RESEARCH ARTICLE

Risk Effects of GST Gene Polymorphisms in Patients with Acute Myeloid Leukemia: A Prospective Study

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Abstract

Glutathione S-transferase (GST) enzyme levels are associated with risk of many cancers, including hematologic tumours. We here aimed to investigate the relationships between GSTM1, GSTT1 and GSTP1 polymorphisms and the risk of AML. Genotyping of GSTs was based upon duplex polymerase-chain-reactions with the confronting-two-pair primer (PCR-CTPP) method in 163 cases and 204 controls. Individuals carrying null GSTT1 genotype had a 1.64 fold risk of acute leukemia relative to a non-null genotype (P<0.05). A heavy risk was observed in those carrying combination of null genotypes of GSTM1 and GSTT1 and GSTP1 Val allele genotypes when compared with those carrying wild genotypes, with an OR (95% CI) of 3.39 (1.26-9.26) (P<0.05). These findings indicate that genetic variants of GST and especially the GSTT1 gene have a critical function in the development of AML. Our study offers important insights into the molecular etiology of AML.

Keywords: Glutathione S-transferase - polymorphism - acute myeloid leukemia - risk

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Introduction

Acute leukemia includes acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) (Mandegary et al., 2011). AML is the most common acute leukemia affecting adult (Jiang et al., 2011). The mechanism of this disease is the abnormal white blood cells accumulating in the bone marrow, and thus interferes with producing normal blood cells.

Despite previous several studies reported the mechanisms of leukemia, the real reasons are still not known. As other cancer, AML is a complex disease caused by the combination effect of environmental and hereditary factors, such as chemical exposure, ionizing radiation and genetics (Gunz et al., 1969; Evans et al., 1972; Le Beau et al., 1986; Thirman et al., 1993). Previous studies reported that DNA damage by the reactive oxygen species in hematopoietic precursor cells influence the carcinogenesis of several cancers, including acute leukemia.

Xenobiotics can be detoxified by phase II enzymes, such as GSTM1, GSTT1 and GSTP1 which have been suggested to be involved in detoxification of polycyclic aromatic hydrocarbons (PAHs) and benzo[a]pyrene (Schneider et al., 2004), which could detoxify carcinogens and reactive oxygen species (Rebeck, 1997). Individuals who have homozygous deletions for GSTM1, GSTT1 and GSTP1 gene have reduced enzyme function. Lack of these enzymes may potentially increase cancer susceptibility due to a decreased ability to detoxify carcinogens such as benzo[a]pyrene-7,8-diol epoxide, the activated form of benzo[a]pyrene. The missing substitution Ile105Val results from an A3G base substitution at nucleotide 313. The Val105 form of the GSTP1 enzyme may be 2-3 times less stable than the canonical Ile105 form (Johansson et al., 1998) and may be associated with a higher level of DNA adducts (Ryberg et al., 1997). Numbers of published studies have focused on GSTM1, GSTT1 and GSTP1 genetic variation with respect to various cancers. A few studies have shown that significant association of GST polymorphisms with AML, and the results are contradictory (Crump et al., 2000; Naoe et al., 2000; Rollinson et al., 2000; D’Alo et al., 2004; Seedhouse et al., 2004; Taspinar et al., 2008; Das et al., 2009; Mandegary et al., 2011). But there is no study reported their association with AML in Chinese population. Whether GSTM1, GSTT1 and GSTP1 polymorphisms are risk factors for AML in Chinese population remains largely uncertain.

Therefore, the aim of the present study was to investigate the relationships between GSTM1, GSTT1 and GSTP1 polymorphisms and the risk of AML, and investigate whether GSTM1, GSTT1 and GSTP1 polymorphisms are novel candidate biomarkers of AML.

Materials and Methods

This case-control and prospective study recruited patients first diagnosed with AML at Shengjing Hospital Affiliated of China Medical University and No.202 Hospital of PLA, Shenyang. *Department of Internal Medical Oncology, General Hospital of PLA, Beijing, China *Equal contributors *For correspondence: liuzg_677@163.com
Hospital of PLA, China, between March 2009 and January 2012. 163 AML patients were consecutively collected between March 2009 and January 2012, and finally 187 patients agreed to participate into this study with a participation rate of 87.2%. The diagnosis of AML is often demonstrated by an increased number of myeloblasts in the bone marrow or peripheral blood according to the WHO criteria. Acute leukemia is diagnosed when a 200-cell differential reveals the presence of 20% or more myeloblasts in a marrow aspirate or in blood (Vardiman et al., 2002). All patients with newly diagnosed primary AML in the hospital were invited for face to face interview within one month after diagnosis.

204 age-matched control subjects (±5 years old) were recruited from individuals attending Shengjing Hospital Affiliated of China Medical University and No.202 Hospital of PLA, China, for routine health examinations. Control subjects with known blood disorders or any other cancers were excluded from the study.

All study participants completed a structured questionnaire during a face-to-face interview with medical staff, and 5 ml venous blood was collected in ethylenediamine tetra-acetic acid (EDTA) coated tubes and stored at -20°C before use.

The study was approved by the Ethics Committee of Shengjing Hospital Affiliated of China Medical University, and all participants provided written informed consent.

**Genomic DNA Exaction**

For DNA extraction, 5 ml blood was provided by each collected subjects. Blood samples were stored at -20°C. DNA was extracted from whole blood or lymphoblastoid cell lines using the Qia-gen Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The genotypes of GSTs (GSTM1, GSTT1 and GSTP1) were determined from DNA directly extracted from whole blood.

**Genotyping of GSTM1, GSTT1 and GSTP1**

The genotyping of GSTs (GSTM1, GSTT1 and GSTP1) was based upon duplex polymerase-chain-reaction with the confronting-two-pair-primer (PCR-CTPP) method. Briefly, the sequences of primers used for polymorphism of GSTM1, GSTT1 and GSTP1 were amplified by using the following primers. The primers of GSTM1 were 5'-GAACCTCCTGAAAAGCTAAGC-3' and 5'-GTTGGGGCTCAAAATATAACGGTG-3'. The primers of GSTT1 were 5'-TTCCTTACTGTTCCTCACATCTC-3' and 5'-TCACCGGATCAGGCCAGCA-3'. The primers of GSTP1 were 5'-ACCCCAGGGCTCTATGGGAA-3' and 5'-TGAGGGCACAAGAAGCCCCT-3'. Each 30μL reaction mixture contained 1.3 U Tag biocatalysts, 1.8 mmol/L Mg\(^{2+}\), 2.4 μL dNTPs, 8 primers, 15 pmol of each primer and 5-8Μl template. The PCR reaction mixture contained 2 mM of MgCl\(_2\), 0.2 mM of dNTP, 1xPCR buffer, 1.2 U of Taq DNA polymerase, 100 ng of DNA template and 5 pmol/ml of both forward and reverse primers in deionized sterile water in 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 30s, at 62°C for 30s, at 72°C for 30s and a final extension at 72°C for 5 min. Amplified products were resolved on a 3% Nusieve agarose gel to determine the presence or absence of 215bp GSTM1, 480bp GSTT1, 337bp products or 268bp β-Globin (Figure 1; Figure 2; Figure 3). Genotyping was conducted without knowing the subjects’ case or control status. For quality control, 10% of the samples were randomly selected to repeat genotyping, and we gained a 100% concordant.

**Statistical Methods**

All analysis was performed by using the STATA statistical package (version 9, STATA, College Station, TX). Hardy-Weinberg equilibrium of alleles at controls was assessed using exact tests. Categorical variables were compared with use of the chi-square test or Fisher’s exact test (when one expected value was <5). Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were used to assess the effect of each gene on AML risk. Unconditional logistic regression was used to estimate ORs and their 95% confidence intervals. A P-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS® version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®.

**Results**

Of the 163 AML cases, 87 (53.4%) were males and
the mean age at diagnosis was 41.3±15.8 years old, while among the 204 cancer-free controls, 112 (54.9%) were males and the mean age were 43.6±12.7 years old. The genotype distributions of the GSTM1, GSTT1 and GSTP1 genotypes between cases and controls are shown in Table 1. The observed genotype frequencies for GSTM1, GSTT1 and GSTP1 genotypes were in Hardy-Weinberg equilibrium among the controls, and the P values were 0.36, 0.12 and 0.19 for GSTM1, GSTT1 and GSTP1, respectively. Null GSTM1 carriers had a 1.20 fold risk of acute leukemia compared with non-null genotype, but no significant association was found (P>0.05). Similarly, compared to the wild Ile/Ile genotype, GSTP1 Val/Val carriers had a non-significant risk of AML, with OR (95% CI) of 1.64 (1.03-2.63) (P>0.05). While, we found those carrying null GSTT1 genotype had a 1.27 fold risk of acute leukemia relative to non-null genotype (P<0.05).

The interaction effects of polymorphisms in GSTT1, GSTM1 and GSTP1 loci on the risk of AML were shown in Table 2. Individuals carrying null GSTM1, null GSTT1 and GSTP1 Val/Val showed a heavy risk of AML when compared with those carrying non-deleted GSTM1 and GSTT1 and GSTP1 Ile/Ile genotypes, with a OR (95% CI) of 3.39 (1.26-9.26) (P<0.05).

## Discussion

In this case-control study, neither the GSTM1 nor GSTP1 deletion polymorphisms were associated with risk of AML. However, our study suggests that a positive association between the GSTT1 null genotype and the risk of AML, and a heavy risk was observed in those carrying combination of null genotypes of GSTM1 and GSTT1 and GSTP1 Val allele genotypes when compared with those carrying wild genotypes. This study indicated GSTs polymorphisms were promising candidate biomarkers for evaluating the AML risk.

GSTs belong to a super-family of detoxification enzymes, which play a role in resisting a large variety of chemical carcinogens and environmental toxicants. The null genotype of GSTM1 and GSTT1 and GSTP Val/Val genotype may lower the expression and activity of genotypes, and these genotypes are known to be associated with increasing incidence of certain cancers, such as head and neck, lung and bladder cancer (Benhamou et al., 2002; Engel et al., 2003; Hashibe et al., 2003). The main reason might be the inefficient carcinogen detoxification and therefore a higher risk of developing cancer. Previous meta-analysis studies indicated that null GSTT1 genotype might contribute to increased risk of various cancers, such as breast cancer, lung cancer, gastric cancer and liver cancer (Ma et al., 2013; Yang et al., 2013a; Yang, 2013b; Zhao et al., 2013).

Previous several studies reported the association between GSTT1 polymorphisms and risk of AML (Crump et al., 2000; Arruda et al., 2001; Liu et al., 2005; Kim et al., 2012), but the results are inconsistent. Crump et al. reported a case-control study conducted in the United States, and the results do not the hypothesis that the GSTT1 gene deletion is related to the risk of AML (Crump et al., 2000). Similarly, Liu reported variation of GSTT1 genotype is not associated with the susceptibility of AML in a Chinese population (Liu et al., 2005). While a case-control study conducted in Brazil with 124 cases reported a moderate association between GSTT1 null genotype and risk of AML (Arruda et al., 2001), and Kim et al. also reported a positive association in Korean population (Kim et al., 2012). In our study, we did not find the association of GSTM1 null and the GSTP1 Val/Val genotypes with AML, while we found GSTT1 null genotype was associated with increased risk of AML. The risk becomes strong when possessing of all three GSTT1 null, GSTM1 null and the GSTP1 Val/Val genotypes, with a with a 3.39-folds risk of AML. The results of GSTs and risk of acute leukemia are conflicting in studies conducted in different ethnicities. The reason might be induced by ethnic difference, case selection and variation of clinical characteristics. Further studies are strong needed to validate the association between variation of GSTT1 and AML risk.

Strengths of this study include comprehensive face-to-face interviews with all cases and controls, and the DNA was gained in all cases and controls. The allele frequencies for all the studied GSTs polymorphisms were similar to those reported from Chinese population (Jing et al., 2012; Song et al., 2012). However, the sample size is relative small in our study, and statistical power is insufficient for detecting the effect of GSTs genes on AML risk.

Therefore, this case-control study indicated that GSTT1 null genotype was associated with AML risk in
a Chinese population. These finding indicate that genetic variants of the GSTT1 gene has a critical function on the development of AML. Our study offers important insights into the molecular etiology of AML.

References


