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Increased risk for acute myeloid leukaemia in individuals with glutathione *S*-transferase mu 1 (GSTM1) and theta 1 (GSTT1) gene defects

Arruda VR, Lima CSP, Grignoli CRE, de Melo MB, Lorand-Metze I, Alberto FL, Saad STO, Costa FF. Increased risk for acute myeloid leukaemia in individuals with glutathione *S*-transferase mu 1 (GSTM1) and theta 1 (GSTT1) gene defects.
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Abstract: *Objectives:* Glutathione *S*-transferases (GST) modulate the effects of exposure to various cytotoxic and genotoxic agents, including those associated with increased risks of the myelodysplastic syndrome (MDS), acute myeloid leukaemia (AML) and aplastic anemia (AA). Both the GST mu 1 (GSTM1) and GST theta 1 (GSTT1) genes have a null variant allele in which the entire gene is absent. In this study, we tested whether null genotypes for the GSTM1 and GSTT1 genes altered the risks for MDS, AML and AA. *Methods:* Genomic DNA from 49 MDS, 38 AML and 37 AA patients and 276 controls was analysed using the polymerase chain reaction (PCR). *Results:* The frequencies of GSTM1 (73.6%) and GSTT1 (34.2%) null genotypes were significantly higher in AML patients than in the controls (36.9 and 18.1%, respectively). A higher frequency of the combined null genotype for both genes was also observed in patients with AML (26.3% compared with 5.0% in the controls). In contrast, no differences in the frequencies of the null genotypes were found among MDS patients, AA patients and the controls. *Conclusion:* Our observation of a 4.7-fold (95% CI: 2.1–11.0) and 2.3-fold (95% CI: 1.0–5.2) increased risk associated with the GSTM1 and GSTT1 null genotypes, respectively, and a 6.6-fold (95% CI: 2.4–7.9) increased risk associated with the combined null genotype presents preliminary evidence that the inherited absence of this carcinogen detoxification pathway may be an important determinant of AML.

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The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterized by abnormal haematopoietic differentiation and maturation. The progression to acute myeloid leukaemia (AML) occurs in about 30% of the cases (1). AML is also a clonal malignant disease of haematopoietic tissue which is characterized by the proliferation of blast cells in the marrow and by the impaired production of normal blood cells. Ten to 20% of cases of AML have a previous history of MDS or evidence of trilineage dysplasia at presentation (2). Substantial morbidity and mortality are associated with MDS and AML (3, 4), and their occurrence has been linked to certain environmental or occupational exposures (3, 5–7).

Aplastic anaemia (AA) is a clonal disorder of the bone marrow which is characterized by a reduction of haematopoietic precursors, fatty replacement of the marrow and pancytopenia. The reduction of functional mass may also be initiated by chemical agents, such as benzene (8). Although not malignant, the overall mortality of adults with AA has been reported to be 65–75% with a median survival of about 3 months if not adequately treated (9).

The ability to metabolize carcinogens varies among humans, and people with a reduced ability to detoxify chemicals may have an increased risk of cancer (10–13). The enzymes of the glutathione *S*-transferase (GST) system catalyze the conjugation of electrophilic molecules of numerous carcinogenic chemicals to glutathione, reducing these noxious agents to less toxic levels. Genes coding for the GST mu 1 (GSTM1) and theta 1 (GSTT1) proteins are polymorphic in humans (14–16) and are absent, or homozygous null, in 10–60% of different ethnic populations (17–21). Previous epidemiological studies have associated the null genotypes for GSTM1 and GSTT1 genes with a high risk for a variety of cancers, including lung, bladder, gastrointestinal tract, skin, cervix, and breast cancer (22–26). However, similar risks of these tumors were found in patients with GSTM1 and GSTT1 null genotypes and those with both alleles in other studies (21). Therefore, there is no consensus on the role of the GSTM1 and GSTT1 gene deffects and cancer risk.

The frequencies of GSTM1 and GSTT1 null genotypes in MDS (27–34) and acute leukaemia (29, 32–37) have been discussed by some investigators, but no consistent conclusions have yet been established. Furthermore, only few reports pointed to the higher frequency of the GST null genotype in AA patients (38–40). Thus, the roles of the GSTM1 and GSTT1 genes in the susceptibility to these diseases remains to be clarified.

Cancer is the second most common cause of death in south-eastern Brazil (41). Environmentally related diseases resulting from exposure to solvents and chemical agents, such as hexachlorobenzene, carbon tetrachloride, perchloroethylene, benzopyrene, 4-nitroquinoline-*N*-oxide, alachlor, atrazine, lindane and methyl parathion, have been described and are a serious health problem in the São Paulo region of Brazil (42–46). For this reason, the identification of GSTM1 and GSTT1 gene polymorphisms in patients with MDS, AML or AA, from an area in which there is a potential exposure to cytotoxic and genotoxic agents, was considered necessary in order to test whether homozygous null genotypes influenced the risk for chemically-induced diseases.

Material and methods

Patients

The case groups consisted of 49 consecutive patients at diagnosis with *de novo* MDS (21 males, 28 females; mean age 59 yr), 38 *de novo* AML patients (22 males, 16 females; mean age 39 yr) and 37 AA patients (19 males, 18 females; mean age 37 yr). The patients were recruited between 1996 and 1997 at the Haematology and Haemotherapy Center of the State University of Campinas. The diagnosis of MDS and AML was reached according to the criteria of the French–American–British Cooperative Group (47, 48). The diagnosis of AA was established in patients who presented pancytopenia and hypocellular bone marrow with fat replacement. Biopsies showed fat accumulations separated by thin strands containing few granulocytes, erythroblasts, lymphocytes, plasma cells and a decreased number or absence of megakaryocytes of characteristic morphology (49, 50). The control group consisted of 276 consecutive newborns from the same university hospital in order to provide a representative group of the general population that seeks medical assistance in this region. Informed consent was obtained from all subjects or their parents.

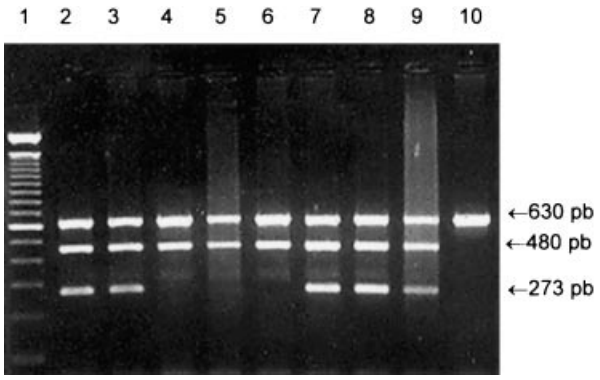

Fig. 1. Multiplex PCR for detection of homozygous null alleles of glutathione *S*-transferase mu 1 (GSTM1) and theta 1 (GSTT1). Ethidium bromide-stained 2% agarose gel showing PCR products of 273 bp and 480 bp corresponding to the normal presence of the allele for the GSTM1 and GSTT1 genes, respectively. The 630 bp PCR fragment was a β-globin gene fragment, including exon 3 and introns 2 and 3, which served as a control for the DNA sample. Lane 1 shows the DNA size marker ladder 100 bp. Lanes 2, 3, 7–9 show the results from individuals with normal GSTM1 and GSTT1 alleles. Lanes 4–6 show the results from individuals with a homozygous deletion in the GSTM1 allele and lane 10 shows a combined deletion of the GSTM1 and GSTT1 alleles.

GSTM1 and GSTT1 gene deletion by multiplex PCR

Genomic DNA was obtained from bone marrow samples from all patients with AML, from peripheral blood in the case of MDS and AA patients and from cord blood in the case of the controls. DNA was extracted using DNAzol^{TM} reagent (Life Technologies). The GSTM1 and GSTT1 genes were amplified by the polymerase chain reaction (PCR) in the same reaction, as was a fragment of the β-globin gene, used as a control for the DNA sample (51). The multiplex PCR was carried out in a mixture of 10 mM Tris–HCl, pH 8.4, 3 mM $MgCl_2$, 50 mM KCl, 0.4 mM of each nucleoside triphosphate,

Table 1. GSTM1 and GSTT1 null genotypes among MDS, AML and AA patients and controls

	MDS ($n=49$)	AML ($n=38$)	AA ($n=37$)	Controls ($n=276$)
GSTM1 null alleles:				
Number (%)	18 (36.7)	28 (73.6)	15 (40.5)	102 (36.9)
OR (95% CI)	0.9 (0.5–1.9)	4.7 (2.1–11.0)	1.1 (0.5–2.4)	
p-value	0.97	<0.0001	0.67	
GSTT1 null alleles:				
Number (%)	7 (14.2)	13 (34.2)	9 (24.3)	44 (18.1)
OR (95% CI)	0.7 (0.2–1.8)	2.3 (1.0–5.2)	1.4 (0.5–3.4)	
p-value	0.51	0.02	0.36	
Combined null alleles:				
Number (%)	3 (6.1)	10 (26.3)	4 (10.8)	14 (5.0)
OR (95% CI)	1.2 (0.2–4.7)	6.6 (2.4–17.9)	2.2 (0.5–8.0)	
p-value	0.48	<0.0001	0.14	

Abbreviations: MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; AA, aplastic anaemia; OR, odds ratio; CI: confidence interval.

500 ng of genomic DNA and 2 U *Taq* polymerase. The reaction involved 30 cycles of incubation at 95 °C (1 min), 62 °C (1 min), and 72 °C (1 min). The GSTM1 and GSTT1 genes were evaluated using primers described by Comstock *et al.* (52) and Pemble *et al.* (17), respectively. The GST genotypes were analysed by electrophoresis in 2.0% agarose gels (Fig. 1) and were scored only if the PCR signal corresponding to the β-globin gene internal control was evident.

Statistical analysis

The statistical significance of the differences between groups was calculated by the chi-square or Fischer's exact test. Crude odds ratios (ORs) were calculated and are given with the 95% confidence intervals (CI). All analyses were performed using the statistical package Epi Info (53).

Results

The frequencies of the GSTM1 and GSTT1 null genotypes, and the comparison of the GST null genotypes among the MDS, AML, AA patients and controls studied are shown in Table 1.

The frequencies of the GSTM1 and GSTT1 null genotypes were significantly higher in AML patients than in the controls. No differences in age and sex distributions were observed among AML patients with homozygous deletion of GSTM1 and those with both alleles were observed (mean age $\pm$ SD: 39.1 ± 17.5 yr vs. 40.3 ± 21.5; male/female: 1.3 vs. 1.5, respectively) ($p = 0.42$ and $p = 0.58$) and among AML patients with GSTT1 null genotype and those with both genes (mean age $\pm$ SD: 38.0 ± 18.3 yr vs. 39.8 ± 19.2; male/female: 1.3 vs. 1.6, respectively) ($p = 0.44$ and $p = 0.51$). Also similar results were found in AML patients with combined null genotype and those with both genes considering the age and the sex distributions (mean age $\pm$ SD: 38.2 ± 19.1 yr vs. 40.1 ± 18.7; male/female: 1.4 vs. 1.6, respectively) ($p = 0.43$ and $p = 0.57$). Individuals with the GSTM1 and GSTT1 null genotypes had an estimated 4.7-fold (95% CI: 2.1–11.0) and 2.3-fold (95% CI: 1.0–5.2) increased risk of AML, respectively. The combined null genotype frequency for both genes among AML patients was also significantly higher than among the controls. An estimated 6.6-fold (95% CI: 2.4–7.9) greater risk of AML was observed in individuals with combined null genotypes.

In contrast, there were no differences in the frequencies of the null genotypes for the GSTM1 and GSTT1 genes among MDS patients, AA patients and the controls. The frequencies of null combined genotypes were also similar among these three groups. Individuals with GSTM1 and GSTT1 null genotypes showed no increase in the risk of developing MDS or AA.

Discussion

Little attention has been paid to the role of inherited genetic susceptibility in the aetiology of haematological diseases such as MDS, AML and AA, although exposure to chemicals in the environment may predispose individuals to these diseases (3, 5–7). In particular, persons with an altered ability to metabolize carcinogens may have an increased risk of cancer (13).

In this study of loci encoding xenobiotic-metabolizing enzymes (GSTM1 and GSTT1) of the GST system in Brazilian patients with MDS, AML or AA, the frequencies of the GSTM1 and GSTT1 null genotypes and combined null genotype among AML patients were significantly higher than in normal individuals. Similar results were obtained by Sasai *et al.* (32) in Japanese patients with therapy-related AML and AML with trilineage dysplasia

and by Davies *et al.* (33) in American patients with AML. In contrast, Basu *et al.* (29) and Whoo *et al.* (34) found no increased risk of AML associated with GSTM1 and GSTT1 null genotypes in British patients with AML and American patients with related-therapy AML, respectively. There are no obvious reasons for the discrepancy among the results obtained in the different studies. A potential problem in assessing the frequencies of GSTM1 and GSTT1 null genotypes in a given disease is the marked variation that these frequencies show between racial groups, being particularly high in some Asian populations (19, 21). The ethnic origin of the Brazilian population is highly heterogeneous, consisting of indigenous Amerindian populations and immigrants from Europe, Africa and Asia (20, 54, 55). However, no differences in the frequencies of the GSTM1 and GSTT1 null genotypes were found in our control group and those obtained by Basu *et al.* (29) and Whoo *et al.* (34) in their control groups. We also did not find difference in the frequencies of the GST genotype in our control group and among caucasian, individuals of African origin and indigenous populations evaluated in a previous study conduced by our group, including only Brazilian individuals with confirmed ethnical origin (20). Thus, the divergence between our results and those previously reported (29, 34) cannot be attributed to the ethnic origin of the populations. On the other hand, variation in the pathogenesis of AML in different countries could be a contributing factor. In Brazil, the workers could be exposed to numerous chemical agents, such as hexachlorobenzene, carbon tetrachloride, perchloroethylene, solvents, benzopyrene, 4-nitroquinoline-*N*-oxide, alachlor, atrazine, lindane and methyl parathion, some of which are known to be metabolized by the enzymes of the GST system (56). Unfortunately there was no reliable data in our patients' records to study the association between chemical agents and AML.

The frequencies of GSTM1 and GSTT1 null genotypes and combined null genotypes were similar in MDS patients and controls. These results agree with reports by Atoyeb *et al.* (28) and Basu *et al.* (29) for British patients, by Preudhome *et al.* (31) for French patients and by Whoo *et al.* (34) for American patients with MDS. However, Chen *et al.* (27), Okada *et al.* (30), Sasai *et al.* (32) and Davies *et al.* (33) reported high frequencies of the GSTT1 null genotype in American and Japanese MDS patients. The reasons for these divergent results are not clear but are unlikely to be caused by the racial heterogeneity of the populations since the frequencies of the GST null genotypes were similar in all control groups. One possible factor could be different causes of MDS in different countries. The exposure to several chemicals (especially exhaust fumes) is associated with a higher incidence of MDS (7, 57, 58) and GST enzymes play a role in the detoxification of some carcinogens, including 1,3-butadiene, methyl bromide and ethylene oxide (17, 59). On the other hand, for some substances such as the solvent dichloromethane, a functional GSTT1 enzyme can form mutagenic metabolites after conjugation with glutathione in certain models (17). Thus, the presence of a functional enzyme, while generally protective, may increase the mutagenic risk of some exposures. These findings suggest that GSTM1 and GSTT1 enzymes should be studied in MDS patients with different exposures to chemicals.

As with MDS, the frequencies of the GSTM1 and GSTT1 null genotypes and combined null genotypes in AA patients and controls were very similar. High frequencies of the GST null genotype were recently reported in two studies conduced in European and Asian patients (38–40). Variation in the pathogenesis of AA in different countries may also explain the differences obtained between our results and those previously described. Since the case number included in this study is small, our results only suggest that the GST system, mediated by the GSTM1 and GSTT1 genes, is probably not important for the aetiology of AA in Brazil.

In conclusion, the 4.7- and 2.3-fold increased risks of AML associated with the GSTM1 and GSTT1 null genotypes present preliminary evidence that the inherited absence of this carcinogen detoxification pathway could be an important determinant of this disease.

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