

Association study between *GSTM1* and *GSTT1* genotypes and other possible risk factors in prostate cancer patients in a population from southwest Bahia, Brazil

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ABSTRACT. Prostate cancer (PCa) is the second most common type of tumor among men over 50 years old and its etiology includes environmental, demographic, and genetic risk factors. We investigated a possible association between *GSTM1* and *GSTT1* polymorphisms, sociodemographic, and clinical factors with PCa in a population of southwestern Bahia, Brazil. The research used a case-control design and was carried out with 268 men aged 50 years or older (134 cases and 134 controls). The mean age was 74 years old (± 7.9) in the case group and 55 years old (± 4.3) in the control group. Polymorphisms were determined by multiplex PCR, followed by electrophoresis. The genotypic frequencies found were 0.45 for *GSTM1* -/- (null), 0.55 for *GSTM1* +/- or +/+ (non-null), 0.37 for *GSTT1* -/- (null) and 0.62 for *GSTT1* +/- or +/+ (non-null). The estimated allele frequencies were: *GSTM1* - (null allele) 0.60 for the

case group and 0.67 for the control group, and *GSTT1* - (null allele) 0.49 for the case group and 0.65 for the control group. These polymorphisms were not significantly associated (P-values 0.68 and 0.21, respectively) with PCa. However, non-white ethnicity (self-reported), sexually transmitted infections, and cigarette consumption were significantly associated with PCa (P-value: 0.03, 0.05 and < 0.01 respectively). Vasectomy exhibited an inverse association (P-value < 0.01), thus behaving as a protective factor for PCa.

Key words: Polymorphism; *GSTM1*; *GSTT1*; Risk factor; Prostate cancer

INTRODUCTION

It is estimated that in two decades the number of new cases for some type of cancer will increase by 70% (World Health Organization - WHO, 2016). In Brazil, non-melanoma skin cancer is the most common type, followed, in men, by prostate cancer (PCa) and, in women, by breast cancer (National Cancer Institute - INCA, 2018). The highest incidence of PCa occurs in South and Southeast regions of the country. Each year, the number of new cases increases throughout the country, which is attributed to extended life expectancy and improvement in diagnosis techniques (Facina, 2016). In Brazil, in 2013, approximately 14,000 deaths were ascribed to this type of cancer, and in 2018, it is estimated that there will be reported 68,220 new cases of PCa (INCA, 2018).

According to American Society Cancer (2018), projections for PCa in the United States, in 2018, there were about 164,690 new cases and 29,430 deaths owing to PCa. The incidence rates of PCa vary among the countries, with highest rates in Australia, New Zealand, North America, and Western Europe while the lowest numbers are in South Central Asian populations (International Agency for Research on Cancer - IARC, 2016).

The emergence of cancer may be related to individual ability to metabolize carcinogenic compounds in routes involving enzymes for xenobiotics elimination (Mannervik et al., 1992). Toxic substances go through different stages of cell detoxification. Among the mechanisms involved, there are enzymes belonging to Glutathione S-Transferases (GSTs) family, which are divided into two large groups, one located at the microsomal membrane and the other present in cytosol. All cytosolic GSTs present genetic polymorphisms in human populations and are subdivided into six classes. Among them, the most studied classes are the *Mu*-class GSTs, whose proteins are encoded by *GSTM1* gene, and the *Theta* class GSTs, encoded by *GSTT1* gene (Silva et al., 2015). *GSTM1* gene, chromosomal location 1p13.3, has three described alleles: two characterized as non-null with functional activity and one null *GSTM1* genotype, described as an allelic version of gene deletion (Wei et al., 2012; Sá et al., 2014). *GSTT1* gene (22q11.23) has two non-null and functional alleles: non-null and functional *GSTT1* and null, non-functional *GSTT1*. The investigation of *GSTM1* and *GSTT1* gene polymorphisms is justified by the potential of these molecular factors to be related to loss of cellular mechanisms for protection against cancer (Yang et al., 2013; Berta et al., 2014; Sá et al., 2014).

The objective of our study was to investigate possible associations between *GSTM1* and *GSTT1* gene polymorphisms, sociodemographic, and clinical factors with PCa in a population of the state of Bahia, Brazil.

MATERIAL AND METHODS

Study sample

We used a case-control design and included 268 male individuals, aged 50 years or older, attended in Vitória da Conquista, Bahia. Among these, 134 belonged to the case group (with diagnosis of PCa by anatomopathological examination) and the other 134 to the control group (healthy individuals, without cancer). Case group individuals were invited to participate in the study from July 2015 to January 2016, at the High Complexity Unit in Oncology (Unidade de Alta Complexidade em Oncologia - UNACON) and the control group members took part in the research from December 2015 to April 2016 at the Southwest Hemotherapy Service (Serviço de Hemoterapia do Sudoeste - SHS). All participants signed an Informed Consent form. The research project was approved by the Research Ethics Committee of the Multidisciplinary Institute of Health of the Federal University of Bahia (IMS-UFBA), under the protocol number 30879614.3.0000.5556, approval opinion 1.325.852.

Data collect

Data and sample collection included: (i) a questionnaire (in which patient information was included) and (ii) a peripheral blood sample. All data and samples were collected at the institution where the research participants were attended. The assembled information was distributed in the following categories: (i) demographic, (ii) life habits, and (iii) health conditions.

Samples of 3 mL from peripheral blood were collected from each patient in EDTA tubes. They were transported in refrigerated boxes to the Laboratory of Cellular and Molecular Biology of the IMS-UFBA to perform genotyping.

Genetic analysis

Genomic DNA was isolated using the Brasília kit (LGC Biotecnologia, SP, Brazil), following the manufacturer's protocol. The samples were amplified by multiplex PCR, using Taq DNAPolimerase (Invitrogen™, SP, Brazil), thermal cycler MyGenie96 thermal block (Bionner, Korea), and three pairs of primers. The primers used were: (i) one for *GSTM1* (sense 5' GAA CTC CCT CAA AAG CTA AAG C 3' e antisense 5' GTT GGG CTC AAA TAT ACG GTG G 3'), (ii) another for *GSTT1* (sense 5' TTC CTT ACT GGT CCT CAC ATC TC 3' e antisense 5' TCA CCG GAT CAT GGC CAG CA 3') described by Kumar et al. (2011), and (iii) the third for *beta-globin* (sense 5' CAA CTT CAT CCA CGT TCA CC 3' e antisense 5' GAA GAG CCA AGG ACA GGT AC 3') (Sá et al., 2014). *β-globin* gene was used as a positive control of the reaction. The negative control (containing all reagents except DNA) was also applied. Reaction conditions used for a final volume of 25 µL were: 1x buffer; 3.5 mM of MgCl₂; 0.2 mM of dNTPs; 0.5 µM of each primer; 0.03 units/µL de Taq DNAPolimerase and approximately 42 ng DNA (per reaction). Thermocycling conditions were: (i) initial denaturation at 94°C for 5 min; (ii) 30 cycles of: 94°C for 1 min, 62°C for 1 min, 72°C for 1 min; and (iii) final extension at 72°C for 7 min. Conditions adapted from Kumar et al. (2011).

PCR products were applied (volume of 15 μ L of the PCR in each channel) in 0.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. The fragments obtained were: (i) 215bp, which identifies *GSTM1* non-null genotype (in homozygosis or heterozygosis); (ii) 459bp, which identifies *GSTT1* non-null genotype (in homozygous or heterozygosis) and (iii) 273bp, corresponding the amplification of the positive reaction control (*β -globin* gene). *GSTM1* null genotype was identified as the absence of the 215bp fragment and the *GSTT1* genotype was null as the absence of the 459bp fragment. Fragment sizes were estimated comparing with molecular weight marker (Universal marker, 100pb-1Kb DNA, LGC Biotechnology, SP, Brazil). Molecular techniques employed by the present work do not distinguish between non-null homozygous genotypes and heterozygous non-null genotypes.

Statistical analysis

The following potential explanatory variables for PCa occurrence were evaluated in this study: demographic, life habits, health status, and *GSTM1* and *GSTT1* genes polymorphisms. Descriptive analysis was performed through (i) relative frequency for categorical variables and (ii) measures of central tendency (as mean and median) for continuous variables. The explanatory variable of the univariate analysis was the conclusive diagnosis of PCa by biopsy. The association between response and explanatory variables was estimated by odds ratio (OR), with 95% confidence intervals (CI). The chi-square test was used with the Yates correction or Fisher's exact test and multivariate analysis to measure the differences, considered significant at $P \leq 0.05$. All analyzes were performed using EPI INFO software, version 7.1.5. The Hardy-Weinberg principle was used to calculate the allelic and genotype frequencies specific to non-null homozygous genotypes (+/+) and heterozygotes (+/-). The frequencies of homozygous null genotypes (-/-) were determined in the present work by genotyping.

RESULTS

The mean age of the research's participants was 74 years old \pm 7.9 for the case group, and 55 years old \pm 4.3 for the control group. In the case group, the most frequent Gleason scores were 7 (46.3%) and 6 (28.4%)

For individuals' genotypes, different genotypic combinations were obtained, they were: (1) *GSTM1* null (-/-) and *GSTT1* non-null (+/+ or +/-) genotypes, (2) *GSTM1* non-null (+/+ or +/-) and *GSTT1* non-null (+/+ or +/-) genotypes, (3) *GSTM1* non-null (+/+ or +/-) and *GSTT1* null (-/-) genotypes, and (4) *GSTM1* null (-/-) and *GSTT1* null (-/-) genotypes.

In the analysis of the genotypes seen in Table 1, the *GSTM1* polymorphism, null genotype (-/-) was present in 35.8% of the individuals in case group and 44.8% of the control group representatives. For *GSTT1* polymorphism, the null genotype (-/-) was detected in 23.9% of the case group participants and in 42.5% of the control group. When we considered double null genotypes (the same individual with deletion for both investigated genes), this event occurred in 8.2% of the case group population and 20.2% of the control group participants. Regarding the presence of PCa cases in the family (considering first - and second - degree kinship - parents, siblings, children, grandparents, uncles, and cousins), 35.8% of the case group reported occurrence, in contrast to 24.6% of the

control group (Table 2). The estimated allele frequencies were: *GSTM1* - (null allele) 0.60 for the case group and 0.67 for the control group, and *GSTT1* - (null allele) 0.49 for the case group and 0.65 for the control group (data not shown in table). The frequencies of the non-null alleles are the complementary frequencies.

Table 1. *GSTM1* and *GSTT1* gene polymorphisms associated with prostate cancer in case and control groups.

Variables	Patients n (%)	Controls n (%)	Odds Ratio (95%CI)	P-value*
<i>GSTM1</i>				
Null	48 (35.8)	60 (44.8)	1.17 (0.68 - 2.01)	0.68
Non-null	86 (64.2)	74 (55.2)	1	
<i>GSTT1</i>				
Null	32 (23.9)	57 (42.5)	0.64(0.35 - 1.19)	0.21
Non-null	102 (76.1)	77 (57.5)	1	

Sample: 268 men (134 cases, 134 controls), age ≥ 50 years old, attended from 2015 to 2016 by the Health System of Vitória da Conquista, Bahia, Brazil. *Statistical significance ($P < 0.05$). Genotype frequencies of homozygous null genotypes were obtained by genotyping (through experiments). Frequencies of non-null genotypes (homozygous and heterozygous) were achieved using the expected allele frequencies attained by the Hardy-Weinberg equation ($p^2 + 2pq + q^2 = 1$).

Table 2. Demographic and clinical aspects related to the prostate cancer for case and control groups.

Variables*	Patients n (%)	Controls n (%)	Odds Ratio (95%CI)	P-value**
Ethnicity¹				
White	20 (15.0)	37 (27.6)	1	0.016
Non-white	113 (85.0)	97 (72.4)	2.16 (1.17 - 3.96)	
Alcohol consumption²				
Yes	118 (88.1)	117 (87.3)	1	1
No	16 (11.9)	17 (12.7)	1.07 (0.52 - 2.22)	
Cigarette consumption³				
Yes	103 (77.4)	77 (58.8)	2.41 (1.41 - 4.11)	< 0.002
No	30 (22.6)	54 (41.2)	1	
Obesity⁴				
Yes	18 (13.4)	23 (17.2)	0.74 (0.38 - 1.46)	0.498
No	116 (86.6)	111 (82.8)	1	
STIs⁵				
Yes	29 (21.8)	14 (10.5)	2.37 (1.19 - 4.73)	0.019
No	104 (78.2)	119 (89.5)	1	
Vasectomy				
Yes	2 (1.5)	17 (12.7)	0.11(0.02 - 0.47)	<0.001
No	130 (98.5)	117 (87.3)	1	
Cases of PCa in the family⁶				
Yes	48 (35.8)	33 (24.6)	1	0.06
No	86 (64.2)	101 (75.4)	1.71(1.0 - 2.90)	

Sample: 268 men (134 cases, 134 controls), age ≥ 50 years old, attended from 2015 to 2016 by the Health System of Vitória da Conquista, Bahia, Brazil. * The maximum number of data lost for each variable was less than 6. ** Univariate analysis (statistical significance P -value < 0.05). Most of the variables described here were self-reported (exceptions are mentioned) in the data collection instrument (questionnaire) by a trained team. Superscript numbers: 1 - Self-reported ethnicity. 2 - Alcohol consumption in the past or in the current days. 3 - Cigarette consumption in the past. 4 - Obesity previously notified to the individual by a healthcare professional. 5 - Presence of Sexually Transmitted Infections (STIs), previously informed by medical report. 6 - First- and second-degree parents (parents, siblings, children, grandparents, uncles, and cousins).

The distribution of the investigated variables (demographic, life habits, health conditions) and the list of this disease data is shown in Table 2 (univariate analysis). Concerned to the variables described, the presence of PCa cases in the family was close to the degree of significance (P-value 0.06; OR 1.71), but it did not compose the final model, which presents non-white ethnicity (P-value 0.016, OR 2.16), cigarette consumption (P-value < 0.002, OR 2.41), STIs (P-value 0.019; OR 2.37), and vasectomy (P-value < 0.001; OR 0.11). These variables were independently associated with PCa in multivariate analysis (Table 3). Vasectomy surgery was presented as a protective factor for the occurrence of PCa.

Table 3. Multivariate analysis of the variables associated with prostate cancer in Vitória da Conquista, Bahia, Brazil.

Variables*	Odds Ratio (95%CI)	P-value**
Non-white Ethnicity (Yes/No) ¹	2.06 (1.09 – 3.90)	0.03
Smoking Habit (Yes/No) ²	2.16 (1.23 – 3.77)	<0.01
STIs (Yes/No) ³	2.07 (1.0 – 4.28)	0.05
Vasectomy (Yes/No)	0.12 (0.03 – 0.05)	<0.01

Sample: 268 men (134 cases, 134 controls), age \geq 50 years old, attended from 2015 to 2016 by the Health System of Vitória da Conquista, Bahia, Brazil. *The maximum number of data lost for each variable was less than 6. **Statistical significance (P < 0.05). Log-likelihood model fit test = 329.578. Most of the variables described here were self-reported (exceptions are mentioned) in the data collection instrument (questionnaire) by a trained team. Superscript numbers: 1 - Self-reported ethnicity. 2 - Cigarette consumption in the past. 3 - Presence of Sexually Transmitted Infections (STIs), previously informed by medical report.

DISCUSSION

In this study, mean age was different between control and case groups (74 years old for the cases vs. 55 years old for controls). This difference was statistically significant (P < 0.01). This data is explained by the individuals' profile attended at the sample collection sites. Control representatives were blood donors and the case ones were individuals being treated for PCa. According to INCA (2018), 75% of PCa cases occur after 65 years old. These data corroborate to the difference in mean age found between the groups in the present work. Agalliu et al. (2006) have also assessed the possible association of *GSTMI* and *GSTT1* with PCa, using case and control groups and encountered age differences as well, where the most frequent age group for control group was 55 - 59 years old and for the cases 60 - 64 years old. Due to this mean age disparity, it was not possible to analyze the element age as a risk factor for PCa.

Perera (1996) has reported that cancer can occur in consequence of accumulation of mutations. Therefore, it is expected that an individual at a later age will have a greater number of accumulated mutations. The results of the present study demonstrate a higher frequency (not statistically significant) of *GSTMI* and *GSTT1* null genotypes in the control group, which indicates that there is no relation between age (higher in the case group) and the mutations analyzed here (present in the null genotypes). Hence, there is no evidence to believe that the mean age difference between the groups influence our study of the association of GST genotypes with PCa.

The polymorphisms of the investigated genes *GSTM1* and *GSTT1* revealed no association with PCa in the studied population. Recent studies of Brazilian populations have analyzed the relation between *GSTM1* and *GSTT1* polymorphism with PCa and corroborate with this behavior. In a study conducted on residents from the south of the country (Londrina, Paraná), no association was established (Rodrigues et al., 2011). Sá et al. (2014) also obtained non-association findings in a sample from southeastern Brazil (Rio de Janeiro, RJ). The absence of association between GST gene polymorphisms and PCa was likewise observed in a population of the city of Rawalpindi, Pakistan (Malik et al., 2015).

Researchers have presented that when an association between GSTs and PCa is detected, most occur with only one of the genes. Meta-analysis studies indicate that only *GSTM1* was associated with PCa (Zengnan et al., 2009; Cao et al., 2015). In a population from north India, both genes were correlated with PCa, although it was verified a higher prevalence of *GSTT1* null genotype (Mittal et al., 2004). In regions of Tunisia and Turkey, *GSTT1* gene was seen to be associated with PCa (Souiden et al., 2010; Berber et al., 2013). The presence of *GSTT1* gene has also been shown to be associated with PCa in a Caribbean population, thus requiring more studies for better understanding (Mallick et al., 2007). The possible connection with GST polymorphisms has not been reported exclusively with PCa, but also with other types of tumor such as colorectal, lung, and bladder (Economopoulos and Sergentanis, 2010; Tamaki et al., 2011; Matic et al., 2013).

Regarding heredity, the findings in this study did not have a statistical association with family history of PCa (first- and second-degree kinship), although P-value of 0.06 demonstrates a probable relation if the level of significance was extended to 90%. Differently, Emeville et al. (2014) has detected a relevant association with a really significant P-value, equivalent to 0.001, in individuals of a Caribbean population.

Non-white ethnicity (self-reported) revealed a significant association with PCa (P-value 0.03). The group that had the highest frequency of non-whites representatives was the case group. The results also indicate that non-white individuals have 2.06 times the chance of developing the disease. The prevalence of PCa varies with geographic location and ethnic composition of the evaluated populations (Berber et al., 2014). Some works have related ethnicity to PCa in certain populations (Kwon et al., 2011; Berber et al., 2013; Emeville et al., 2014; Cao et al., 2015). Jamaicans and Caribbeans of African descent have the highest incidences of PCa in the world; which may be attributed in part to heredity (Facina, 2016). In most researches, black men were more likely to develop PCa compared to non-black men (Kheirandish and Chinegwundoh, 2011).

Cigarette consumption was statistically related with PCa in the analyzed sample (P-value < 0.01), with a 2.16-fold chance of developing the disease for individuals who had smoked. The findings acquired here corroborate to the researchers conducted by Huncharek et al. (2010), who have reported the same linkage. The possible explanation for this association would be that exposure to toxic substances in smoke, such as polycyclic aromatic hydrocarbons, increases the risk of mutations (Leitzmann and Rohrmann, 2012).

Another risk factor associated with PCa was the history of STIs (P-value 0.05). Individuals who reported STIs present 2.07 times the chances of having this type of cancer. Studies by Cheng et al. (2010) found an association between STD and PCa, while Caini et al. (2014) research have pointed to gonorrhea as the STI most related to the tumor. Although, the mechanisms of relationship between emergence of PCa and STDs still require further research to be elucidated. Regarding vasectomy variable, the results revealed a

protective association with PCa (P-value < 0.001). This relation was not found by many studies, being absent in Ganesh et al. (2011) and Tyagi et al. (2010)'s researches. It is possible that, concerned to other studied variables, vasectomy has a frequency more influenced by the stochasticity of a small sample.

Considering populations from northeastern Brazil, research on molecular epidemiology involving GSTs and cancer genes was performed by Arruda et al. (1998), Gattás et al. (2004), Silva et al. (2014), and Santos (2017).

Arruda et al. (1998) analyzed the possible relationship of different ethnic groups (including blacks) and the prevalence of *GSTM1* and *GSTT1* with environmental carcinogenesis in populations from different Brazilian regions, including Salvador, Bahia. In this study, the authors have verified the prevalence of null genotype for *GSTM1* among Caucasians (55%), followed by Brazilian blacks (33%), and homogenous distribution of *GSTT1* among Caucasians and African descendants. Gattás et al. (2004) while studying different ethnicities (identified by the interviewer) from São Paulo and Salvador, Bahia, have not identified an association between *GSTM1* and *GSTT1* with cancer risk (cancer in general, not specifically PCa). However, these authors have detected a significantly higher frequency of *GSTM1* null genotype among whites than non-whites (mulattoes and blacks) in the São Paulo group.

Silva et al. (2014) evaluated the association between *GSTM1* and *GSTT1* genes with upper aerodigestive tract cancer in Ilhéus and Itabuna (State of Bahia). They encountered frequencies of null *GSTM1* greater in the case group than in the control, and null *GSTT1* among controls than in the cases; however, no statistical significance was found. Santos (2017) conducted a research on the association between *GSTM1* and *GSTT1* gene polymorphisms with the susceptibility and prognosis of PCa in Piauí, in Northeast Brazil. Both null *GSTM1* and null *GSTT1* demonstrated to be statistically associated with the presence of PCa, being the *GSTM1* null genotype as protective and null *GSTT1* as a risk factor; although, when both genotypes were analyzed in association, there was no statistical significance.

This is the first study on molecular epidemiology and risk factors for PCa in the southwest region of Bahia. The different association of variables among the populations can be explained due to their particular characteristics. Because of this variation, the study of gene polymorphisms and their relation with cancer should be performed in specific populations. Moreover, our study confirms putative factors for PCa that other publications have revealed, though we recommend additional studies to confirm these findings, due to sampling limitations in our investigation.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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