoma,[8] esophageal carcinoma,[9] and cervical carcinoma,[10] among others. In most studies, polymorphisms were analyzed in association with known environmental risk, like smoking. Hematologic malignancies were included in similar studies with evidence pointing to associations between acute lymphocytic leukemia (ALL) and GST polymorphism genotypes.[11,12]

Acute lymphocytic leukemia (ALL) is the most common form of malignancy in childhood. ALL is more common in males than in females with a noted peak of incidence between ages 2 and 5 years. Three to four out of 100,000 white children are affected. In the United States, it accounts for approximately three quarters of diagnosed cases of leukemia annually.[13] Local data on the incidence of ALL is not available. However, in the Pediatric Hematology Section of the Philippine General Hospital, an average of 22 new cases are seen yearly.

Race was long recognized as an important factor in the risk assessment of ALL with this malignancy being more common in the white population. Racial differences in

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susceptibility and even treatment outcome have been shown to be present.[14-19] Survival variability by race and ethnicity was also demonstrated with ALL children from white and Asian/Pacific Islander populations having better survival rates compared to black, Hispanic, and American Indian/Alaskan natives.[16,17]

To our knowledge, this is the first study comparing the frequency and possible association between common GST polymorphisms and ALL in a Filipino population. The authors also describe the demographic features of pediatric ALL patients in the UP-PGH Medical Center. This paper may provide additional baseline data to the currently few studies[20,21] on the frequency of GST polymorphisms in the Filipino population. It will also help future research that attempts to elucidate the differences in ALL susceptibility and survival variability among races with levels of detoxifying enzymes as a parameter.

Methods

Subjects

Patients, 18 years of age and below, diagnosed with ALL through morphology and/or flow cytometry and undergoing treatment at the Department of Pediatrics in the University of the Philippines-Philippine General Hospital (UP-PGH) Medical Center from January 2007 to June 2007 were included in the study population. Age- and sex-matched subjects without chronic illness, malignancy nor signs of a hematologic disorder, seen at the UP-PGH Out Patient Department, were included as controls. Demographic characterization of ALL patients was done by chart review and follow- up interviews.

Informed consent was obtained from both patients and controls or their guardians before the study. All samples were collected in accordance with local institutional review board or medical ethics committee guidelines.

Genotyping for GSTM1, GSTP1, GSTT1

Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) from peripheral blood of both patients and controls.

A 220-bp fragment from GSTM1 was PCR-amplified using the primers F: 5′- GAA CTC CCT GAA AAG CTA AAG C-3′ and R: 5′- GTT GGG CTC AAA TAT ACG GTG G - 3′. A 312-bp fragment from the CYP1A1 gene was co-amplified as internal control using the primers CYP1A1_f: 5′- GAA CTG CCA CTT CAG CTG TCT-3′ and CYP1A1_r: 5′- CAG CTG CAT TTG GAA GTG CTC - 3′.

A 442-bp fragment from GSTP1 was PCR-amplified using the primers GSTP1_f: 5′-GTA GTT TGC CCA AGG TCA AG - 3′ and GSTP1_r: 5′- AGC CAC CTG AGG GGT AAG - 3′. The resulting PCR product was digested with 2.5U *Al26wI* for 4h at 37°C.

A 480-bp fragment from GSTT1 was PCR-amplified using the primers F: 5′- TTC CTT ACT GGT CCT CAC ATC TC - 3′ and R: 5′- TCA CCG GAT CAT GGC CAG CA - 3′. A 312-bp fragment from the CYP1A1 gene was co-amplified as internal control using F: 5′- GAA CTG CCA CTT CAG CTG TCT-3′ and R: 5′- CAG CTG CAT TTG GAA GTG CTC-3′.

Each PCR reaction mixture contained: 100-200 ng genomic DNA, 1X PCR Buffer, 2mM MgCl2, 200 uM DNTPs, 0.01 uM of each primer, and 0.75 U Taq Polymerase. The amplification profile is as follows: initial denaturation (5 min, 94°C), 35 cycles of denaturation (1 min, 94°C), annealing (50s, 65°C), and extension (1 min, 72°C), followed by final extension (5 min, 72°C). All PCR products and digests were resolved in a 2% agarose gel.

Statistical analysis

Frequencies of polymorphisms of ALL patients and controls were statistically done using the Matched-Odds Ratio formula.

Results

From January 2007 to June 2007, 50 patients with ALL were seen for follow-up consult or for chemotherapy in the Hematology-Oncology Outpatient Clinic of the Department of Pediatrics, University of the Philippines-Philippine General Hospital. There was a 1.6:1 male preponderance observed with 80% of patients falling in the 1-9 year age group. Of the 26 patients with ALL who had immunophenotying done, 84% had B lineage. Four of 10 who had cytogenetics studies performed had chromosomal abnormalities. The majority of the patients had no known family history of malignancy. Pallor was the most frequent presenting symptom (Table 1).

The GSTT1, GSTP1 and GSTM1 gene polymorphisms are shown in Figures 1-3. The Ile/Ile GSTP1 polymorphism is the most frequent genotype in ALL patients (52%). The control group frequency is 42%. The heterozygous Ile/Val

Table 1. Characteristics of the study population of 50 children with ALL

Gender		
	Male	31
	Female	19
Age at Diagnosis		
	<1 Year	0
	1-9 Years	40
	10-19 Years	10
Phenotype/Lineage		
	T-Lineage	4
	B-Lineage	22
	Unspecified	24
Cytogenetics		
	Normal	6
	Abnormal	4
	Not Done	40
Family History of Malignancy		
	Present	7
	Absent	43
Presenting Sign/Symptom		
	Pallor	18
	Bleeding and Pallor	10
	Bleeding	8
	Fever	9
	Lymphadenopathy	3
	Bone Pain	2

genotype has similar frequencies in both case and control groups. The Val/Val genotype is more frequently seen in the control group (16% vs. the 2% in ALL group). The GSTM1 null genotype is more frequently observed in both controls and cases. The GSTT1 wild type polymorphism is more frequently observed in the control group than the null genotype but the reverse is true for the ALL group (Table 2).

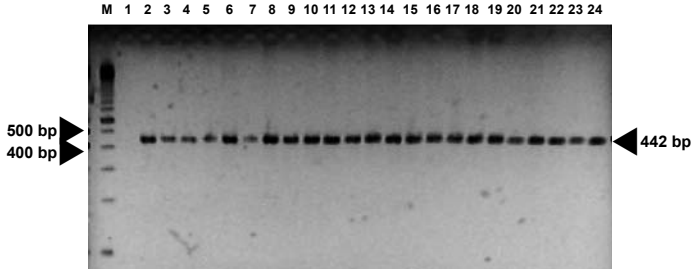

Figure 1A. *Undigested 442 bp PCR products* of the GSTP1 gene of ALL patients (lanes 3-15) and normal controls (lanes 16-24). M represents the 100 bp marker. Lane 1, blank. Lane 2, represents a heterozygote control.

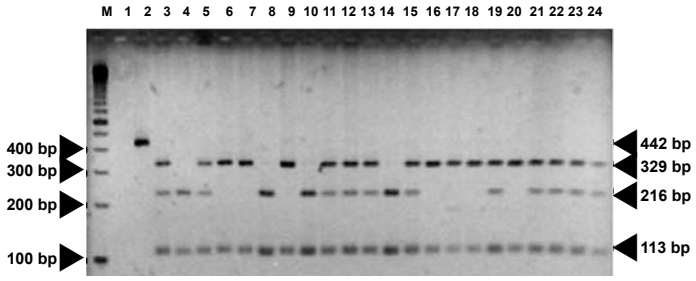

Figure 1B. *Confirmation of the GSTP1 polymorphism* in ALL patients (lanes 3-15) and normal controls (lanes 16-24) by Al26wI digestions of amplified genomic DNA. Lane 2 represents the undigested amplicon. Lanes 6, 7, 9, 16-18, and 20 show the wild type allele (329, 113 &/or 107 bp). Lanes 3, 5, 11-13, 15, 19, 21-24 show the heterozygous mutant genotype (329, 216, 113 &/or 107 bp) while lanes 4, 8, and 14 show the homozygous mutant genotype (216, 113 &/or 107 bp). M, 100 bp ladder; Lane 1 Blank.

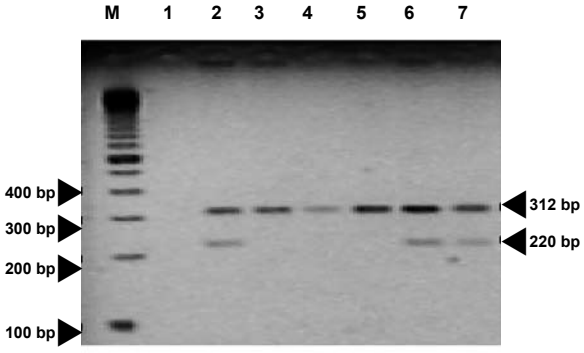

Figure 2. *GSTM1 gene polymorphism.* M is the 100 bp molecular weight marker. Lane 1 is the negative control. Lanes 2, 6 and 7 represent the wild type allele as shown by the presence of the 220 bp band. Lanes 3-5 represent the null alleles. The 312 bp band is the CYP1A1 exon 7 which serves as the internal control for the PCR run.

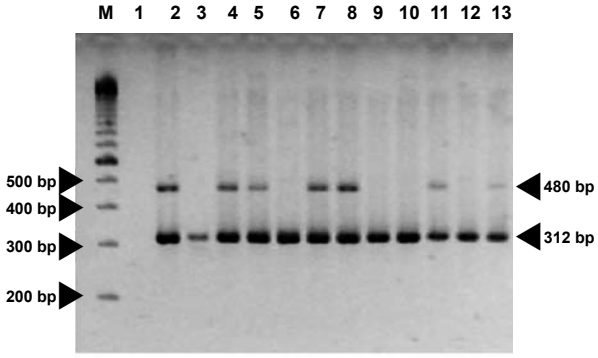

Figure 3. *GSTT1 gene polymorphism.* M is the molecular weight marker; lane 1 is the negative control. Lane 2 is the normal control. Lanes 4, 5, 7, 8, 11, 13 represent the wild type alleles as shown by the presence of the 480 bp band. Lanes 3, 6, 9, 10, 12 represent the null alleles. The 312 bp band is the CYP1A1 exon 7 which serves as the internal control.

Table 2. Distribution of GSTT1, GSTM1 and GSTP1 genotypes in ALL cases (n = 50) and controls (n = 50)

GENOTYPE		ALL cases (%)	Controls (%)	TOTAL
GSTP1	Ile/Ile	26 (52)	21 (42)	47
	Ile/Val	23 (46)	21 (42)	44
	Val/Val	1 (2)	8 (16)	9
GSTM1	Present	16 (32)	18 (36)	34
	Null	34 (68)	32 (64)	66
GSTT1	Present	23 (46)	30 (60)	53
	Null	27 (54)	20 (40)	47

Matched Odds Ratio showed that a trend towards protection from having ALL with the presence of the common GSTT1 and GSTM1 wild polymorphisms with OR 0.59(95% CI: 0.24-1.36) and OR 0.86 (95% CI: 0.36-2.00), respectively. Having the common GSTP1 polymorphism, however, was shown to be a risk factor to having ALL with OR 1.7 (95% CI: 0.74-4.15) (Table 3).

Table 3. Matched Odds Ratios for GSTT1, GSTM1, and GSTP1

a. GSTM1

Patient \ Control	+	–
+	4	12
–	14	20

b. GSTT1

Patient \ Control	+	–
+	13	10
–	17	10

c. GSTP1

Patient \ Control	+	–
+	13	117
Others	10	10

*others- heterozygous Ile/Val/and Val/Val

Discussion

The demographic characteristics of the ALL patients in this study follow the usual characteristics reported in the literature, namely: male preponderance with a male to female ratio of 1.5:1; the majority diagnosed within

the 1-9 age group; and predominant B-cell lineage on phenotyping.[13,14] Immunophenotying and cytogenetics/ chromosomal analysis were done only in a minority of the patients, primarily due to unavailability of funds. Both of these parameters are routinely done in developed countries. These are used for better risk assessment and prognostication. These also help in tailoring chemotherapeutic protocol to be implemented on a specific patient. In our ALL patients, morphologic classification was used for risk assessment and prognostication when immunophenotyping was not available.

Much interest has been generated with the possibility of using detoxifying enzymes as biomarkers for risk assessment of various malignancies. The ultimate goal of investigations such as this study is to improve cure rates or treatment outcomes by tailoring treatment based on one's genetic makeup.[22] With those patients having the genotype associated with high risk given more intensive therapy. Previous studies have shown that there is marked geographical and ethnic variation in the distribution of genes for polymorphic GSTs.[24] Reports on genomic frequencies for these enzymes are available for blacks and Caucasian populations with a few studies reported on Japanese, Chinese and Indian populations, and even fewer studies on the Malay race. The previous two local studies on GST polymorphisms in cancer were done on adult population and involved solid tumors.[20,21] This is the first study to involve a Filipino pediatric population with a hematologic malignancy.

The frequencies of GSTM1 and GSTT1 null genotypes in this study are noticeably different and higher than previous reports.[7, 8, 12, 14, 15, 20, 21, 23] The frequencies of null genotypes are even higher than those reported by Davis et al for the white population in which ALL occurred at relatively high incidence. The frequencies are similar to the Japanese genotypes reported by Imanishi et al, also for GSTM1 and GSTT1. GSTP1 genotypes are similar overall with those reported on the Chinese population[23] and on the Filipino population by a local study on colorectal cancer.[21]

Surprisingly, the local study on an adult population[21] showed much lower frequencies, with GSTM1 null at 26.2% for patients with colorectal cancer and 34% for the control. Likewise with GSTT1 null genotype at 31.8% for the cancer cases and 9%for the control. These differences need to be validated in a bigger study population.

This study reports a trend towards protection from having ALL with presence of the common GSTT1 and GSTM1 polymorphisms and, hence, an increased risk with GSTM1 and GSTT1 null genotype. Although this trend has been demonstrated in most solid tumors in the adult population, conflicting results were reported by previous studies on ALL. Krajinovic et al[25] reported an increase risk of ALL with GSTM1 null genotype but not with the GSTT1 null genotype. In a report by Davies et al,[12] however, no association was noted for both null genotypes. The GSTP1 common polymorphism was shown in this study to be a risk factor for having ALL, contrary to another report by Krajinovic et al.[26] As pointed out by Modal et al,[27] the differences in the associations or lack of them in the previous studies may be explained by other factors like the heterogeneity in environmental toxin exposure and the effect of other detoxifying genes.

Differences in the frequencies of GST polymorphisms were noted between the control and patients with ALL. This study reports a trend towards protection from having ALL with presence of the common GSTT1 and GSTM1 polymorphisms while the common GSTP1 polymorphism apparently increases risk for ALL. This observation is based on a limited number of subjects and needs to be confirmed in a larger population study.

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