Exposure to environmental pollutants and genetic variants related to oxidative stress and xenobiotic metabolism—Association with prostate cancer

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ABSTRACT: This study assessed whether genetic variants coding for certain enzymes involved in xenobiotic detoxification, antioxidant defences and DNA repair, along with exposure to environmental chemicals, were associated with an increased prostate cancer (PCa) risk. The study population consisted of 300 men (150 PCa cases and 150 controls) which underwent prostate biopsy as their serum prostate specific antigen (PSA) levels were greater than 4 ng/ml. Genetic variants in *GSTM1, GSTP1, SOD2*, *CAT*, *GPX1*, *XRCC1* were determined and data for chemical exposures was obtained through a structured questionnaire and by biomonitoring in a subsample of cases and controls. High serum PSA levels were associated with a greater risk of PCa, while physical exercise appears to exert a protective effect against its development. In addition, elevated urinary levels of certain organic pollutants, such as benzo(a)pyrene (BaP), bisphenol A (BPA), and ethyl-paraben (EPB), were associated with an increased risk of PCa.

Keywords: Genetic Polymorphisms; Prostate cancer; Environmental exposure; Biomonitoring, Organic pollutants

Abbreviations: BaP, Benzo[*a*]pyrene; BPA, Bisphenol-A; CAT, Catalase; CNV, DNA copy number variations; CI, Confidence interval; EDCs, Endocrine disrupting chemicals; EDTA, Ethylenediaminetetraacetic acid; EPB, Ethylparaben; GPX, Glutathione peroxidase; GST, Glutathione S-transferase; HWE, Hardy-Weinberg equilibrium; N, Number; NPh, Naphthalene; OR, Odds Ratio; PAHs, polycyclic aromatic hydrocarbons; PCa, Prostate cancer; PCR, Polymerase chain reaction; PSA, Prostate specific antigen; ROS, Reactive oxygen species; SNP, Single nucleotide polymorphism; SOD, Superoxide dismutase; TNM, Tumour, Node, Metastasis Cancer staging system; XME, Xenobiotic metabolizing enzymes; XRCC1, X-ray repair cross-complementing 1.

1. Introduction

Prostate cancer (PCa) is one of the most common cancers among men in Europe and the third leading cause of cancer-related mortality (Cirne et al., 2022). Numerous risk factors have been associated with PCa development, with non-modifiable factors, such as age, family history, genetic factors, and ethnicity being the best known. PCa, along with nasopharyngeal cancer, showed the fastest increasing trend in early-onset cancers from 1990 to 2019, and this trend was related to changes in diet, lifestyle, and pollution (Zhao et al., 2023). However, the burden of the disease can only be reduced by acting on modifiable risk factors (e.g., obesity, smoking, alcohol intake, sedentarism, and dietary habits) which are less known (Bergengren et al., 2023; Cirne et al., 2022). Furthermore, chronic exposures to low levels of environmental chemicals present in air, water, soil, dust, or consumer products have been associated with PCa risk (Cohen and Jefferies, 2019; Iwasaki et al., 2023), particularly in occupational settings such as farmers and fire fighters, which are exposed to agriculture chemicals and combustion byproducts, respectively (Krstev and Knutsson, 2019; Pardo et al., 2020).

Exposure to environmental carcinogens produces excess oxidative stress that may cause changes in the DNA base sequence, damage tumour suppressor genes, enhance the expression of proto-oncogenes and induce malignant transformation of cells (Lavender et al., 2015). Reactive oxygen species (ROS) generated endogenously or exogenously (e.g., as a result of smoking, alcohol consumption, inadequate diet, excessive physical stress, or environmental pollution) are usually neutralized by complex antioxidant mechanisms in healthy organisms. Antioxidant enzyme systems are pivotal to protect cells from increased ROS production and only when these defense systems are overwhelmed, oxidative stress and subsequent oxidative damage occur. Due to their high reactivity, excess production of ROS and other free radicals

may damage cells by oxidation of cell membranes, modification of proteins, and changing the DNA structure. All these events may drive cellular malignant transformation capable of initiating prostate carcinogenesis (Drozdz-Afelt et al., 2022).

The major antioxidant defense system of the body consists of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). Glutathione S-transferase (GST) is also considered as a crucial antioxidant enzyme that catalyzes the formation of glutathione S-conjugates between GSH and certain electrophilic substrates (Singh and Reindl, 2021). A decrease in SOD activity has been reported in PCa patients, indicative of an altered antioxidant system (Drozdz-Afelt et al., 2022).

Inherited differences in the activity of xenobiotic metabolizing enzymes (XME) have been described as a major factor in genetic susceptibility to cancer because of deficiencies in environmental carcinogen detoxification pathways. An increased risk of PCa has been reported in individuals with a null genotype for *GSTM1* (Benabdelkrim et al., 2018). On the other hand, exposure to environmental chemicals may produce DNA damage which if not repaired may increase the risk of PCa (Koutros et al., 2011). DNA repair gene variants may thus play an important role in the development of different types of cancer, especially environmentally induced cancer (Mishchuk-Reka et al., 2020). *XRCC1* (X-ray repair cross-complementing group 1) is a component of the base excision repair pathway of damaged DNA during replication or because of exposure to environmental agents or ionizing radiation (Park et al., 2009). Polymorphic variants of this gene (e.g., *XRCC1* Arg399Gln polymorphism, rs25487) may impact the protein functionality and increase the risk of cancer (Mishchuk-Reka et al., 2020). Although previous studies reported that *XRCC1* rs25487 may be a prognostic and predictive biomarker for susceptible men, the available evidence is restricted only to Asians (Noureddini et al., 2018), but not to Caucasians (Alvarez-Gonzalez et al., 2023).

Exposure to environmental organic pollutants, assessed through biomonitoring of phenols and parabens in urine samples has been reported as risk factors of PCa in US men (NHANES data 2005–2015) (Alwadi et al., 2022). Besides, higher urinary levels of bisphenol A (BPA), an endocrine disrupting chemical (EDC) ubiquitously present in the environment, have been observed in PCa patients as compared to controls, suggesting that urinary BPA levels may have prognostic value for PCa (Fang et al., 2022). Polycyclic aromatic hydrocarbons (PAHs) are pollutants widely distributed in the environment that induce toxic effects through genotoxic metabolites, which are generated by *CYP1A1* and oxidative stress pathways (Ranjit et al., 2016). Long-term exposure to PAHs has been associated with cancer at different sites, such as skin, lungs, bladder, etc. (Sampaio et al., 2021). In a previous study, we found higher urinary levels of benzo[*a*]pyrene (BaP) and BPA in patients with bladder cancer compared to controls,

thus supporting a relationship between exposure to these pollutants and cancer risk (Martín-Way et al., 2022).

Since tumors are complex diseases that usually involve the concurrence of multiple risk factors, in particular environmental agents and genetic mutations, a beneficial modification of lifestyle factors and minimizing exposure to environmental pollutants may contribute to reduce the risk of developing PCa, or slow its progression, increase survival and decrease disease recurrence (Pernar et al., 2018). This study therefore assessed the potential association of genetic variants in enzymes coding for antioxidant defenses, XME, and DNA repair as well as exposure to environmental pollutants with PCa and its aggressiveness in an attempt to gain information on possible non-invasive biomarkers of exposure and susceptibility.

2. Material and methods

2.1. Study population

Participants in this study consisted of 300 men aged (43–91 years) with suspicion of PCa that met the criteria for prostate biopsy (prostate specific antigen (PSA) levels ≥4.0 ng/ml) and recruited in Urology outpatient Service (Hospital Universitario Virgen de las Nieves-HUVN, Granada, Spain) between 2012 and 2014, and between 2018 and 2023 (Table 1). All individuals underwent a systematic 20-core ultrasound guided biopsy for PCa diagnosis to limit the false negative rate. Those with a positive biopsy for PCa (n=150) were analyzed for T stage and Gleason score and classified according to the D′Amico risk group classification (low, intermediate, and high risk) regarding tumour aggressiveness. Individuals with negative biopsy (n=150) were considered as controls. Study participants agreed to complete a structured epidemiological questionnaire to assess environmental exposures (detailed in Section 2.3). A sub-sample of individuals (30 patients and 30 controls from the cohort recruited between 2018 and 2023) provided urine samples for biomonitoring of environmental pollutants. Prior to recruitment, subjects provided written informed consent after being informed of the study objectives and that they had the opportunity to withdraw from the research at any time without consequences. This study was approved by the Research Ethics Committee of the Granada Centre (CEI-Granada internal codes 1638-N-18 and 1503-M2–20) according to the Ethical Declaration of Helsinki.

2.2. DNA isolation and genotyping assays

A peripheral blood sample was obtained from all individuals, collected in EDTA-coated tubes, and stored frozen at -20 ºC until genomic DNA extraction. 300 µL of blood were used for DNA extraction using the RealPure SSS" DNA Extraction Kit (Durviz, Spain). Buccal swabs were collected from individuals from whom blood samples were lacking and stored frozen at -20 ºC until genomic DNA extraction. In these samples, DNA was extracted according to the method described by Gómez-Martín, et al. (2015) and based on salt extraction techniques. DNA purity and concentration were assessed by fluorescence using Qubit™ 3.0 (Invitrogen™ from Thermo Scientific, USA), and the nanodrop 2000 system (Thermo Scientific, USA). The ratio of absorbance at 260/280 nm was used as a quality control for DNA purity. The extracted DNA was stored at -20 ºC until genotyping analysis.

Six gene variants of genes encoding XME, cellular antioxidant defenses, and DNA-repair enzymes were selected based on the findings from a prior meta-analysis that investigated the association of gene variants related to oxidative stress, xenobiotic detoxification, and DNA repair enzymes, with the risk of PCa (Álvarez-González et al., 2023). These genes included *GPX1* (rs17650792), *SOD2* (rs4880, encoding Mn-SOD), *CAT* (rs1001179), *GSTM1* CNV, *GSTP1* (rs1695), and *XRCC1* (rs25487); see Supplementary Table 1 for probe details. Fig. 1 maps the role of the enzymes encoded by these genes against cellular stressors potentially involved in prostate carcinogenesis. DNA genotyping was performed using TaqMan® Genotyping Master Mix (Applied Biosystems, USA), which contained all essential components (except probes, templates, and water) for polymerase chain reaction (PCR). Quantitative PCRs (qPCRs) were performed in the QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems™) and analysed with the software QuantStudio™ Real-Time PCR v1.3.1. Supplementary Table 2 shows the manufacturer's thermal cycling conditions. A validation assay using Sanger sequencing was conducted on a randomly selected 3% of the genotyped samples for each individual SNP.

2.2. Data collection of lifestyle and environmental factors

A detailed epidemiological questionnaire was administered to the study participants by urologists during outpatient urology consultations. Information on lifestyle factors (including smoking, alcohol consumption, and physical exercise); and exposure to occupational and environmental factors (such as dust, smoke, pesticides, and other chemicals potentially associated with PCa) was collected, as reported elsewhere (Martínez-González et al., 2020). This information was collected in a dichotomous manner (yes/no) except for physical exercise,

which was assessed as weekly hours dedicated to this activity and then coded as a binary variable: < 6 h/week or ≥ 6 h/week.

2.4. Urinary levels of organic pollutants

The concentration of 18 environmental pollutants, including 4 parabens (methyl-, ethyl-, propyl- and butyl-paraben), 4 bisphenols (BPA, BPAF, BPF and BPS), and 10 PAHs (naphthalene, acenaphthene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo(a) pyrene, benzo(e)pyrene and indene) was determined in urine samples using the analytical procedure described elsewhere (Vela-Soria et al., 2014) with minor modifications (Martín-Way et al., 2022). Briefly, this consisted of the simultaneous extraction of those analytes from 4 ml of urine samples to which α-naphthol was added as internal standard. Subsequently, urine samples were subjected to enzymatic digestion with β-glucuronidase and sulfatase and extracted using an efficient liquid–liquid microextraction method. The extract was derivatized with a mixture of 50% N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), 25% pyridine and 25% acetic acid and then analyzed by gas chromatography–mass spectrometry (GC–MS). The instrumental analysis was performed on an Agilent 6890 series gas chromatograph system coupled to a 5973-mass spectrometer using a HP-5MS capillary column. GC-MS data acquisition was performed using selected Ion Monitoring (SIM) mode.

2.5. Statistical analysis

The association of PCa with sociodemographic data, lifestyles, gene variants, environmental and occupational exposures, and indicators of PCa aggressiveness was analyzed by chi-square test ($\chi 2$) or Fisher exact tests for small sample size. Logistic regression analysis (unadjusted and adjusted) was used to assess the independent effect of each of the genetic and environmental factors studied on PCa risk. Odds Ratios (OR) and 95% confidence intervals (95% CI) were calculated and the criterion for significance was set at $p < 0.05$. The urinary levels of the chemical pollutants studied were compared by cancer status (PCa and controls) and by the selected genetic variants, sociodemographic factors, lifestyle and chemical exposures using the non-parametric Kruskal-Wallis test. All analyses were performed using SPSS v.28 statistical package (IBM Corporation, USA). Genotype analyses, Hardy–Weinberg equilibrium (HWE) and Linkage disequilibrium (LD) analyses were performed using the online SNPStats software (Solé et al., 2006). SNPs were considered to be in Linkage disequilibrium (LD) when they had an r^2 value > 0.5.

3. Results

The main features of the study population stratified by PCa and controls are shown in Table 1. Statistically significant differences were observed for age, PSA, physical exercise, and usual work as a farmer, and near-significant differences for exposure to smoke and/or dust. As compared to controls, PCa individuals were significantly older, had PSA levels above 10 ng/ml, performed less physical exercise and were more likely exposed to smoke and/or dust and worked in agriculture.

Table 1

From the 6 genetic variants analysed (*GSTM1* CNV, *GSTP1* (rs1695), *SOD2* (rs4880), *GPX1* (rs17650792), *CAT* (rs1001179), and *XRCC1* (rs25487), only the rs4880 in *SOD2* gene showed statistically significant differences, with G allele showing a protective role against PCa, while carriers of AA genotype had an increased risk of PCa. Near-significant differences were observed for *GPX1* and *XRCC1*, with carriers of G allele of *GPX1* and of T allele of *XRCC1* showing an increased risk of PCa (Table 2).

Table 2

We further examined the association between the gene variants studied according to clinical parameters of tumour aggressiveness (serum PSA level, Gleason score, TNM stage and D'Amico risk classification) (Table 3). Statistically significant differences were found only for *CAT* and near-significant associations for *CAT*, *GPX1* and *GSTM1*. Carriers of T allele of *CAT* had a greater odds of having a PSA level above 10 ng/ml, a Gleason score above 7; and less odds of having a high score in Amico risk classification. Carriers of A allele of the *GSPX1* polymorphism had a higher odds of having a PSA levels above 10 ng/ml and carriers of the null genotype for *GSTM1* had a near-significantly greater chance of having an advanced-stage tumour (T3/T4).

Table 3

The results of multiple logistic regression analyses performed to identify predictors of PCa risk are shown in Table 4. When the regression model was adjusted for the covariates that were statistically significant (or near-significant) in the crude analysis shown in Tables 1 and 2, only PSA levels ≥10 ng/ml and performing physical exercise ≥ 6 h/week remained significant in the model. Despite using a backward selection process to find the optimal combination of predictors, the above two variables were retained as significant risk and protective factors, respectively.

Table 4

Of the 18 pollutants analysed in urine samples only three showed levels above the detection limit (BaP, BPA and ethyl-paraben -EPB-). The urinary concentration of these pollutants was compared by PCa status, sociodemographic factors, lifestyles, environmental exposures, usual work activity and the gene variants studied (Table 5).

Table 5

Significant higher levels of BaP, BPA and EPB were observed in individuals with PCa compared to controls. In addition, BaP levels were significantly greater in those who performed physical exercise and worked in the construction industry. Regarding BPA, significantly higher levels were observed in individuals exposed to dust (as well as to those exposed to dust and/or smoke) and other chemical agents (e.g., pesticides, asbestos, etc), near-significantly higher levels in those who worked in agriculture and a near-significant inverse association with alcohol consumption. Likewise, significantly higher levels of BPA were observed in carriers of T allele of the *XRCC1* rs25487 and near-significantly in carriers of T allele of *CAT* rs1001179. In relation to EPB, individuals with BMI < 25 kg/m2 had significantly greater levels as compared to those with BMI between 25 and 30 kg/m^2.

Table 6 shows the results of the multiple logistic regression analysis to identify potential predictors of PCa risk in the subsample of individuals with data for urinary concentrations of pollutants. Models were adjusted for the covariates that were found statistically significant in the multiple logistic regression analysis shown in Table 4 (i.e., PSA above or below 10 ng/ml and performance of physical exercise). Interestingly, the three pollutants (BaP, BPA and EPB) were associated with an increased risk of having PCa, as were PSA levels above 10 ng/ml. Conversely, the performance of physical exercise failed to be significantly associated with a lower PCa risk. The significant associations found remained when a backward selection procedure was used with minimal changes in the size of the effect.

Table 6

4. Discussion

This study assessed the association of certain genetic factors and environmental exposures with PCa risk. We found that higher urinary levels of BaP, BPA, and EPB were associated with an increased risk of PCa. However, no clear association was observed with the genetic variants studied, albeit *CAT* appears to play a role in the aggressiveness of PCa.

The crude analysis of factors potentially related to PCa showed significant associations with age, PSA levels above 10 ng/ml, performance of physical exercise and agricultural job. These findings are consistent with previous studies indicating an increased risk associated with

advanced age (Daniyal et al., 2014; Belkahla et al., 2022) and elevated PSA levels, which on the other hand increase with age (David and Leslie, 2024), and a protective effect of physical activity, which may reduce the risk of PCa (Liu et al., 2011), as occurs with cancer at other sites. The relationship of age with PCa risk may be related to the increase in ROS that occurs with aging, which can lead to a greater number of mutations in DNA and contribute to the development of the disease (Sikka, 2003). According to the American Cancer Society, serum levels of PSA greater than 10 ng/ml increase the chance of having PCa by more than 50 % (ACS, 2021), whereas our study found a more than 3-fold increased risk. The association between agriculture and PCa risk has also been reported in other studies (Koutros et al., 2013; Togawa et al., 2021; Ragin et al., 2013), and it is probably due to exposure to certain environmental factors in agricultural settings.

The performance of regular physical activity has a protective effect on PCa risk, disease progression, and mortality, while the underlying mechanisms are not yet defined (Brookman-May et al., 2019). Several mechanisms have been proposed for the potentially beneficial effects of exercise on prostate carcinogenesis, including reduction in oxidative stress, improvement in the expression of anti-inflammatory markers, and activation of proapoptotic pathways (Capece et al., 2020). An increased gene expression of XME, such as GSTM1 in prostate tissue, has also been claimed, as it contributes to the neutralization of ROS and other reactive molecules (Gómez-Martín et al., 2019).

Antioxidant enzymes like SOD, GPx and GST work together in the neutralization of harmful and highly-reactive molecules like ROS. Functional SNPs of these and other enzymes involved in redox homeostasis can disrupt the pro-oxidant-antioxidant balance, leading to accumulation of ROS and further oxidative damage (Djokic et al., 2022). In our study, genetic factors such as SNPs in *SOD2* (rs4880), *GPX1* (rs17650792) and *XRCC1* (rs25487) were significantly or near-significantly associated with PCa in the crude analysis; however, these associations no longer remained significant in the multivariate-adjusted analysis. The missense substitution A16V (C47>T) may allow greater access of *SOD2* to the mitochondrial matrix, which results in an increased catalytic activity thus enhancing antioxidant defenses (Bastaki et al., 2006). Anyway, in a recent meta-analysis, we found a modest increased risk for this variant (OR: 1.08 [95% CI: 1.01–1.15]) (Álvarez-González et al., 2023). As for T allele in *XRCC1* (rs25487), our previous meta-analysis found no significant association with PCa risk (Álvarez-González et al., 2023). Changes in DNA repair genes can lead to variations in the precision of DNA repair processes, and persistence of unrepaired DNA damage would result in genetic instability eventually leading to unregulated prostate cell growth and carcinogenesis. Regarding *GPX1* (rs17650792), the gene product is part of the enzymatic antioxidant defense, preventing

oxidative damage to DNA, proteins and lipids by detoxifying hydrogen and lipid peroxides that may contribute to PCa development (Erdem et al., 2012). Again, our previous meta-analysis found no significant association between *GPX1* (rs1050450) and PCa risk (Álvarez-González et al., 2023).

The biomonitoring of 18 organic pollutants measured in this study found detectable levels of BPA and DEP, two well-known EDCs, and BaP, one of the best-known carcinogenic PAH. These three organic pollutants were significantly elevated in urine of individuals with PCa compared to controls. Since EDC can interfere with hormonal pathways and the body's hormonal balance, these chemicals may play a role in the development of hormone-dependent cancers, including PCa (Pellerin et al., 2021; Lacouture et al., 2022). Furthermore, these pollutants have also been associated with an increased risk of other genitourinary tumours, such as bladder cancer (Martín-Way et al., 2022; Pellerin et al., 2021). The association of BPA with PCa is consistent with previous studies (Tarapore et al., 2014; Salamanca-Fernández et al., 2021). While exposure to parabens (methyl, propyl and to a lower extent, ethyl-paraben) has been associated with an increased risk of lung cancer (Mao et al., 2023), to the best of our knowledge no association has been reported so far with PCa. Parabens and their metabolites may interfere the synthesis of steroid hormones, which may afford biological plausibility to the development of hormone-dependent cancers (Bessonneau et al., 2021), like PCa. Parabens have been also linked to telomer shortening and further genomic instability, ultimately increasing the risk of cancer, as occurs with breast cancer (Finot et al., 2017).

In our study, higher urinary levels of BPA were observed in individuals exposed to various environmental chemicals, such as dust and other chemical agents, as well as in those working in agriculture. BPA is a common industrial component widely used in plastics, resins and other consumer products (Ribeiro et al., 2017; Li et al., 2010). Workers handling pure BPA are considered to have the highest risk of occupational exposure (Heinälä et al., 2017). The greater exposure to BPA might increase the risk of hormone-dependent tumours, and high concentrations of this chemical have been shown to drive PCa cells toward hormone refractoriness (Bilancio et al., 2017). BPA was the only organic pollutant showing associations with the gene variants studied. Significantly greater urinary levels of BPA were found in individuals with the dominant genotype of *XRCC1* (rs25487) for T allele, which is in line with a previous study (Zhou et al., 2021). BPA has been shown to increase ROS levels and suppress DNA repair, which could have important implications for susceptibility to genetic damage and risk of disease, including cancer (Gassman et al., 2015).

As for BaP, significantly increased levels were observed in individuals performing physical exercise and in those who work in the construction sector. Since BaP is a well-known air

pollutant, present in tobacco smoke and vehicle and factory emissions, the more time spent outdoors or in areas with higher BaP concentrations, such as exercising in urban environments or in areas with higher pollution levels, the greater the exposure to BaP (Tainio, 2015; Annesi-Maesano, 2017), as captured by urine biomonitoring. The same applies to individuals working in construction, who are more exposed to this pollutant while performing their job tasks (McCormick et al., 2022). Mechanistically, BaP has been shown to exert effects on proliferation, DNA strand breaks and mutagenic activity in prostate cells (PC-3) (Gao et al., 2020), thus supporting an association between BaP exposure and impaired prostate cell health.

This study has some limitations that could affect the interpretation of the results. First, the subsample size of individuals who contributed urine samples to measure organic pollutants levels limits the representativeness of the results, which cannot be extrapolated to the general population. In addition, since the pollutants studied were assessed at the same time as PCa diagnosis, the lack of temporal concordance prevents drawing causal conclusions. Furthermore, the pollutants studied were not evaluated in terms of accumulation over time, which may limit the full understanding of the long-term exposure of individuals to these substances. Notwithstanding that, this study provides additional evidence for understanding the association of the environmental factors assessed with PCa risk. Future studies are needed to replicate and strengthen these findings.

5. Conclusion

This study found that exposure to BaP, BPA and EPB was associated with PCa, suggesting that these organic pollutants might be considered as chemical risk factors. Furthermore, physical activity had a protective role in PCa risk. However, none of the genetic variants studied can be considered as genetic risk factors for PCa in our study population. Future research into genetic factors should be performed using massive genomic analyses, rather than focusing solely on a few individual genes. Data from these studies should be integrated with alterations in whole-transcriptome analysis following exposure to the studied chemicals to better understand how altered expression of genetic variants by these chemicals contributes to PCa. Moreover, this study supports the implementation of preventive interventions aimed at reducing exposure to several pollutants, which would contribute to reduce the prevalence of PCa.

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CRediT authorship contribution statement

Beatriz Álvarez-González: Data curation, Investigation, Methodology, Writing – original draft. Antonio F. Hernandez: Conceptualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. Alberto Zafra-Gómez: Formal analysis, Methodology, Validation. Luis Javier Martínez-González: Conceptualization, Funding acquisition, Project administration, Supervision. María Jesús Álvarez-Cubero: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft. Fernando Vázquez-Alonso: Conceptualization, Investigation, Resources. Lucia Chica-Redecillas: Data curation, Formal analysis, Investigation, Methodology. Sergio Cuenca-López: Data curation, Formal analysis, Investigation, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors do not have permission to share data.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2024.104455.

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Figure Captions

Fig. 1. Summary of the physiological role of gene variants encoding xenobiotic metabolising enzymes (XME), antioxidant defence systems and DNA repair systems at cellular level.

Table 1
Characteristics of the study population and comparison of PCa cases and controls by sociodemographic features, lifestyles, environmental exposures and employment.

	PCa n (%)	Control n (%)	OR (95% CI)	*p*-value*
Age (mean ± SD)	70.7 ± 9.1	67.7 ± 9.6	-	0.003
PSA (ng/ml)	74 (40.9%)	107	Ref.	<0.001
< 10	74 (63.8%)	(59.1%)	2.55	
>10		42 (36.2%)	(1.56–4.12)	
BMI (kg/m^2)				0.576
<25	22 (48.9%)	23 (51.1%)	Ref.	
25–30	43 (42.2%)	59 (57.8%)	0.76	0.449
			(0.38–1.54)	
>30	38 (49.4%)	39 (50.6%)	1.02	0.961
			(0.49–2.13)	
Lifestyles				
Smoking habit				0.287
No	46 (52.3%)	42 (47.7%)	Ref.	
Yes	30 (42.3%)	41 (57.7%)	0.67	0.209
			(0.36–1.25)	
Former smoker	55 (53.9%)	47 (46.1%)	1.07	0.820
			(0.60–1.89)	
Alcohol consumption				0.382
No	92 (52.9%)	82 (47.1%)	Ref.	
Yes	41 (47.1%)	46 (52.9%)	0.79	
			(0.47–1.33)	
Physical exercise				0.028
No (<6 h/week)	64 (59.8%)	43 (40.2%)	Ref.	
Yes (≥ 6 h/week)	62 (45.6%)	74 (54.4%)	0.56	
			(0.34–0.94)	
Environmental exposures				
Smoke				0.168
No	86 (47.5%)	95 (52.5%)	Ref.	
Yes	39 (57.4%)	29 (42.6%)	1.49	
			(0.85–2.61)	
Dust				0.142
No	63 (46%)	74 (54%)	Ref.	
Yes	62 (55.4%)	50 (44.6%)	1.46	
			(0.88–2.41)	
Smoke and/or dust				0.075
No	54 (44.3%)	68 (55.7%)	Ref.	
Yes	70 (55.6%)	56 (44.4%)	1.57	
			(0.95–2.60)	
Other chemical agents				0.454
No	78 (48.4%)	83 (51.6%)	Ref.	
Yes	47 (53.4%)	41 (46.6%)	1.22	
			(0.73–2.05)	
Employment				
Mining				0.578
No	126 (49.8%)	127 (50.2%)	Ref.	
Yes	1 (33.3%)	2 (66.7%)	0.50	
			(0.05–5.63)	
Yes	1 (33.3%)	2 (66.7%)	0.50	
			(0.05–5.63)	
Agriculture				0.050
No	90 (46.2%)	105 (53.8%)	Ref.	
Yes	37 (60.7%)	24 (39.3%)	1.80	
			(1.00–3.23)	
Construction				0.270
No	105 (48.2%)	113 (51.8%)	Ref.	
Yes	22 (57.9%)	16 (42.1%)	1.48	
			(0.74–2.97)	
Industry				0.291
No	122 (50.4%)	120 (49.6%)	Ref.	
Yes	5 (35.7%)	9 (64.3%)	0.55	
			(0.18–1.68)	

Table 2
Distribution and comparative analysis of the gene variant studied in PCa cases and controls.

	PCa n−150 (%)	Control n−150 (%)	OR (95% CI)	*p*-value[a]
GSTM1 CNV				0.296
Null	80 (53%)	71 (47%)	Ref.	
Functional	68 (46.9%)	77 (53.1%)	0.78 (0.50–1.24)	
GSTP1 rs1695				0.654
AA	65 (47.4%)	72 (52.6%)	0.73 (0.36–1.50)	0.395
AG	64 (51.2%)	61 (48.8%)	0.85 (0.41–1.76)	0.661
GG	21 (55.3%)	17 (44.7%)	Ref.	
AG + GG vs AA	65 (47.4%) vs 85 (52.1%)	72 (52.6%) vs 78 (47.9%)	1.21 (0.77–1.90)	0.417
AG + AA vs GG	21 (55.3%) vs 129 (49.2%)	17 (44.7%) vs 133 (50.8%)	0.79 (0.40–1.56)	0.488
SOD2 rs4880				**0.031**
GG	38 (49.4%)	39 (50.6%)	0.56 (0.30–1.05)	0.070
AG	58 (45.3%)	70 (54.7%)	0.48 (0.27–0.84)	0.010
AA	54 (63.5%)	31 (36.5%)	Ref.	
AG + AA vs GG	38 (49.4%) vs 112 (52.6%)	39 (50.6%) vs 101 (47.4%)	1.14 (0.68–1.92)	0.627
AG + GG vs AA	54 (63.5%) vs 96 (46.8%)	31 (36.5%) vs 109 (53.2%)	0.51 (0.30–0.85)	**0.010**
GPX1 rs17650792				0.120
AA	62 (60.2%)	41 (39.8%)	1.99 (1.01–3.94)	**0.047**
AG	62 (51.2%)	59 (48.8%)	1.39 (0.72–2.68)	0.332
GG	22 (43.1%)	29 (56.9%)	Ref.	
AG + GG vs AA	62 (60.2%) vs 84 (48.8%)	41 (39.8%) vs 88 (51.2%)	0.63 (0.39–1.04)	**0.069**
AG + AA vs GG	22 (43.1%) vs 124 (55.4%)	29 (56.9%) vs 100 (44.6%)	1.64 (0.89–3.02)	0.117
CAT rs1001179				0.410
TT	10 (62.5%)	6 (37.5%)	1.38 (0.48–3.95)	0.554
CT	43 (47.8%)	47 (52.2%)	0.76 (0.45–1.26)	0.278
CC	97 (54.8%)	80 (45.2%)	Ref.	0.202
CT + CC vs TT	10 (62.5%) vs 140 (52.4%)	6 (37.5%) vs 127 (47.6%)	0.66 (0.23–1.87)	0.436
CT + TT vs CC	97 (54.8%) vs 53 (50%)	80 (45.2%) vs 53 (50%)	0.83 (0.51–1.34)	0.434
XRCC1 rs25487				0.151
CC	50 (47.6%)	55 (52.4%)	0.82 (0.40–1.71)	0.599
CT	76 (60.3%)	50 (39.7%)	1.38 (0.67–2.81)	0.383
TT	21 (52.5%)	19 (47.5%)	Ref.	
CT + TT vs CC	50 (47.6%) vs 97 (58.4%)	55 (52.4%) vs 69 (41.6%)	1.55 (0.95–2.53)	**0.082**
CT + CC vs TT	21 (52.5%) vs 26 (54.5%)	19 (47.5%) vs 105 (45.5%)	1.09 (0.55–2.13)	0.811

The genotype frequency of normal control group met the Hardy-Weinberg equilibrium and SNPs were not in linkage disequilibrium (LD).

[a] Chi-squared test (χ^2).

Table 3
Effects of the genetic variants studied on serum levels of PSA and clinical parameters of PCa aggressiveness.

Variants	Genotype	PSA Level		p-value*	Gleason score		p-value*	T Stage (TNM)		p-value*	D'Amico risk classification			p-value*
		<10 ng/ml	≥10 ng/ml		<7	≥7		T1-T2	T3-T4		Low	Medium	High	
GSTM1	Null	42 (57.5%)	37 (50.7%)	0.407	33 (51.6%)	46 (56.1%)	0.585	42 (52.5%)	8 (88.9%)	**0.072**	22 (53.7%)	25 (52.1%)	33 (56.9%)	0.879
	Functional	31 (42.5%	36 (49.3%)		31 (48.4%)	36 (43.9%)		38 (47.5%)	1 (2.6%)		19 (46.3%)	23 (47.9%)	25 (43.1%)	
GPX1	AA+AG	56 (78.9%)	66 (90.4%)	**0.054**	52 (83.9%)	70 (85.4%)	0.805	69 (86.3%)	8 (100%)	0.588	34 (82.9%)	38 (80.9%)	51 (89.5%)	0.439
	GG	15 (21.1%)	7 (9.6%)		10 (16.1%)	12 (14.6%)		11 (13.8%)	0 (0%)		7 (17.1%)	9 (19.1%)	6 (10.5%)	
CAT	CT+TT	20 (27%)	32 (43.2%)	**0.039**	17 (26.2%)	35 (42.2%)	**0.043**	29 (35.4%)	2 (22.2%)	0.713	10 (23.8%)	22 (45.8%)	21 (35.6%)	**0.093**
	CC	54 (73%)	42 (56.8%)		48 (73.8%)	48 (57.8%)		53 (64.6%)	7 (77.8%)		32 (76.2%)	26 (54.2%)	38 (64.4%)	
SOD	AG+GG	44 (59.5%)	51 (68.9%)	0.230	43 (66.2%)	52 (62.7%)	0.659	51 (62.2%)	6 (66.7%)	0.999	28 (66.7%)	30 (62.5%)	37 (62.7%)	0.898
	AA	30 (40.5%)	23 (31.1%)		22 (33.8%)	31 (37.3%)		31 (37.8%)	3 (33.3%)		14 (33.3%)	18 (37.5%)	22 (37.3%)	
GSTP1	AG+GG	46 (62.2%)	38 (51.4%)	0.184	38 (58.5%)	46 (55.4%)	0.711	51 (62.2%)	5 (55.6%)	0.729	26 (61.9%)	26 (54.2%)	32 (54.2%)	0.695
	AA	28 (37.8%)	36 (48.6%)		27 (41.5%)	37 (44.6%)		31 (37.8%)	4 (44.4%)		16 (38.1%)	22 (45.8%)	27 (45.8%)	
XRCC1	CT+TT	52 (71.2%)	44 (61.1%)	0.198	45 (70.3%)	51 (63.0%)	0.353	53 (67.1%)	5 (55.6%)	0.489	28 (66.7%)	31 (67.4%)	37 (63.8%)	0.919
	CC	21 (28.8%)	28 (38.9%)		19 (29.7%)	30 (37.0%)		26 (32.9%)	4 (44.4%)		14 (33.3%)	15 (32.6%)	21 (36.2%)	

* Chi-square test (or Fisher's exact test for small-sized samples).

Table 4
Multiple logistic regression analysis of PCa risk a.

Variable	Full model[a]			Backwards variable selection[b]		
	OR	95% CI	*p-value*	OR	95% CI	*p-value*
Age (years)	1.02	0.99 – 1.06	0.219			
PSA (≥ 10 *vs.* < 10 ng/ml)	2.96	1.50 – 5.85	0.002	3.43	1.80 – 6.54	<0.001
Physical exercise (≥ 6 *vs.* <6 h/wk)	0.55	0.29 – 1.05	0.069	0.55	0.30 – 1.03	0.060
Working in Agriculture (Yes *vs.* No)	1.44	0.66 – 3.13	0.358			
Exposure to smoke and/or dust (Yes *vs.* No)	1.35	0.72 – 2.54	0.351			
SOD2 rs4880 (AG+GG *vs.* AA)	0.63	0.32 – 1.26	0.192			
GPX1 rs17650792 (G *vs* AA)	0.82	0.43 – 1.58	0.556			
XRCC1 rs25487 (T *vs.* CC)	1.27	0.67 – 2.42	0.458			

[a] The full model was adjusted for all the explanatory variables that showed a statistically significant association in the crude analysis depicted in Tables 1 and 2

[b] Method used for selecting predictor variables in logistic regression models

Table 5
Association between urinary levels of BaP, BPA and EPB with PCa and socio-demographic, lifestyles, environmental exposures, job and genetic factors.

	Benzo(a)pyrene (BaP) Median (IQR)	Bisphenol A (BPA) Median (IQR)	Ethyl-paraben (EPB) Median (IQR)
Cancer			
Yes (n=30)	66.7 (37.0–94.3) **	20.0 (13.1–27.4) **	22.9 (4.7–48.3)*
No (n=30)	22.8 (17.8–45.6)	10.0 (3.6–15.4)	12.0 (1.5–21.5)
Age			
< 70 (n=25)	33.0 (21.9–61.6)	13.7 (3.7–17.9)	12.8 (1.0–25.9)
≥ 70 (n=35)	40.7 (16.7–74.6)	15.0 (9.5–22.9)	12.3 (2.8–39.0)
PSA (ng/ml)			
< 10 (n=34)	36.5 (21.6–63.3)	13.8 (8.20–17.9)	12.6 (1.0–25.7)
≥ 10 (n=26)	39.1 (15.3–78.4)	18.7 (7.3–23.3)	13.7 (3.3–42.6)
BMI (kg/m2)			
< 25 (n=5)	37.4 (11.0–89.4)	31.5 (5.7–37.5)	41.9 (12.9–48.0) *
25–30 (n=18)	44.7 (20.8–68.7)	13.7 (7.1–16.8)	6.4 (1.0–16.3)
>30 (n=21)	32.7 (19.1–46.8)	15.2 (7.3–18.0)	13.3 (4.1–26.8)
Physical exercise			
≥ 6 h/week (n=37)	53.5 (27.4–82.1) **	13.7 (8.9–23.9)	13.3 (3.7–40.5)
< 6 h/week (n=22)	27.0 (17.6–42.8)	16.0 (3.7–21.9)	12.4 (1.0–28.1)
Tobacco consumption			
Yes (n=37)	34.5 (21.4–53.9)	13.7 (6.8–19.4)	13.3 (4.1–44.8)
No (n=23)	61.4 (19.7–93.2)	16.0 (8.3–25.9)	9.2 (1.2–21.2)
Alcohol consumption			
Yes (n=48)	35.9 (21.3–67.0)	13.7 (7.6–18.1)*	12.6 (1.9–26.1)
No (n=12)	51.1 (12.8–116.1)	26.6 (10.4–39.5)	19.2 (2.4–37.8)
Exposure to Smoke			
Yes (n=21)	53.5 (28.2–68.1)	15.2 (7.9–21.0)	11.1 (1.5–43.0)
No (n=37)	34.5 (19.4–70.7)	13.8 (6.8–22.2)	12.8 (2.0–26.8)
Exposure to Dust			
Yes (n=27)	40.7 (23.0–63.8)	16.9 (12.8–26.0)*	15.5 (4.3–41.9)
No (n=31)	35.9 (20.8–92.4)	10.3 (3.8–17.9)	11.5 (1.2–27.2)
Exposure to Smoke and/or Dust			
Yes (n=31)	49.2 (31.3–74.6)	15.9 (11.6–25.9) #	18.5 (4.3–44.8)
No (n=26)	33.8 (19.3–63.9)	10.0 (3.7–18.0)	10.8 (1.0–22.1)
Exposure to other chemical agents			
Yes (n=22)	51.8 (30.4–68.1)	17.2 (11.9–29.1)*	15.1 (1.2–45.7)
No (n=36)	34.5 (19.3–70.8)	13.5 (4.4–18.1)	12.4 (3.0–27.0)
Work activity in Agriculture			
Yes (n=17)	47.0 (38.0–66.0)	19.6 (13.1–26.0) #	21.2 (1.4–43.4)
No (n=42)	33.8 (17.8–70.6)	13.7 (5.7–19.3)	11.9 (2.4–25.7)
Work activity in Construction			
Yes (n=7)	54.3 (49.2–89.6) *	19.2 (10.3–26.0)	18.5 (9.3–46.9)
No (n=52)	35.2 (19.3–67.4)	14.3 (7.3–21.2)	11.9 (1.4–29.9)
Work activity in Industry			
Yes (n=5)	68.1 (10.5–130.6)	17.4 (1.8–39.6)	11.1 (3.6–33.1)
No (n=54)	38.0 (21.0–65.9)	14.3 (8.1–21.6)	12.6 (1.5–31.7)
GSTM1			
Null (n=36)	43.7 (20.9–70.8)	14.9 (8.7–20.1)	12.6 (1.9–35.3)
Functional (n=24)	35.2 (19.2–59.6)	13.5 (5.7–25.2)	13.6 (1.6–26.6)
GSTP1			
AG+GG (n=33)	38.5 (22.1–83.9)	15.0 (8.8–22.4)	13.3 (1.2–40.5)
AA (n=27)	34.5 (18.2–61.4)	13.7 (5.5–19.7)	12.4 (2.8–24.9)
SOD2			
AG+GG (n=32)	36.7 (19.1–64.9)	13.8 (7.3–20.1)	12.8 (1.9–26.1)
AA (n=21)	48.2 (28.7–92.4)	17.7 (11.7–27.3)	23.3 (4.9–50.3)
GPX1			
AG+GG (n=32)	36.7 (18.4–63.2)	13.6 (4.2–27.7)	14.9 (1.1–29.9)
AA (n=21)	35.9 (30.9–81.5)	14.5 (10.0–20.0)	12.3 (4.8–41.3)
CAT			
CT+TT (n=22)	41.3 (19.1–75.6)	17.5 (11.4–26.6) #	13.7 (4.7–25.7)
CC (n=37)	38.5 (21.0–68.1)	13.4 (6.8–19.4)	12.4 (1.4–37.3)
XRCC1			
CT+TT (n=32)	41.8 (17.3–68.1)	17.2 (12.7–25.6) **	11.3 (1.1–33.4)
CC (n=27)	34.5 (21.1–70.9)	9.5 (3.2–17.9)	15.0 (3.8–35.6)

U Mann-Whitney test and Kruskal-Wallis test: #p<0.1
IQR: Interquartile range (25–75th percentiles)

Table 6
Multiple logistic regression analysis of PCa risk in the subsample of individuals for whom urinary data for pollutants were available [a].

Variable	Full model (All explanatory variables)[b]			Backwards variable selection[b]		
	OR	95% CI	*p-value*	OR	95% CI	*p-value*
PSA (≥10 *vs.* <10 ng/ml)	1.55	1.04 – 2.97	0.031	1.49	1.04 – 2.12	0.029
Physical exercise (≥ 6 *vs.*<6 h/wk)	0.16	0.01 – 3.91	0.257			
Benzo(a)pyrene	1.12	1.03 – 1.22	0.009	1.09	1.03 – 1.15	0.002
Bisphenol A	1.32	1.09 – 1.59	0.004	1.28	1.09 – 1.51	0.003
Ethyl paraben	1.07	1.00 – 1.15	0.047	1.08	1.01 – 1.16	0.035

[a] Covariates used for model adjustment were those showing a statistically significant association in the multivariate analysis shown in Table 4 and the three pollutants showing a significant association with PCa risk (Table 5).
[b] Method used for selecting predictor variables in logistic regression models

Figure 1