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Influence of *p53* codon 72 exon 4, *GSTM1*, *GSTT1* and *GSTP1*B* polymorphisms in lung cancer risk in a Brazilian population

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Summary
Purpose: Glutathione *S*-transferases (GST) modulates the effects of various cytotoxic and genotoxic agents, particularly those derived from benzo[*a*]pyrene, which is one of the main tobacco carcinogens. Both the mu 1 (*GSTM1*) and theta 1 (*GSTT1*) genes have a null variant allele in which the entire gene is absent. The *GSTP1*B* allele has an A to G transition at nucleotide 313 (codon 105) in exon 5, causing a change of isoleucine (Ile) to valine (Val), which affects the electrophile binding site of GSTP1 and results in an enzyme with reduced activity. Polymorphisms in these metabolizing enzymes may alter the response to benzo[*a*]pirene-induced DNA

Abbreviations: A, adenine; bp, base pairs; BPDE, benzo[*a*]pyrene diolepoxide; CI, confidence interval; dGMP, deoxyguanosine phosphate; DNA, deoxyribonucleic acid; dNTP, deoxynucleotides; EDTA, ethylene diamine tetracetic acid; G, guanine; GST, glutathione *S*-transferase; GSTM1, gene mu1 of glutathione *S*-transferase; GSTP1, gene pi 1 of glutathione *S*-transferase; GSTT1, gene theta 1 of glutathione *S*-transferase; HCl, acid chlorhydrique; I, isoleucine; Ile, isoleucine; KCl, potassium chloride; LC, lung cancer; $MgCl_2$, magnesium chloride; mM, millimole; ng, nanogram; μl, microliter; NSCLC, non-small cell lung cancer; OR, odds ratio; PCR, polymerase chain reaction; pmol, picomole; RFLP, restriction fragment length polymorphism; SCLC, small cell lung cancer; TNM, tumor, nodes and metastasis; U, unit; Val, valine; VV, valine/valine.

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Metabolism *p53* codon 72 polymorphism; Polymorphism; Susceptibility; Xenobiotics

damage. Polymorphisms in *p53* may also modulate the risk of lung cancer (LC) carcinogenesis. The aim of our study was to measure the frequency of *GSTM1*, *GSTT1*, *GSTP1*B* and *p53* gene polymorphisms in a Brazilian population and determine the possible contribution of these genetic variations to LC risk.

Patients and methods: Genomic DNA was obtained from 200 Brazilian patients with LC and 264 blood donors (control group). All samples were analyzed by PCR and PCR-RFLP to determine *GSTM1*, *GSTT1*, *GSTP1*B* and *p53* codon 72 genotypes. Multiple logistic regressions were used to adjust for confounding factors in this case—control study.

Results: No statistical significance was observed between *GSTM1*, *GSTT1* and *GSTP1*B* genetic polymorphisms, either isolated or combined, with LC incidence in the studied population. However, our data showed a higher frequency of *p53* codon 72 A/P plus P/P genotype in African-Brazilian than Caucasian-Brazilian patients with LC, and we also found a higher frequency of the P/P genotype of the *p53* gene in non-smokers compared to smokers with LC.

Conclusions: Genetic polymorphisms of GST and *p53* codon 72 did not increase the risk of LC in Brazilian patients. The A/P plus P/P genotype of *p53* codon 72 is more common in LC patients with African ethnical background and the P/P genotype more prevalent in non-smoking related LC.

1. Introduction

Tobacco smoke is the main risk factor for lung cancer (LC) development. However, not all populations are equally susceptible to tobacco-related carcinogens [1]. Thus, the identification of genes responsible for lung carcinogenesis susceptibility may allow us to perform screening programs and chemoprevention trials in subgroups of heavy smokers.

The glutathione *S*-transferases (GSTs), forming a family of enzymes catalyzing the detoxification of wide range of eletrophilic substrates, play a significant role in phase II biotransformation of xenobiotics. This detoxification is achieved by the conjugation of xenobiotics with glutathione, which facilitates the neutralization of their eletrophilic centre. Conjugated xenobiotics can be eliminated by urine or bile either directly or by subsequent intermediate stages of transformation in which *N*-acetylases and transpeptidases take their places [2].

Inter-individual variability in Glutathione *S*-transferase (GST) enzyme activity can also influence the susceptibility to cancers, especially in those with environmental determinants, such as LC [3,4]. Some genetic variants in *GST* genes, such as the *GSTM1* null polymorphism, are known to abolish enzymes activity. Individuals with *GSTM1* null genotype have been reported to have higher levels of polycyclic aromatic hydrocarbon —dGMP adducts in lung tissues, which can induce genetic mutations [5].

The *GSTT1* gene, located on chromosome 22q11.23, has also been found to have a deletion polymorphism that results in absence of enzymatic activity [6]. *GSTT1* is involved in the metabolism of smaller compounds found in tobacco smoke, such as monohalomethane and ethylene oxide [7].

GSTP1 is known to metabolize many carcinogenic compounds, among them benzo[*a*]pyrene diolepoxide (BPDE), which is one of the most important carcinogenic metabolites derived from tobacco smoke [8]. Given that *GSTP1* is the most abundant GST isoform in the lung [9], it is anticipated to be of particular importance in the detoxification of inhaled carcinogens. The *GSTP1*B* allele has an A to G transition at nucleotide 313 (codon 105) in exon 5, causing a change of isoleucine (Ile) to valine (Val), and affecting the electrophile binding site of the GSTP1 enzyme. It has been reported to result in an enzyme with reduced activity and it is possible that deficient or reduced activity of this enzyme may result in an increased susceptibility to cancer [10]. The *Val* variant has generally lower activity towards polycyclic aromatic hydrocarbon diol epoxides, especially BPDE, and has been shown to have a lower detoxification potential leading to a greater risk of neoplasic transformation [11].

The *p53* gene is located on chromosome 17p13 and encodes a 53 kDa protein which plays a critical role in cell growth control. Variant alleles of codon 72 in exon 4 encode either arginine [Arg-CGC] or proline [Pro-CCC]. Polymorphic variants differ in biochemical and biological properties. An association between the presence of the *p53* Pro*72* allele and an increased risk of LC development has been suggested [12—14]. However, further validation is required and the mechanism of this possible relationship is still not well understood. Ethnic differences in the frequency of Arg72 and Pro72 alleles warrant analysis of the potential clinical relevance of this polymorphism in various populations. Other studies have also shown that the Pro72 allele confers a worse prognosis in LC patients [15,16].

The aim of our study was to estimate and compare the frequency of *GSTM1*, *GSTT1*, *GSTP1*B* and *p53* gene polymorphisms in a Brazilian population and determine the possible contribution of these genetic variations to LC risk.

2. Subjects and methods

2.1. Study subjects

The study population included 200 patients with LC (144 men and 56 women; mean age $\pm$ S.D.: 64.0 $\pm$ 9.7 years) seen at the Pulmonary unit of School Hospital at the State University of Campinas (UNICAMP) from January 2004 to December 2006.

The control group consisted of 264 blood donors (160 men and 104 women) selected in the same Hospital.