



Association of *GSTT1* and *GSTM1* variants with acute myeloid leukemia risk

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ABSTRACT. We aimed to investigate the relationships between polymorphisms of the glutathione S-transferases (GSTs) *GSTM1*, *GSTT1*, and *GSTP1* and the risk of developing acute myeloid leukemia (AML). A total of 206 AML cases and 231 controls were collected for our study. The genotyping of GSTs (*GSTM1*, *GSTT1*, and *GSTP1*) was based upon the duplex polymerase chain reaction with the confronting two-pair primer (PCR-CTPP) method. Individuals carrying null *GSTT1* and *GSTM1* genotypes had a 1.52- and 1.78-fold increased risk of developing acute leukemia, respectively, compared to non-null genotype carriers ($P < 0.05$). A high risk was observed in those carrying a combination of null genotypes of *GSTM1* and *GSTT1* with *GSTP1*-Val allele genotypes when compared with those carrying wild-type genotypes, with an odds ratio (95% confidence interval) of 3.62 (1.53-8.82) ($P < 0.05$). These findings indicate that genetic variants of *GSTT1* and *GSTM1* significantly increase the risk of developing AML. Our study offers important insights into the molecular etiology of AML.

Key words: Glutathione S-transferase; Polymorphisms; Acute myeloid leukemia

INTRODUCTION

Acute myeloid leukemia (AML) is a blood cancer of myeloid type cells and is the most common acute leukemia that affects adults (Mandegary et al., 2011). In AML, abnormal white blood cells accumulate in the bone marrow, and thus interfere with the production of normal blood cells. Although the etiology of AML is still unclear, it is generally considered that various factors may contribute to its pathophysiology, such as ionizing radiation, genetics, and chemical exposure (Gunz and Veale, 1969; Evans and Steward, 1972; Le Beau et al., 1986; Thirman et al., 1993).

Glutathione S-transferases (GSTs) are a family of cytosolic enzymes that play an important role in the detoxification of various exogenous and endogenous reactive species (Ketterer, 1998; Hengstler et al., 1998). *GSTM1*, *GSTT1*, and *GSTP1* have been suggested to be involved in the detoxification of polycyclic aromatic hydrocarbons (PAHs) and benzo[*a*]pyrene (Schneider et al., 2005), which can detoxify carcinogens and reactive oxygen species (Rebbeck, 1997). Individuals who have homozygous deletions in the *GSTM1*, *GSTT1*, or *GSTP1* genes have reduced enzyme function. The absence of these enzymes may potentially increase cancer susceptibility because of a decreased ability to detoxify carcinogens, such as benzo[*a*]pyrene-7, 8-diol epoxide, the activated form of benzo[*a*]pyrene. Previous studies indicated that genetic variants of *GSTM1*, *GSTT1*, and *GSTP1* are associated with an increased risk of developing various cancers (Ozerkan et al., 2013; Wang et al., 2013; Silva et al., 2013). A few studies have shown a significant association of GST polymorphisms with AML risk, but with contradictory results (Seedhouse et al., 2004; Taspinar et al., 2008; Das et al., 2009; Mandegary et al., 2011; Kim et al., 2012; Dunna et al., 2013). However, no study has yet reported their association with AML in a Chinese population. We aimed to investigate whether *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms represent novel candidate biomarkers of AML.

MATERIAL AND METHODS

Subjects

We recruited 243 patients who were first diagnosed with AML at the Affiliated Hospital of Xinxiang Medical University between March 2009 and December 2012 for this prospective case-control study. Ultimately, 206 patients agreed to participate for an overall participation rate of 84.8%. AML was diagnosed by an increased number of myeloblasts in the bone marrow or peripheral blood according to the World Health Organization (WHO) criteria. Acute leukemia could be diagnosed when a 200-cell differential count revealed the presence of 20% or more myeloblasts in a marrow aspirate or in the blood (Vardiman et al., 2002).

We selected 231 control subjects from the Affiliated Hospital of Xinxiang Medical University who had no known blood disorders or other cancers. The demographic and clinical characteristics of patients were collected from medical records and a self-designed questionnaire.

Genomic DNA extraction

All patients were asked to provide 5 mL blood samples. We collected 5 mL venous blood samples in ethylenediamine tetra-acetic acid (EDTA)-coated tubes and stored samples at -20°C before use. DNA was extracted from whole blood or lymphoblastoid cell lines using

the Qiagen Blood Mini Kit (Qiagen; Hilden, Germany) following the manufacturer protocol. The genotypes of GSTs (*GSTM1*, *GSTT1*, and *GSTP1*) were determined from DNA that was directly extracted from whole blood. The genotyping of GSTs (*GSTM1*, *GSTT1*, and *GSTP1*) was based upon the duplex polymerase chain reaction (PCR) with the confronting two-pair primer (CTPP) method. The PCR mixture contained 2 mM MgCl₂, 0.2 mM dNTP, 1X PCR buffer, 1.2 U Taq DNA polymerase, 100 ng DNA template, 5 fM forward primer, and 5 fM reverse primer in deionized sterile water in a total volume of 50 mL. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 62°C for 30 s, 72°C for 30 s and then a final extension at 72°C for 5 min.

Statistical methods

Statistical analyses were performed using Stata 8.0 (StataCorp; College Station, PA, USA). We compared demographic characteristics between cases and controls with the Student's *t*-test and chi-squared test. Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of *GSTM1*, *GSTT1*, and *GSTP1* genotypes within the control group using the chi-squared test. Unconditional logistic regression was used to estimate the odds ratios (ORs) and associated 95% confidence intervals (CIs) for the risk of developing AML. A *P* value < 0.05 was considered to represent a statistically significant difference. All statistical analyses were performed using SPSS® version 11.0 for Windows® (SPSS Inc.; Chicago, IL, USA).

RESULTS

Among the 206 AML cases, 114 (55.4%) were males and 92 (44.6%) were females. The mean age at diagnosis was 43.5 ± 16.4 years. Among the 231 cancer-free controls, 136 (58.9%) were males and 95 (41.1%) were females. The mean age of the control patients was 46.2 ± 15.1 years. The genotype distributions of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in the control group conformed to Hardy-Weinberg equilibrium (*P* values were 0.27, 0.22, and 0.37, respectively). Null *GSTM1* carriers had a 1.52-fold greater risk of developing acute leukemia compared with non-null genotype individuals, and a significant association was detected (*P* = 0.04; OR = 1.52, 95%CI = 1.03-2.36) (Table 1). Similarly, we found that individuals carrying null *GSTT1* alleles had a moderately increased risk of developing AML when compared to non-null genotype individuals, with an OR (95%CI) of 1.78 (1.16-2.81). However, we did not find that *GSTP1* polymorphisms could influence the risk of developing AML (*P* > 0.05).

Table 1. Effect of GSTs genotypes on the risk of acute myeloid leukemia (AML).

Genotype	AML	%	Control	%	χ^2	P value	OR (95%CI) ¹
<i>GSTM1</i>							
Present	92	44.7	124	53.7			
Null	114	55.3	107	46.3	3.74	0.04	1.52 (1.03-2.36)
<i>GSTT1</i>							
Present	133	64.5	176	76.3			
Null	73	35.5	55	23.7	7.11	0.007	1.78 (1.16-2.81)
<i>GSTP1</i>							
Ile/Ile	90	43.7	104	45.1			
Ile/Val	71	34.6	82	35.5	0.01	0.99	1.0 (0.66-1.61)
Val/Val	45	21.7	45	19.4	0.32	0.57	1.21 (0.72-2.05)

¹Adjusted for gender and age.

The interaction effects of polymorphisms in *GSTT1* and *GSTM1* loci on the risk of developing AML are shown in Table 2. Individuals carrying intact *GSTM1* and null *GSTT1* alleles showed a moderately increased risk of developing AML when compared to those carrying intact *GSTM1* and *GSTT1* genotypes, with an OR (95%CI) of 2.34 (1.30-4.23) ($P < 0.001$). Similarly, we found that a null *GSTM1* and intact *GSTT1* allele combination presented a 1.91-fold increased risk of developing AML and a 3.62-fold increased risk of having cancer.

Table 2. Combination effect of *GSTT1* and *GSTM1* polymorphisms on the risk of acute myeloid leukemia (AML).

<i>GSTM1</i>	<i>GSTT1</i>	AML	%	Controls	%	OR (95%CI) ¹	P value
Present	Present	41	19.9	81	37.7	-	-
Present	Null	51	24.8	43	35.3	2.34 (1.30-4.23)	<0.001
Null	Present	92	44.7	95	14.3	1.91 (1.16-3.16)	0.006
Null	Null	22	10.7	12	12.8	3.62 (1.53-8.82)	0.001

¹Adjusted for gender and age.

DISCUSSION

In this case-control study, we found that *GSTM1* and *GSTT1* deletion polymorphisms were both individually associated with an increased risk of developing AML, and a greater risk was observed in individuals carrying a combination of null *GSTM1* and *GSTT1* genotypes when compared with individuals carrying wild-type genotypes. Our data indicate that *GSTM1* and *GSTT1* polymorphisms could be used as candidate biomarkers for assessing the risk of developing AML.

GSTs are a well-known family of multifunctional enzymes that can detoxify a variety of electrophilic compounds. Recent studies indicated that genetic polymorphisms in *GSTM1* and *GSTT1* may play a role in the development of various cancers, such as head and neck, lung, and bladder cancer (Benhamou et al., 2002; Engel et al., 2002; Hashibe et al., 2003). The main reason might be inefficient carcinogen detoxification, resulting in an increased likelihood of developing cancer. Two recent studies indicated that *GSTM1* and *GSTT1* polymorphisms may confer an increased risk of developing AML (Ouerhani et al., 2011; Dunna et al., 2013), and two studies conducted in western countries showed that *GSTM1* and *GSTT1* polymorphisms affect the prognosis of AML (Borst et al., 2012; Zareifar et al., 2013). Our data are in agreement with these previous studies.

The strengths of this study include comprehensive face-to-face interviews and access to DNA for all cases and controls. The allele frequencies for all of the GST polymorphisms studied were similar to those reported for the Chinese population previously (Jing et al., 2012; Song et al., 2012). However, there were several limitations to our study. First, cases and controls were not age- and gender-matched, although we used further statistical adjustment to minimize potential biases for these factors. Second, some risk predictors of AML, such as exposure to carcinogens, were not included in our analysis. Therefore, large population-based studies with different ethnicities are warranted to further investigate the impact of GST polymorphisms on AML susceptibility.

In conclusion, this case-control study indicated that null *GSTT1* and *GSTM1* genotypes are associated with an increased AML risk in a Chinese population. These findings indicate that genetic variants of *GSTT1* and *GSTM1* have critical functions in the development of AML. Our study offers important insights into the molecular etiology of AML.

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