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Short Communication

# Polymorphisms in the *glutathione S-transferase theta* and *mu* genes and susceptibility to myeloid leukemia in Brazilian patients

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## Abstract

The null genotype for *glutathione S-transferase* (*GST*, EC 2.5.1.18) gene polymorphisms is considered a risk factor for leukemia in different populations. In this work we investigated the *GSTT1* and *GSTM1* polymorphisms using multiplex PCR in 53 patients with chronic myeloid leukemia (CML), 23 with acute promyelocytic leukemia (APL) and 304 apparently healthy controls. In this association study we found that the $GSTT1^{null}$ genotype was more frequent in our group of APL patients than in the control group [OR = 2.75 (95% CI = 1.10-6.88)], providing evidence that a deletion in the *GSTT1* gene could be a risk factor for this type of leukemia.

*Key words:* acute promyelocytic leukemia, chronic myeloid leukemia, GSTM1, GSTT1, gene polymorphism.

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Leukemias are complex diseases determined by a combination of several factors. It has been shown that DNA damage in hematopoietic precursor cells is directly linked to the risk of acute leukemia in adults (Rollinson *et al*., 2000) and may results from an interaction between reactive species generated by environmental or endogenous metabolites (Rollinson *et al*., 2000; Dalo *et al*., 2004). Human cells possess metabolic systems to eliminate toxic agents and several enzymes are responsible for the degradation of these xenobiotics, one systems being the glutathione S-transferase (GST, EC 2.5.1.18) group of enzymes which detoxify environmental carcinogens by conjugation with glutathione (Crump *et al.,* 2000). The GST group is known to be coded for by 16 genes in six *GST* subfamilies, known as alpha (*GSTA*), mu (*GSTM*), omega (*GSTO*), pi (*GSTP*), theta (*GSTT*) and zeta (*GSTZ*). Two widespread genetic polymorphisms that involve deletions in *GSTT1* and *GSTM1* have been reported to lead to loss of enzyme activity (Bolufer *et al.,* 2007) and have been investigated in many different populations, including those from Japan (Naoe *et al.,* 2000), Italy (D'alo *et al*., 2004) and Spain (Bolufer *et al.,* 2007). Furthermore, several studies have proposed that susceptibility to acute and chronic myeloid leukemia (AML and CML respectively) could be related to *GSTT1* and/or *GSTM1* deletions (Rollinson *et al*., 2000; Mondal *et al*., 2005; Ye and Song, 2005.

Brazil is the largest country in South America, with a highly heterogeneous population due to several waves of immigration which have resulted in cultural, socioeconomic and ethnic diversity. In the Northeastern Brazilian state of Bahia, which has a highly mixed population of mainly African descent, the largest city is Salvador (population 2.7 million; RIPSA, 2006), 86% of the local population being of African, or European and African, descent. A recent study by Barreto *et al.* (2006) reported that the Bahian population shows a high prevalence of pediatric acute promyelocytic leukemia (APL), which accounted for 21% of all AML patients (n = 105) evaluated between 1995 and 2004). Ribeiro and Rego (2006) reported that patients with a Latin American background (*i.e.* some Amerindian genetic input) were much more likely to have APL (18.2%) than were white (7.7%) or black (10.3%) patients without a Latin American background. Based on this, we decided to investigate the frequency of *GSTT1* and *GSTM1* polymorphisms among patients with APL and CML from the city of Salvador.

We investigated a total of 76 patients with myeloid leukemia, 53 (30 (57%) male, 23 (43%) female; mean age 41 y ± a standard deviation (SD) of 21 y) with CML and 23 (11 (48%) male, 12 (52%) female; mean age 14 y ± 6.8 y) with APL which were selected at diagnosis between 2000

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and 2003 from several institutions that attend patients with malignant diseases. Most of the APL cases were from a pediatric oncological institute (Clínica de Oncologia, Salvador, Bahia). The control group consisted of 304 (131 (43.2%) male, 173 (56.8%) female ; mean age 29 y ± 9.5 y) was composed of apparently healthy individuals randomly chosen from the staff of a private clinical laboratory (Labcheckup, Pituba, Salvador, Bahia). Both patients and control individuals all came from Bahia. The diagnoses of leukemia were made according to clinical, morphological and molecular criteria and the study was approved by the Oswaldo Cruz Research Foundation's Human Research Ethics Committee. Bone marrow and peripheral blood samples were obtained only after an informed consent form was signed.

We extracted RNA, using Trizol$^{R}$ (Gibco-BRL, Life Technologies, USA), and DNA, using Genomic Blood DNA Purification Kits (Amersham Pharmacia Biotech, USA), from bone marrow cells and peripheral blood leukocytes according to the guidelines of the manufacturer. The translocations t(9;22)(q34;q11) for CML and t(15;17)(q22; q12-21) for APL patients were investigated using the reverse transcriptase polymerase chain reaction PCR (RT-PCR) method according to the methodology of Biernaux *et al.* (1995) and Miller *et al.* (1993). The *GST* polymorphisms were assessed using the multiplex PCR method described by Arruda *et al.* (2001), using the β-globin gene as an internal control.

Descriptive analyses included genotype frequencies and the odds ratio (OR) as an estimate of relative risk, with 95% confidence intervals (CI). Chi-square test using the Yates correction or the Fisher's exact test were applied and differences were considered significant at the p level. All analyses were carried out using the EPI INFO software, version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

All 53 CML patients had the t(9;22)(q34;q11) translocation and all 23 APL patients had the t(15;17)(q22; q11-21) translocation. The frequencies of the *GSTT1* and the *GSTM1* genotypes in both patients and controls are presented in Table 1. Although we found similar patterns for the GST multiplex PCR it is possible that the loss of heterozygosity in peripheral blood or bone marrow cells may have increased the frequencies of the *GSTM1*$^{null}$ and *GSTT1*$^{null}$ genotypes. However, the frequency distributions of these polymorphisms were in agreement with the Hardy-Weinberg equilibrium. No association was found between the *GSTT1* or *GSTM1* deletions and CML risk in the group studied but there was an increased APL risk for the *GSTT1*$^{null}$/*GSTM1*$^{normal}$ (OR = 2.75, 95% CI = 1.1 to 6.88) and *GSTT1*$^{null}$/*GSTM1*$^{null}$ (OR = 3.61, 95% CI = 1.37 to 9.51) genotypes (Table 1).

Several studies associating the presence of the *GSTM1* and *GSTT1* polymorphisms with lymphoid and myeloid leukemias have been performed (Crump *et al*., 2000; Naoe *et al*., 2000; Dalo *et al.*, 2004; Mondal *et al*., 2005; Bolufer *et al*., 2007). Our analyses showed that the *GSTT1* gene deletion was significantly higher among Brazilian APL patients than controls but no association was observed between APL susceptibility and the isolated *GSTM1*$^{null}$ genotype, although when the *GSTM1*$^{null}$/*GSTT1*$^{null}$ genotype was considered there was a significant difference between the APL group and the control group. The APL type of disease is a French-American-British (FAB, Bennett *et al.,* 1976) classification subtype leukemia, also named AML-M3, frequently presenting the t(15;17)(q22;q12-21) translocation. We suppose that the deletions may lead to lack of detoxification of electrophilic compounds and/or a higher DNA damage ratio, which could contribute to the development and proliferation of leukemia.

Rollinson *et al.* (2000) observed similar results, but found a weaker association between the *GSTM1* or *GSTT1* deletions and increased risk to AML, the same FAB-subtype leukemia, in British adults. The differences between these studies may be related to age variation or to the diverse genetic backgrounds of the patients. Arruda *et al.* (2001) found an association between the development AML and the *GSTM1*$^{null}$ or *GSTT1*$^{null}$ genotypes among Brazilian individuals, but they did not separate the FAB subgroups.

Ye and Song (2005) performed a systematic review of several studies of *GST* gene polymorphisms in relation to the risk of acute leukemia, their results suggesting that the *GSTM1*$^{null}$ and *GSTT1*$^{null}$ genotypes are not associated with AML. Our results, however, surprisingly showed a differ-

**Table 1** - The *glutathione S-transferase* (*GST*) *GSTT1* and *GSTM1* polymorphism genotype distribution plus the odds ratio (OR) and 95% confidence intervals (CI) for acute promyelocytic leukemia (APL) patients (n = 23) and chronic myeloid leukemia (CML) patients (n = 53) as compared to apparently healthy control individuals (n = 304).

| GST genotype | APL, n (%) | CML, n (%) | Control, n (%) | APL *vs.* controls | | CML *vs.* controls | |
|---|---|---|---|---|---|---|---|
| | | | | OR (95% CI) | p value | OR (95% CI) | p value |
| GSTM1$^{normal}$/GSTT1$^{normal}$ | 7 (30.4) | 28 (52.8) | 165 (54.2) | 1* | | 1* | |
| GSTM1$^{null}$/GSTT1$^{normal}$ | 7 (30.4) | 15 (28.3) | 100(33.0) | 0.89 (0.32 to 2.40) | 0.80$^{\dagger}$ | 0.83 (0.48 to 1.45) | 0.61$^{\dagger}$ |
| GSTM1$^{normal}$/GSTT1$^{null}$ | 5 (21.7) | 8 (15.1) | 25 (8.2) | 2.75 (1.10 to 6.88) | 0.04$^{\ddagger}$ | 1.75 (0.90 to 3.38) | 0.09$^{\dagger}$ |
| GSTM1$^{null}$/GSTT1$^{null}$ | 4 (17.5) | 2 (3.8) | 14 (4.6) | 3.61 (1.37 to 9.51) | 0.02$^{\dagger}$ | 1.85 (0.47 to 7.22) | 0.31$^{\dagger}$ |
| Total | 23 | 53 | 304 | | | | |

*Reference group (OR=1.0); $^{\dagger}$Chi-square test/Yates correction; $^{\ddagger}$Fisher's exact test.

ent pattern of *GSTT1* polymorphism frequency among the APL patient group. Since the GSTT1 enzyme is responsible for the detoxification of environmental xenobiotics these results may be associated with high rates of APL in the investigated population. Barragan *et al.* (2007) recently suggested the influence of *GST* deletions on treatment follow up after chemotherapy in adult non-promyelocytic patients.

We observed similar frequencies in CML patients and controls but Lourenco *et al.* (2005) found a lower frequency of the *GSTT1*$^{null}$ genotype in CML Brazilian patients who were both in the blast crisis or in the chronic phase, while Mondal *et al.* (2005) observed an increase in the *GSTT1*$^{null}$ genotype in CML patients from India.

Our population was composed of an admixture of Amerindian, African and European-derived subjects, and the *GST* polymorphisms is known to exhibit different frequencies according to ethnic group (Gattas *et al.,* 2004). Our results were different from those for individuals of European-descent from the Southeastern Brazilian state of São Paulo reported by Gattas *et al.* (2004), who found frequencies of 22.3 for the *GSTT1*$^{null}$ and 55.4% for the t*GSTM1*$^{null}$ genotypes. This difference can be explained by the high percentage of African genes present in Salvador population.

In conclusion, our analyses suggest that the *GSTT1* genetic background might be an important marker for APL risk, at least in Salvador.

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