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Original Article

GSTM1 gene expression and copy number variation in prostate cancer patients—Effect of chemical exposures and physical activity

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Abstract

Background: Many etiological factors have been related to prostate cancer (CaP) development, progression, and survival, such as age, population origin, geographic area, occupational exposures, and nutrition and lifestyle factors. However, physical activity affords health benefits to cancer patients, including those with CaP. Glutathione S-Transferases enzymes have been linked to CaP because of their role in the detoxification of a wide variety of potential carcinogens, steroid hormones and xenobiotics. Among the different glutathione S-transferases isoforms, null genotype for *GSTM1* has been associated with an increased risk of CaP, although data are controversial. As the relationship between copy number variation and gene expression of *GSTM1* in CaP remains unexplored, this study analyzed *GSTM1* gene expression and/or dosage effect on CaP risk and aggressiveness. The potential protective role of physical activity was also explored.

Methods: Three hundred and seventeen patients (159 non-CaP and 158 CaP) were recruited from the Service of Urology (Hospital Virgen de las Nieves, Granada, Spain) over the period 2012 to 2014 and were followed-up until January 2018 to ensure a correct classification of control and patients. Individuals were classified in each group based on histological analysis of tissue biopsy, along with data on PSA level, Gleason score and T stage in patients with biopsies positive for CaP. Individuals with a negative biopsy were considered as controls. All controls underwent a systematic 20-core ultrasound guided biopsy in order to limit the false negative rate. Genomic DNA was extracted from peripheral blood to determine the exact copy numbers of *GSTM1*, and RNA was extracted from prostate tissue samples to determine *GSTM1* gene expression. Both analyses were performed using the qPCR method. A questionnaire was administered to all patients to assess environmental exposures, lifestyle, and physical activity. The association of GSTM1 copy number variation and expression with the rest of variables was assessed by chi-square test and the Mann-Whitney test. Multiple logistic regression was used to assess which factors were associated with the risk of CaP.

Results: The presence of 1 or 2 copies of the *GSTM1* gene was not less prevalent in CaP compared to non-CaP patients; however, a significant decreased *GSTM1* gene expression was observed in CaP tissue relative to non-CaP tissue ($P = 0.003$). CaP patients with environmental exposure to dust and smoke, and smoking habit had a significantly decreased *GSTM1* gene expression (and near-significantly decreased for living in urban areas) as compared to non-CaP patients with the same exposures. In addition, physical activity was significantly associated with a lower risk of CaP ($P = 0.006$) and with increased *GSTM1* gene expression ($P = 0.002$).

Conclusions: A reduced *GSTM1* gene expression in prostate tissue was observed in CaP patients with some environmental chemical exposures. Intriguingly, physical activity might play a protective role against CaP development, possibly as a result of increasing *GSTM1* gene expression in prostate tissue. However, this observation warrants further confirmation.

Keywords: Prostate cancer; *GSTM1*; CNV; Gene expression; Physical activity

1. Introduction

Risk factors involved in the development of prostate cancer (CaP) are extraordinarily diverse, and include

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chemicals found in occupational and environmental settings, such as pesticides, cadmium, rubber industry chemicals, dust, smoke, and nutritional products, among others [1]. GST isoforms are known phase II enzymes involved in the metabolism of carcinogens (harmful electrophilic compounds), steroids, and several anticancer drugs; and their activities have been implicated as a drug resistance mechanism. Among the 8 classes of GST enzymes, GSTM1, GSTT1, and GSTP1 show genetic polymorphisms leading eventually to nonfunctional enzymes [2]. In particular, GSTM1 and GSTT1 isoforms have been reported to play a protective role against the development of cancer at different sites, such as oral, colorectal, lung, breast, and prostate [3–5]. While *GSTM1* and *GSTT1* null genotypes may contribute to CaP development, GSTM1 has proven to be a stronger candidate risk factor [6]. Individual variations in the catalytic activity of GSTM1, among other GST isoforms, contribute to regulate the clearance of toxic metabolites and might be partially responsible for individual variation in CaP progression, thus having a prognostic value [7]. So far, there is limited information about the *GSTM1* gene-dosage effect on cancer development, that is, whether having 2 alleles affords greater protection than having 1 or none. If so, individuals with null genotype for *GSTM1* would be at an increased risk for developing cancer, although there are controversial data among different populations. A large meta-analysis showed that the *GSTM1* null genotype was significantly associated with CaP among Eurasians and Americans, but not among Europeans and Africans [6]. Furthermore, as *GSTM1* gene expression is down-regulated in human and murine CaP [8], it is important to characterize not only the genetic variation in *GST* genes but also their gene expression in CaP tissue.

GST isoforms have been studied in relation to several environmental exposures [9]; however, data concerning lifestyle habits are scarce and suggest that nutrition and physical activity play a protective role in CaP. Dietary recommendations to prevent CaP generally involve a greater intake of vegetables and fruits, and a reduced intake of red meat and saturated fat. Furthermore, physical activity has been reported to play a protective effect on CaP by modulating hormone levels, preventing obesity, enhancing immune function, and reducing oxidative stress [10,11]. Epidemiological and clinical studies have shown that higher levels of exercise are associated with a 10% to 30% decrease in the incidence of CaP [12].

This study sought to determine the *GSTM1* gene-dosage effect on gene expression, CaP development, and CaP aggressiveness as well as the contribution of environmental exposures and physical activity to the risk of CaP in Spanish patients. GSTM1 was selected over other GST polymorphisms because separate meta-analyses reported that GSTM1 has been proven as a stronger CaP candidate risk factor [6,13,14]. Furthermore, *GSTM1* gene has been found underexpressed in CaP [15]. To the best of our knowledge, this is the first study assessing the association between *GSTM1* gene expression and physical activity in CaP patients.

2. Materials and Methods

2.1. Study population

The study population consisted of 317 Spanish males of Caucasian origin (mean age 68.4 ± 8.6 years) with prostate-specific antigen (PSA) values >4 ng/ml subjected to prostate biopsy. Data were collected over the period 2012 to 2014; and patients were followed-up until January 2018. *GSTM1* gene expression was studied in fresh prostate tissue samples and *GSTM1* CNV genotyping was performed in blood samples.

All study subjects were informed and provided a written informed consent before being enrolled. The study was approved by the Research Ethics Committee of Granada following Helsinki ethical declaration.

2.2. Prostate tissues

Fresh prostate tissue was obtained by needle biopsy from all men enrolled in the study. Prostate tissue samples were subjected to pathological examination in the Anatomic Pathology Service of Hospital Virgen de las Nieves (Granada, Spain). CaP was pathologically diagnosed in 158 patients, whereas 159 subjects were free from malignant cells in prostate biopsies. The subgroup of CaP patients was further classified according to Gleason score, PSA level, and T stage. Individuals with a negative biopsy were considered as controls, who then underwent a systematic 20-core ultrasound guided biopsy in order to reduce the false negative rate. A small amount of prostate tissue was stored at −80°C immediately after removal for gene expression analysis.

2.3. GSTM1 copy number and gene expression

GSTM1 CNV was measured in DNA extracted from blood samples using the Real Pure Extraction DNA SSS kit (Durviz, Valencia, Spain) and DNA was quantified using NanoDrop spectrophotometer. *GSTM1* copy number was determined using a TaqMan Gene Copy Number Assay (Hs03947236_cn, Applied Biosystems, Foster City, CA). RNase P gene was run in the same quantitative PCR (qPCR) as a reference gene to determine the target gene in each sample. qPCR data were exported into the CopyCaller Software (Life Technologies).

For the *GSTM1* gene expression assay, RNA was extracted from prostate tissue samples by using TRIzol reagents. RNA concentration and quality were measured with a NanoDrop spectrophotometer. One hundred nanograms of total RNA was retrotranscribed into cDNA with QuantiTect Reverse Transcription Kit (Qiagen) and a SYBR Green Real-Time PCR experiment was carried out.

Variations in cDNA content were normalized to a reference gene (*GAPDH*). The primer sequences used for *GSTM1* were 5′-GGACGCCTTCCCAAATCTGA-3′ (forward) and 5′-GCTGAGTATGGGCTCCTCAC-3′ (reverse); and for *GADPH* were 5′-ATCACCATCTTCCAGGAGCGAGA-3′ (forward) and 5′-CATGGTTCACACCCATGACGAACA-3′ (reverse). The fold change in gene expression was estimated using the $2^{-\Delta\Delta Ct}$ method [16].

Gene expression analysis was performed in triplicate by the relative quantitation method and the Applied Biosystems 7500 Real-Time PCR Systems. For the CNV assay, blood samples were amplified in triplicate on the Applied Biosystems 7900HT Fast Real-Time PCR System.

2.4. Environmental exposures and lifestyle collection

A detailed epidemiological questionnaire was administered to the study population (CaP patients and non-CaP patients) by urologists to collect information on lifestyle and occupational and environmental risk factors. Lifestyle factors examined included alcohol and smoking habits (with indication of grams of alcohol use per week and pack-years of tobacco smoking), eating habits and physical exercise. Occupational risk factors included workplace exposure to hazardous chemicals (agriculture, livestock, construction industry, and chemical industry) and lifetime years devoted to these activities. Regarding environmental risk factors, these included exposure to airborne dust particles (dust, dirt, soot, liquid droplets from road traffic or domestic heating systems emitted into the air and suspended in the atmosphere, silica, wood dust, talc containing asbestiform fibers), smoke (from gasoline or diesel fumes emitted into the air, coal tar fumes, bitumen fumes, fire, combustion emissions, tobacco smoke), other chemicals present in the environment or used in homes, such as, pesticides, fertilizers, household cleaning products, organic solvents, dyes, paints, chemicals used in wood treatment, varnish, metal elements (lead, cooper, zinc, iron…), and radiation (ionizing radiation, ultraviolet radiation). Then, these exposures were treated as dichotomous variables (i.e., environmental exposure to dust and smoke) or categorical variables (i.e., exposure to pesticides, exposure to other chemical agents, or lack of exposure to any hazardous chemical). Information on clinical staging parameters, such as Tumor, Node, and Metastasis classification and Gleason score, was also gathered.

2.5. Statistical analysis

The Mann-Whitney test was used to test the association of *GSTM1* CNV with disease status, Gleason score (codified as <7 or ≥7) and T staging (1–4). The relative changes in *GSTM1* gene expression across *GSTM1* genotypes, as well as by CaP status, environmental exposures, and physical activity were also assessed by the Mann-Whitney test. Differences in *GSTM1* gene expression across physical activity categories were assessed by the Kruskall-Wallis test. Multiple logistic regression analysis was used to assess which factors might be determinants of CaP risk. Models were adjusted for *GSTM1* gene expression, *GSTM1* CNV, PSA levels, and physical activity categories. Odds ratios and 95% confidence intervals were calculated and the criterion for significance was set at $P < 0.05$. All analyses were performed using the SPSS v.20 statistical package.

3. Results

The general characteristics of the study population are shown in Table 1. The frequency of null genotype of *GSTM1* gene (homozygous deletion, that is, 0 copies) was similarly distributed between CaP and non-CaP patients (51.3% vs. 50.3%, respectively). Individuals carrying the *GSTM1* null genotype had a significantly lower *GSTM1* gene expression in prostate tissue than those having 1 or 2 copies ($P = 0.000166$; Fig. 1). Also, CaP patients showed a significantly lower gene expression of *GSTM1* than non-CaP patients ($P = 0.003$). No significant differences were observed between CaP and non-CaP patients in relation to the diverse environmental exposures assessed; however, CaP patients reported significantly less physical activity than non-CaP individuals (data not shown). *GSTM1* gene expression in prostate tissue varied according to chemical exposures and physical activity. In particular, CaP patients with a number of chemical exposures had lower *GSTM1* gene expression than non-CaP patients (Fig. 2), with differences being statistically significant for exposure to environmental dust and smoke ($P = 0.042$) and for tobacco smoking habit ($P = 0.005$). Near-significant differences were observed for individuals living in urban areas with >10,000 inhabitants ($P = 0.060$). No statistically significant

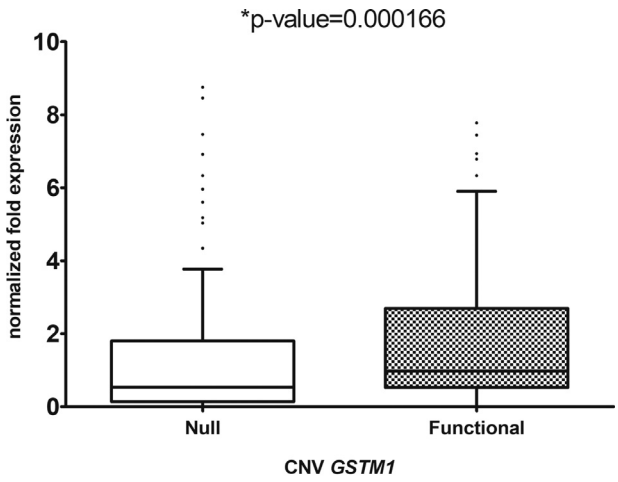


Fig. 1. Box plot of GSTM1 gene expression in individuals carrying null and functional (1 or 2 copies) genotype of GSTM1. CNV were measured by relative quantification using qPCR technique. The statistical analysis has been performed using Mann-Whitney test.

association was observed between gene-dosage effect of *GSTM1* with clinical aggressiveness of CaP, as measured by Gleason score ($P = 0.601$) and T staging system ($P = 0.822$). Intriguingly, a significantly increased *GSTM1* gene expression was observed in individuals reporting more physical activity ($P = 0.002$), with gene expression being greater as long as the level of physical activity was higher ($P = 0.004$; Fig. 3).

Table 2 shows the results of the multiple logistic regression analysis adjusted for potential risk and protective factors. Relevant associations were found for PSA levels (as individuals with ≥10 ng/ml had almost 2.3 more risk of having CaP), and for physical activity (as the risk of CaP risk decreased by almost half in patients reporting more than 7 h/wk of physical activity).

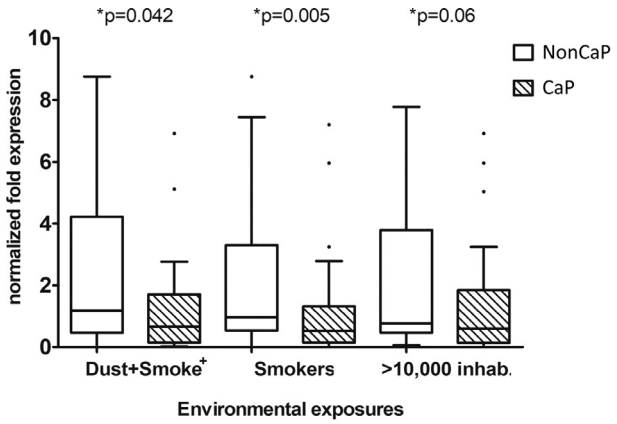
4. Discussion

To the best of our knowledge, this is the first study addressing the association of *GSTM1* genetic polymorphism and gene expression with the risk of CaP. Although we have found differences in *GSTM1* gene expression among patients carrying 1 or 2 copies of *GSTM1* functional allele, the number of copies was not linked to the risk of CaP as no differences were found between null genotype for *GSTM1* and functional genotype (irrespective of having 1 or 2 copies) in relation to the occurrence of CaP. This finding does not support a gene-dosage protective effect among our CaP patients. In contrast, 2 meta-analysis found an increased risk of CaP among individuals carrying the null genotype for *GSTM1* [8,17]. The multifactorial origin

Table 1
Characteristics of the study population.

	Non-CaP, *n* (%)	CaP, *n* (%)	*P* value[a]
Age			0.011
<50	1 (0.6)	3 (1.9)	
50–59	33 (20.8)	19 (11.9)	
60–69	65 (40.9)	58 (36.5)	
70–79	53 (33.3)	57 (35.9)	
>80	7 (4.4)	22 (13.8)	
GTSM1 polymorphism			0.865
GSTM1 null	79 (50.3)	80 (51.3)	
GSTM1 functional	78 (49.7)	76 (48.7)	
Smoking habit			0.634
Never smokers	47 (34.3)	42 (31.6)	
Smokers	90 (65.7)	91 (68.4)	
Alcohol consumption			0.701
Never drinkers	107 (79.9)	106 (77.9)	
Current drinkers	27 (20.1)	30 (22.1)	
Residence area			0.629
Rural (<10,000 inhabitants)	47 (33.8)	41 (31.1)	
Urban (>10,000 inhabitants)	92 (66.2)	91 (68.9)	
Environmental exposure to dust and smoke			0.703
No	63 (48.5)	59 (46.1)	
Yes	67 (51.5)	69 (53.9)	
Exposure to other environmental agents			0.566
No	59 (44.4)	56 (42.4)	
Other chemicals	46 (34.6)	41 (31.1)	
Pesticides	28 (21.0)	35 (26.5)	
Physical Activity			0.028
No	41 (37.3)	65 (54.2)	
Low level	22 (20.0)	21 (17.5)	
High level	47 (42.7)	34 (28.3)	
PSA level			<0.001
<10	112 (70.0)	86 (53.4)	
10–20	46 (28.8)	36 (22.4)	
≥20	2 (1.2)	39 (24.4)	
T stage			–
T1	–	63 (63.6)	
T2	–	28 (28.3)	
T3 and T4	–	8 (8.1)	
Gleason score			–
<7	–	80 (50.3)	
≥7	–	79 (49.7)	

[a] Chi-square test.

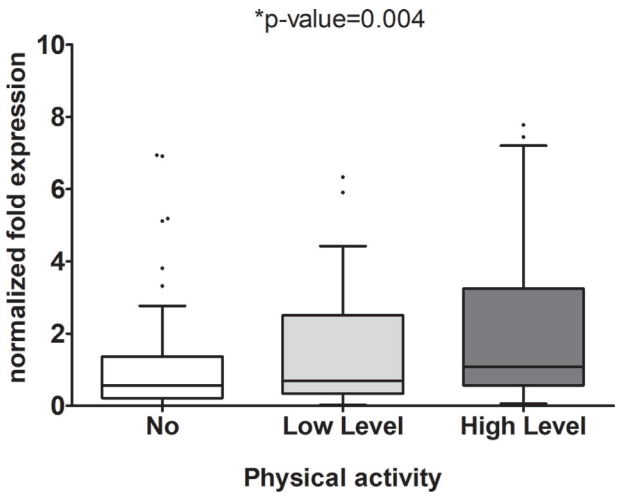


*Mann-Whitney test
+Environmental dust and smoke

Fig. 2. Box plot of GSTM1 gene expression in non-CaP and CaP patients with a number of chemical exposures (environmental dust and smoke, smoking habit, and living in urban areas with >10,000 inhabitants). Expression levels were measured by relative quantification using qPCR technique. The statistical analysis has been performed using Mann-Whitney test.

of CaP, including distinct environmental and dietary exposures across populations, may account for the different functional impact of *GSTM1* CNV on CaP.

The controversial data regarding the expression of GSTs and CaP development may be related to genetic, environmental, and dietary factors, which may differ across populations. Since GST superfamily members are detoxifying enzymes involved in the metabolization of numerous chemical carcinogens, differences in the inter- and intrapopulation distribution of genetic polymorphisms encoding enzymes from those classes, and in the exposure profile to



*Kruskal-Wallis test

Fig. 3. Dose-response relationship between *GSTM1* gene expression and different categories of physical exercise ("no": without physical activity; "moderate": up to 6 hours per week; "high": ≥7 hours per week). GSTM1 expression showed statistically significant differences across the 3 categories of physical exercise. Expression levels were measured by relative quantification using qPCR technique. The statistical analysis has been performed using Kruskal-Wallis test.

Table 2
Multiple logistic regression analysis of CaP risk adjusted for several factors.

Predictors	OR	95% CI	*P*
GSTM1 gene expression[a]	0.92	0.81–1.04	0.158
GSTM1 CNV	1.07	0.58–1.99	0.823
PSA	2.29	1.23–4.27	0.009
Physical activity	0.57	0.31–1.04	0.068

GSTM1 CNV: 0: null genotype; 1: functional genotype. PSA: 0: <10 ng/ml; 1: ≥ 10 ng/ml. Physical activity: 0: < 7 h/wk; 1: ≥ 7 h/wk.
[a] Fold change.

environmental or dietary chemicals across populations, may partially account for the controversy on the role of GSTs and risk of CaP [18]. In addition, the factors mentioned above can contribute to increase heterogeneity in the meta-analyses conducted so far. In relation to environmental exposures, *GSTM1* genetic polymorphism failed to be significantly associated with exposure to airborne dust, smoke, pesticides, and alcohol in CaP patients. Conversely, exposure to these and other contaminants (e.g., diesel exhaust and agricultural chemicals) has been previously associated with CaP [9]. Conflicting data have also been reported for the relationship between *GSTM1* null genotype and cancer aggressiveness in several tumors; however, in our study, *GSTM1* polymorphism was not significantly associated with cancer aggressiveness defined by clinical stage at diagnosis or Gleason score, which is in line with other studies [19–21].

As expected, CaP patients with 1 and 2 copies of *GSTM1* had an increased gene expression relative to patients lacking any copy. In addition, *GSTM1* gene expression was lower in CaP than in non-CaP, as also found other studies [22,23]. However, the most relevant finding in our study was the decreased *GSTM1* gene expression in CaP patients exposed to various environmental chemicals, which was not observed in patients without CaP. This finding is in agreement with a previous study utilizing gene expression array technology [15] where *GSTM1* was identified as one of the few genes underexpressed in CaP, leading to a significant reduction in the detoxification capability of cells. On the other hand, hypermethylation of the promoter region of the *GSTP1* gene has been observed in CaP tissue but not in healthy tissue, thus leading to protein underexpression. While this gene is methylated in about 70% to 80% of CaP cases, it is usually hypomethylated in benign prostatic hyperplasia [24]. The lower expression of GST proteins in CaP is considered detrimental, as either endogenous or exogenous reactive molecules could not be properly detoxified, thus contributing to cancer progression.

The association found in this study between physical activity and a lower risk of CaP (Table 2) supports previous findings [25,26], although a recent meta-analysis suggested no obvious association between leisure-time activity and risk of CaP [27]. However, this study reports for the first

time an increased *GSTM1* gene expression in relation to physical activity (Fig. 3). A positive trend was observed as the higher level of physical activity (≥7 hours per week) the greater gene expression. Although emerging data provide strong evidence of a lesser risk of CaP following physical activity, the molecular and cellular pathways associating physical activity and CaP still remains unknown. It has been suggested that physical activity (in particular aerobic exercise of moderate-to-high intensity) might reduce the risk of CaP and disease progression by favorably altering the immune function and signaling pathways relevant to carcinogenesis, including oxidative stress [26,28]. In fact, reduced levels of oxidative stress may be a potential mechanism through which physical activity protect against CaP, or delay CaP progression. Magbanua et al. [29] reported that vigorous physical activity modulated the Nuclear factor-erythroid 2-related factor 2 (Nrf2)-mediated oxidative stress response pathway. Moreover, acute aerobic exercise elicits activation of nuclear Nrf2, regardless of the exercise intensity [30]. Nrf2 is an important stress-responsive transcription factor that is activated during oxidative stress [31]. Once activated, Nrf2 upregulates antioxidant and anti-inflammatory cellular responses, leading to the expression of a number of detoxifying phase II enzymes, including glutathione S-transferases isoenzymes [30,32]. Therefore, the association found in this study between physical activity and a lower risk of CaP can partially be explained by inducing the expression of antioxidant enzymes such as GSTM1.

The differences found between non-CaP and CaP patients represent one limitation of this study. These differences can be accounted for the difficulty in finding appropriate fresh prostate tissue samples from control individuals lacking CaP. Accordingly, we collected fresh tissue samples from men who underwent a biopsy, which was negative for CaP and failed to develop any clinical symptom of CaP, unless until the latest clinical follow-up (January 2018). This represents the major strength of our study as the periodic clinical follow-up of the study population enables an accurate diagnosis for both control and CaP patients.

5. Conclusions

In summary, our study suggests that some environmental factors (i.e., exposure to airborne dust and smoke, tobacco-smoking habit, and to a lesser extent living in urban areas with >10,000 inhabitants) are linked to the occurrence of CaP, thus underpinning the contribution of environmental factors to the risk of CaP. Importantly, these associations were not observed in individuals lacking CaP but with the same type of exposures. Notably, the most relevant finding of this study was the apparent protective role played by physical activity against CaP development. The lower risk of CaP in individuals with greater physical activity might result from the induction of metabolic enzymes and up-regulation of the Nrf2 pathway as an attempt to counteract the adverse effects of reactive molecules and oxidative stress linked to environmental exposures. Overall, GSTM1 appears to afford protection against CaP in individuals exposed to certain environmental factors.

Conflict of interest

Authors declare that they have no conflict of interest.

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References

[1] Bidgoli SA, Jabari N, Zavarhei MD. Prostate-specific antigen levels in relation to background factors: are there links to endocrine disrupting chemicals and AhR expression? Asian Pac J Cancer Prev 2014;15:6121–5.

[2] Safarinejad MR, Shafiei N, Safarinejad SH. Glutathione S-transferase gene polymorphisms (GSTM1, GSTT1, GSTP1) and prostate cancer: a case-control study in Tehran, Iran. Prostate Cancer Prostatic Dis 2011;14:105–13. https://doi.org/10.1038/pcan.2010.54.

[3] Duggan C, Ballard-Barbash R, Baumgartner RN, Baumgartner KB, Bernstein L, McTiernan A. Associations between null mutations in GSTT1 and GSTM1, the GSTP1 Ile(105)Val polymorphism, and mortality in breast cancer survivors. Springerplus 2013;2:450. https://doi.org/10.1186/2193-1801-2-450.

[4] Ntais C, Polycarpou A, Ioannidis JP. Association of GSTM1, GSTT1, and GSTP1 gene polymorphisms with the risk of prostate cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2005;14:176–81.

[5] Rotunno M, Lam TK, Vogt A, Bertazzi PA, Lubin JH, Caporaso NE, et al. GSTM1 and GSTT1 copy numbers and mRNA expression in lung cancer. Mol Carcinog 2012;51:E142–50. https://doi.org/10.1002/mc.21890.

[6] Malik SS, Kazmi Z, Fatima I, Shabbir R, Perveen S, Masood N. Genetic polymorphism of GSTM1 and GSTT1 and risk of prostatic carcinoma—a meta-analysis of 7,281 prostate cancer cases and 9,082 healthy controls. Asian Pacific J Cancer Prev 2016;17:2629–35. https://doi.org/10.7314/APJCP.2016.17.5.2629.

[7] Acevedo CA, Quiñones LA, Catalán J, Cáceres DD, Fullá JA, Roco AM. Impact of CYP1A1, GSTM1, and GSTT1 polymorphisms in overall and specific prostate cancer survival. Urol Oncol Semin Orig Investig 2014;32:280–90. https://doi.org/10.1016/j.urolonc.2013.05.010.

[8] Gong M, Dong W, Shi Z, Xu Y, Ni W, An R, et al. Genetic polymorphisms of GSTM1, GSTT1, and GSTP1 with prostate cancer risk: a meta-analysis of 57 studies. PLoS One 2012;7:e50587. https://doi.org/10.1371/journal.pone.0050587.

[9] Ahluwalia M, Kaur A. Modulatory role of GSTT1 and GSTM1 in Punjabi agricultural workers exposed to pesticides. Environ Sci Pollut Res 2018: 1–6. https://doi.org/10.1007/s11356-018-1459-7.

[10] Torti DC, Matheson GO. Exercise and prostate cancer. Sport Med 2004;34:363–9. https://doi.org/10.2165/00007256-200434060-00003.

[11] Young-McCaughan S. Potential for prostate cancer prevention through physical activity. World J Urol 2012;30:167–79. https://doi.org/10.1007/s00345-011-0812-y.

[12] Ballon-Landa E, Parsons JK. Nutrition, physical activity, and lifestyle factors in prostate cancer prevention. Curr Opin Urol 2017: 1. https://doi.org/10.1097/MOU.0000000000000460.

[13] Cao D-L, Ye D-W, Dai B, Zhang H-L, Shen Y-J, Zhu Y, et al. Association of glutathione S-transferase T1 and M1 polymorphisms with prostate cancer susceptibility in populations of Asian descent: a meta-analysis. Oncotarget 2015;6:35843–50. https://doi.org/10.18632/oncotarget.5346.

[14] Cai Q, Wang Z, Zhang W, Guo X, Shang Z, Jiang N, et al. Association between glutathione S-transferases M1 and T1 gene polymorphisms and prostate cancer risk: a systematic review and meta-analysis. Tumour Biol 2014;35:247–56. https://doi.org/10.1007/s13277-013-1030-6.

[15] Chetcuti A, Margan S, Mann S, Russell P, Handelsman D, Rogers J, et al. Identification of differentially expressed genes in organ-confined prostate cancer by gene expression array. Prostate 2001;47:132–40. https://doi.org/10.1002/pros.1056.

[16] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods 2001;25:402–8. https://doi.org/10.1006/meth.2001.1262.

[17] Zhao Y, Deng X, Song G, Qin S, Liu Z. The GSTM1 null genotype increased risk of gastric cancer: a meta-analysis based on 46 studies. PLoS One 2013: 8. https://doi.org/10.1371/journal.pone.0081403.

[18] Lampe JW. Diet, genetic polymorphisms, detoxification, and health risks. Altern Ther Health Med 2007;13:S108–11.

[19] Agalliu I, Langeberg WJ, Lampe JW, Salinas CA, Stanford JL. Glutathione S-transferase M1, T1, and P1 polymorphisms and prostate cancer risk in middle-aged men. Prostate 2006;66:146–56.

[20] Kwon DD, Lee JW, Han DY, Seo IY, Park SC, Jeong HJ, et al. Relationship between the glutathione-S-transferase P1, M1, and T1 genotypes and prostate cancer risk in Korean subjects. Korean J Urol 2011;52:247–52. https://doi.org/10.4111/kju.2011.52.4.247.

[21] Lima MM, Oliveira MNL, Granja F, Trindade ACG, de Castro Santos LEM, Ward LS. Lack of association of GSTT1, GSTM1, GSTO1, GSTP1 and CYP1A1 polymorphisms for susceptibility and outcome in Brazilian prostate cancer patients. Folia Biol (Praha) 2008;54:102–8:FB2008A0017 [pii].

[22] Butler MW, Hackett NR, Salit J, Strulovici-Barel Y, Omberg L, Mezey J, et al. Glutathione S-transferase copy number variation alters lung gene expression. Eur Respir J 2011;38:15–28. https://doi.org/10.1183/09031936.00029210.Glutathione.

[23] Bostwick DG, Meiers I, Shanks JH. Glutathione S-transferase: differential expression of alpha, mu, and pi isoenzymes in benign prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma. Hum Pathol 2007;38:1394–401. https://doi.org/10.1016/j.humpath.2007.02.008.

[24] Martignano F, Gurioli G, Salvi S, Calistri D, Costantini M, Gunelli R, et al. GSTP1 methylation and protein expression in prostate cancer: diagnostic implications. Dis Markers 2016;2016:4358292. https://doi.org/10.1155/2016/4358292.

[25] Shephard RJ. Physical activity and prostate cancer: an updated review. Sport Med 2016: 1–19. https://doi.org/10.1007/s40279-016-0648-0.

[26] Wekesa A, Harrison M, Watson RW. Physical activity and its mechanistic effects on prostate cancer. Prostate Cancer Prostatic Dis 2015;18:197–207. https://doi.org/10.1038/CaPn.2015.9.

[27] Liu F, Wang J, Wu H-L, Wang H, Wang J-X, Zhou R, et al. Leisure time physical activity and risk of prostate cancer: a dose-response meta-analysis. Minerva Urol Nefrol 2018;70:152–61. https://doi.org/10.23736/S0393-2249.17.02874-0.

[28] Thomas RJ, Kenfield SA, Jimenez A. Exercise-induced biochemical changes and their potential influence on cancer: a scientific review. Br J Sports Med 2017;51:640–4. https://doi.org/10.1136/bjsports-2016-096343.

[29] Magbanua MJM, Richman EL, Sosa EV, Jones LW, Simko J, Shinohara K, et al. Physical activity and prostate gene expression in men with low-risk prostate cancer. Cancer Causes Control 2014;25:515–23. https://doi.org/10.1007/s10552-014-0354-x.

[30] Done AJ, Newell MJ, Traustadóttir T. Effect of exercise intensity on Nrf2 signalling in young men. Free Radic Res 2017;51:646–55. https://doi.org/10.1080/10715762.2017.1353689.

[31] Nguyen T, Sherratt PJ, Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. Annu Rev Pharmacol Toxicol 2003;43:233–60. https://doi.org/10.1146/annurev.pharmtox.43.100901.140229.

[32] Ho HK, White CC, Fernandez C, Fausto N, Kavanagh TJ, Nelson SD, et al. Nrf2 activation involves an oxidative-stress independent pathway in tetrafluoroethylcysteine-induced cytotoxicity. Toxicol Sci 2005;86:354–64. https://doi.org/10.1093/toxsci/kfi205.