MOLECULAR ANALYSIS OF *GSTP1* ILE105VAL POLYMORPHISM AND ITS ASSOCIATION WITH GASTRIC CANCER SUSCEPTIBILITY

Abstract: This study was aimed at examining the association between *GSTP1* Ile105Val polymorphism and gastric cancer in the city of Macapá, state of Amapá, Amazon, Brazil. Overall 160 DNA samples, 62 cases and 98 controls, were examined using polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) with the restriction enzyme *BsmAI* to detect polymorphism. Our results revealed that 79.0% (X^2=86,077, p<0.0001) of the cases with gastric cancer had the ile105val genotype compared to 7.1% of control samples. *GSTP1* polymorphism frequency in exon 5 in cases and controls was 83.8% and 15.3%,respectively, for the AG+GG alleles (p <0.0001, X^2=73. 347). Our results suggest that *GSTP1* Ile105Val polymorphism may contribute to gastric cancer susceptibility in the study population because the mutation in the *GSTP1* gene alters the enzymatic function of GSTP1, which might lead to the development of the disease.

Keywords: *GSTP1*, genetic polymorphisms, gastric cancer

INTRODUCTION

Glutathione S-transferases (GSTs) are the most important enzymes of the phase II of xenobiotic metabolization, which detoxifies several cytotoxic compounds (17). They are involved in the metabolism of carcinogens, drugs, and reactive oxygen species (ROS), playing a protective role against oxidative DNA damage. They comprise five distinct classes of enzymes known as: α (GSTA1-GSTA4), σ (GSTS), μ (GSTM1 – GSTM5), π (GSTP1), θ (GSTT1e GSTT2), and ζ (GSTZ1) (4, 5). Polymorphisms of these genes affect the function of enzymes in this group, especially the polymorphism that occurs in exon 5 of the *GSTP1* gene in nucleotide 1,587, substituting A for G (Ile105val, rs200139798) (9,14).

The activity of the enzyme GSTP1 is affected by the substitution of the amino acid isoleucine at position 105 for valine, located in the hydrophobic region, the substrate binding site, with considerable effects depending on the type of chemical interaction with environmental factors (7,23). Several epidemiological studies have suggested an association between *GSTP1* polymorphism and cancer risk, such as breast cancer, prostate cancer, colon cancer, lung cancer, and gastric cancer (17, 26).

Gastric carcinogenesis is a multifactorial process, influenced by interactions between genetic susceptibility and environmental factors. Growing evidence suggests that interactions among various susceptibility genes associated with hereditary cancer may affect an individual's risk of developing gastric cancer (12,19,28). They are also known as risk-modifying genes, particularly those with allelic polymorphisms responsible for a deficient metabolism of environmental carcinogens and/or DNA damage repair induced by oxidative stress (11, 13,14).

In this case-control study, we examined whether *GSTP1* Ile105Val polymorphism in exon 5 may be associated with gastric cancer susceptibility in patients in the city of Macapá, Amazon region, Brazil.

MATERIALS AND METHODS

Study Population

Overall 160 DNA samples, 62 patients and 98 controls, were analyzed for the identification of *GSTP1* Ile105Val polymorphism. All participants were selected from March 2019 to December 2019, in the city of Macapá, state of Amapá, Amazon, Brazil. The study was approved by the Ethics Committee on Human Research of the Federal University of Amapá, CAAE#: 07398519.3.0000.0003. All participants signed the Informed Consent Form in writing in accordance with the Declaration of Helsinki.

Molecular Analysis

Genomic DNA was extracted with the bioscience® extraction kit. Genotype identification was carried out with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism analysis (PCR-RFLP), following Uddin et al, (2014) with modifications.

The PCR product was digested with the restriction enzyme *BsmAI*, incubated overnight at 55^0 C, followed by electrophoresis in agarose gel at 2.5% and staining with ethidium bromide. The digested product of 176 bp had the following pattern: heterozygous (genotype A/G, Ile/Val) with fragments of 176, 93, 83 pb and homozygous (G/G, Val/Val), with fragments of 93.83 bp.

Statistic analysis

All statistical analyses were performed using the software BioEstat (Ayres, M. Pará, Brazil). The Hardy-Weinberg equilibrium (HWE) using the frequencies of all alleles and genotypes and the general characteristics among patients with gastric cancer and cancer-free controls were compared with the chi-square test ($\chi 2$). The odds ratios test (ORs) and 95% confidence intervals (95% CI) of unconditional logistic regression were used to evaluate the possible associations between *GSTP1* variants and the risk of gastric cancer. Significance was set at 0.05.

RESULTS

Frequency Distribution of the *GSTP1* Ile150Val genotype

The distribution of the *GSTP1* Ile105Val genotype was significantly different between the two groups. The percentage of the Ile/Val polymorphic allele was 79.0% (X^2=86,077, p<0.0001) in gastric cancer samples (table 1) when compared with the control samples.

Table1: Distribution of the *GSTP1* genotype in gastric cancer and control samples

Genotype	Cases %	Control%	OR (95%CI)	*p*	X^2
Ile/Ile	10(16.0)	83(84.6)	Reference		
Ile/Val	49(79.0)	7(7.1)	58,1000 (20.7749-162.4247)	<0.0001	86,077
Val/Val	3(4.8)	8(8.1)	3,1125 (0.7083-13.6766)	0.2781	2.454
Total	62	98			

Table2 presents a comparison of the polymorphism in exon 5, altering the genotype A/A to A/G and G/G. In the controls, 15.3% had the base change that alters the function of the GSTP1 protein, while in the samples of gastric cancer cases, the percentage was 83.8% (X^2 =73,347, p <0.0001).

Table 2: Polymorphism frequency in exon 5 of the *GSTP1* gene in the case and control groups

Groups	Total	AA	AG+GG	*p*	X^2
Controls	98	83(84.6%)	15(15.3%)	Ref.	
Cases	62	10(16%)	52(83.8%)	<0.0001	73,347

DISCUSSION

In the last 20 years, there has been a remarkable progress in understanding the role of genetic and environmental factors in the etiology of gastric cancer. Members of the GST family are multifunctional and multigenic products. They are versatile enzymes and participate in the nucleophilic attack of the sulfur atom of glutathione in electrophilic centers of several endogenous and xenobiotic compounds. Among the main GST genes, *GSTP1* has an important significance in cancer diagnosis because it is abundantly expressed in tumor cells (28, 6, 30).

Some studies have observed a relationship between some polymorphisms of this gene and their involvement in some important cellular functions. The best known of them is the role as a phase II enzyme that catalyzes the S conjugation of glutathione (GSH) with a wide variety of electrophilic compounds, including many mutagens, carcinogens, anticancer agents and their metabolites (28, 25,8,21). The enzymatic activity of GSTP1 is influenced by the polymorphism in amino acids at position 105, the hydrophobic substrate binding site, which can affect catalytic activity, nucleophilic addition, and epoxide conjugation. The detoxification activity of the enzyme is reduced because of the presence of 105Val in the GSTP1 protein; thus, carcinogens that enter the human body might further affect other genes, increasing the risk of gastric cancer (26, 22, 15).

In the present case-control study, our findings revealed that the *GSTP1* ile105Val genotype was significantly associated with gastric cancer susceptibility in the study population. This corroborates the results by Wang X et al, 2017 that reported that the variant genotype GSTP1 Ile105Val was closely associated with an increased risk of gastric cancer in the Chinese population. Zhang Y et al, 2012, also associated the amino

acid 105Val and its interaction with smoking, alcohol consumption, and especially *H. pylori* infection thus increasing the risk of gastric cancer. These findings suggest new pathophysiological pathways for the development of gastric cancer.

The study also analyzed the frequency of AG+GG variant genotypes and their association with gastric cancer and observed that in the study population, these variant genotypes account for 83.8% of gastric cancer patients and 15.3% of the control group. The presence of these AG+GG variant genotypes may increase gastric cancer susceptibility in carriers.

In conclusion, our results suggest that the *GSTP1* Ile105Val polymorphism may contribute to gastric cancer susceptibility in the study population because the mutation in the *GSTP1* gene alters the enzymatic function of GSTP1, favoring the action of mutagenic and carcinogenic agents in the body.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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