No contribution of GSTM1 and GSTT1 null genotypes to the risk of neutropenia due to benzene exposure in Southeastern Brazil

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Abstract

Exposure to benzene has been associated with haematological diseases such as neutropenia (NEB) and acute myeloid leukaemia (AML). We tested whether the null genotypes of the GSTM1 and GSTT1 genes, involved in benzene inactivation, altered the risk for NEB in southeastern Brazil. Genomic DNA from 55 NEB patients and 330 controls was analysed by multiplex-polymerase chain reaction. The frequency of the GSTM1, GSTT1 and combined null genotypes was similar in patients and controls (GSTM1, 27.3% vs. 38.8%, p = 0.16; GSTT1, 25.5% vs. 19.7%, p = 0.24; GSTM1/GSTT1, 12.7% vs. 6.7%, p = 0.26; respectively). The distribution of genotype classes in NEB patients was similar to normal controls, suggesting that GSTM1 and GSTT1 null genotypes make no specific contribution to the risk of NEB. As the GSTM1 and GSTT1 null genotypes were previously associated with increased risk for AML in Brazil and elsewhere, we hypothesise that different thresholds of chemical exposure relative to distinct GSTM1 and GSTT1 genotypes may determine whether AML or NEB manifests in benzene exposed individuals from southeastern Brazil. Although indicative, our results still require support by prospective and large scale epidemiological studies, with rigorous assessment of daily chemical exposures and control of the possible contribution of other polymorphic genes involved in benzene metabolism.

Key words: neutropenia, glutathione S-transferase, GSTM1, GSTT1.

Received: November 27, 2008; Accepted: May 13, 2009.
drug-related neutropenia, as previously recommended by our group (Lima et al., 2006), were also required for NEB diagnosis. The control group consisted of 330 blood donors (247 men, 83 women; 174 Caucasians, 156 African-Brazilians; mean age 51 ± 3 years) without a consistent history of benzene exposure. They were recruited from the same university hospital in order to provide a representative group of the general population that seeks medical assistance in the region. All procedures were carried out according to the principles of the institutional guidelines and all patients and controls provided written informed consent.

Genomic DNA from peripheral blood of patients and controls was analysed by multiplex-polymerase chain reaction for identification of GST genotypes (Arruda et al., 2001). The GST genotypes were analysed after electrophoresis on 2.0% agarose gels (Figure 1), using the β-globin gene as internal control.

Differences between groups were analysed by means of chi-squared or Fishers exact tests. For analysing the associations with NEB, univariate and multivariate analyses were used throughout, in order to obtain odds ratio (OR), adjusted or not for age, gender and ethnic origin, and their corresponding 95% confidence intervals (CI). The statistical package Epi Info was used to perform all these analyses.

The frequencies of the GSTM1, GSTT1 and combined GSTM1 and GSTT1 null genotypes were similar in patients and controls. Patients with the distinct genotypes of the GSTM1 and GSTT1 genes exhibited similar distribution to normal controls, suggesting that GST genotypes make no significant contribution to NEB, under the chemical exposures encountered in this study (Table 1).

In Brazil, workers are exposed predominantly to solvents, such as benzopyrene, hexachlorobenzene, ethylene oxide, dichloromethane and epoxybutanes, which are metabolised by the GSTM1 and GSTT1 enzymes (Ruiz et al., 1994; Queiroz et al., 1997; Hayes et al., 2005). The chemicals have been consistently associated with benzene for patients in either group. Taking these results together, we hypothesise that different thresholds of chemical exposure relative to distinct GST genotypes may determine whether NEB or AML arises in chemical hazard exposed individuals from southeastern Brazil. Thus, those highly exposed to chemicals and homozygous for the null GST alleles may develop AML, since this seems to be more dependent on the GST pathway of carcinogen metabolism, whilst those individuals with less exposition to chemicals may be less dependent on carcinogen inactivation by the GST isoenzymes, and therefore more prone to present the benign form of occupational disease, NEB, without mediation of GST genotypes. Although indicative, these results must, however, be confirmed by prospective studies with Vaughan et al., 2005). These data supported the association of both GST null genotypes with increased risks for AML previously found by our group (Arruda et al., 2001). On this basis, the GST null genotypes were also expected to be associated with increased NEB risk. Surprisingly we found similar frequencies of the GST genotypes in our NEB patients and controls.

Unfortunately, there was no available data concerning the levels of benzene exposure of the NEB patients in this study and of the AML patients in our previous study (Arruda et al., 2001). We assumed similar exposures to benzene for patients in either group. Taking these results together, we hypothesise that different thresholds of chemical exposure relative to distinct GST genotypes may determine whether NEB or AML arises in chemical hazard exposed individuals from southeastern Brazil. Thus, those highly exposed to chemicals and homozygous for the null GST alleles may develop AML, since this seems to be more dependent on the GST pathway of carcinogen metabolism, whilst those individuals with less exposition to chemicals may be less dependent on carcinogen inactivation by the GST isoenzymes, and therefore more prone to present the benign form of occupational disease, NEB, without mediation of GST genotypes. Although indicative, these results must, however, be confirmed by prospective studies with Vaughan et al., 2005). These data supported the association of both GST null genotypes with increased risks for AML previously found by our group (Arruda et al., 2001). On this basis, the GST null genotypes were also expected to be associated with increased NEB risk. Surprisingly we found similar frequencies of the GST genotypes in our NEB patients and controls.

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![Figure 1 - Multiplex-PCR for detection of the wild and null alleles of the glutathione S-transferase mu1 (GSTM1) and theta1 (GSTT1) genes. Ethidium bromide-stained 2% agarose gel showing products of 273 bp and 237 bp.](Image 314x602 to 546x722)

### Table 1 - GSTM1 and GSTT1 genotypes in 55 patients with neutropenia due to exposure to benzene and 330 controls.

|       | GSTM1 |      | GSTT1 |      | GSTM1/GSTT1 |
|-------|-------|------|-------|------|-------------|
|       | Present n (%) | Null n (%) | Present n (%) | Null n (%) | Both present n (%) | One null n (%) | Both null n (%) |
| Cases | 40 (72.7) | 15 (27.3) | 41 (74.5) | 14 (25.5) | 33 (60.0) | 22 (40.0) | 7 (12.7) |
| Controls | 202 (61.2) | 128 (38.8) | 265 (80.3) | 65 (19.7) | 159 (48.2) | 149 (45.1) | 22 (6.7) |
| OR (CI 95%) | 1.0 (ref) | 0.59 (0.31-1.11) | 1.0 (ref) | 1.39 (0.72-2.71) | 1.0 (ref) | 0.71 (0.40-1.28) | 1.53 (0.60-3.88) |
| P value | 0.13 | 0.37 | 0.24 | 0.34 | 0.26 |

| OR* (CI 95%) | 1.0 (ref) | 0.61 (0.31-1.21) | 1.0 (ref) | 1.54 (0.75-3.14) | 1.0 (ref) | 0.74 (0.39-1.38) | 0.82 (0.65-4.81) |

| P value | 0.16 | 0.24 | 0.24 | 0.34 | 0.26 |

n: number of cases; OR: odds ratio; *: adjusted OR by age, gender, and ethnic origin; CI: confidence interval.
larger samples of NEB and AML patients and controls, with rigorous assessment of daily chemical exposures, and control of the influence of other polymorphic genes involved in benzene metabolism (Aydin-Sayitoglu et al., 2006).

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Associate Editor: Peter L. Pearson

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