Steroid 5 alpha-reductase 2 enzyme variants, biomass exposure and tobacco use in Mexican patients with prostate cancer

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Abstract. The presence of the genetic variants of the steroid 5-alpha reductase 2 enzyme, which is encoded by the *SRD5A2* gene, has been associated with an increased risk of developing prostate cancer among certain ethnic groups. However, these molecular studies have not been conducted on the Mexican population. The analysis of the genetic variants, rs9282858 and rs523349, was performed in 101 males with prostate cancer and 100 healthy controls classified as males without prostate abnormalities (n=60) and males with benign prostatic hyperplasia (n=40), to identify a probable association with this cancer type in the Northeast Mexican population. An association was identified between prostate cancer and biomass exposure [P=0.012; odds ratio (OR), 2.89; confidence interval (CI)=1.21-6.88] and tobacco use (P=0.028; OR=1.88; CI=1.07-3.31), while no association was observed between cancer development and the rs9282858 variant, or between a protective effect and the rs523349 variant. Notably, an association was identified between rs523349 and biomass exposure (P=0.013, OR=3.17; CI=1.23-8.17 for the G risk allele, and OR=0.32, CI=0.12-0.81 for the C protective allele) using the dominant genetic model. To the best of our knowledge, the present study was the first of its type to investigate the Mexican population with prostate cancer.

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Introduction

Prostate cancer (PCa) is the second most common type of malignancy among males worldwide; in 2018, the World Health Organization estimated more than 1.3 million new prostate cancer cases and over 359,000 mortalities due to this disease (1). In Mexico, PCa is the most common and fatal cancer type among males, accounting for >10,000 new cases and 5,000 mortalities during the same period of time (1). PCa is usually diagnosed in the fifth decade of life, with a variable rate of progression that largely depends on genetic and environmental factors, as well as the patient's lifestyle (2).

Androgens are necessary for the correct development and function of the prostate gland; however, they also serve a critical role in driving the growth of early-stage PCa (3). Androgen action, mediated by the androgen receptor (AR), leads to the activation of target genes that stimulate the proliferation and inhibit the apoptosis of cancer cells (3,4). Free testosterone diffuses across the membranes of target cells located within the prostatic tissue acting as a substrate for the steroid 5 alpha-reductase 2 enzyme (encoded by the *SRD5A2* gene), which converts testosterone to dihydrotestosterone (DHT), a more potent metabolite that activates the AR (5). Once activated,

the AR is translocated to the nucleus where it dimerizes with another AR and activates target genes that promote cell proliferation (3,4,6,7) (Fig. 1).

Certain DNA variants in the genomic sequence of the *SRD5A2* gene alter the catalytic activity of steroid 5-alpha reductase 2, which may increase the risk of PCa development (8-11). In this context, certain variants have been associated with an increased risk of developing PCa, including TA dinucleotides in the 3'-UTR region (12,13), rs9332964 (14,15), rs928258 (9) and rs523349 (16,17). The rs928258 and rs523349 variants have been screened mainly across different ethnic groups.

The rs9282858 (p.Ala49Thr or A49T) variant results from a single nucleotide substitution, a G by an A (GCC/ACC), causing an amino acid change from an alanine to a threonine. It has been reported that this change increases the catalytic activity of the enzyme by 5-fold (14,18). The ENSEMBL database reports this variant as a) benign in ClinVar, b) deleterious in SIFT, c) benign in PolyPhen, and d) likely benign in CADD (http://www.ensembl.org/Homo_sapiens/Variation/Mappings?db=core;r=2:31580256-31581256;v=rs9282858;vdb=variation;vf=57248637).

By contrast, the rs523349 (p.Val89Leu or V89L) variant results from a single nucleotide substitution, a C by a G (CTA/GTA), causing an amino acid change from a valine to a leucine. It has been reported that this change reduces the enzyme activity by 30% (14,16,19). The ENSEMBL database reports this variant as a) benign in ClinVar, b) tolerated substitution in SIFT, c) benign in PolyPhen, and d) likely benign in CADD (http://www.ensembl.org/Homo_sapiens/Variation/Mappings?db=core;r=2:31580136-31581136;v=rs523349;vdb=variation;vf=54157055).

As in a number of other diseases, the molecular findings on populations of European descent cannot be assumed for all populations. To implement a precision medicine model, molecular studies must be performed in diverse ethnic groups of interest. Genomic and genetic data of populations of non-European descendants remain under represented (20).

The Mexican population is genetically diverse (21); therefore, a more detailed study of the population structure alongside geographical data is required to assess the frequency and prevalence of genetic diseases in native and Mexican-mestizo populations (21-23). In diseases, including prostate cancer, this information may aid in diagnosis, prognosis, and treatment (21,22).

In Mexico, PCa is a national health problem, but molecular studies regarding PCa in this population are limited. Certain studies with regard to *AR*, *VDR* (24), *VEGF* (25), *ATP6*, and *ND3* (26) genetic variants have been made. However, to the best of our knowledge, there are no previous reports that analyze the presence of the genetic variants, A49T and V89L, of the steroid 5 alpha-reductase 2 enzyme in the Mexican-mestizo population. Therefore, a molecular analysis of these genetic variants and examination of relevant clinical data was performed in the present study to identify a possible association with the development of PCa on the Mexican-mestizo population.

Materials and methods

Study design. The protocol was approved by the Ethics and Research Committee of the School of Medicine of the Universidad Autónoma de Nuevo León (no. UR16-00007). This protocol was performed by the Biochemistry and Molecular Medicine Department using convenience sampling. Participants were enrolled between January 2018 and December 2019 through the Urology and Oncology Services from the 'Dr. José Eleuterio González' University Hospital of the Universidad Autónoma de Nuevo León, and each patient provided written informed consent to participate in the present study.

Recruited participants were classified into three groups according to subsequent analyses: PCa cases (n=101; median age, 70 years; age range, 64.5-75.0 years) and non-PCa subjects (n=100; median age, 58 years; age range, 48-67 years) composed of males without prostate abnormalities (n=60) and subjects with benign prostatic hyperplasia (BPH; n=40). The PCa cases were males diagnosed with PCa regardless of the time elapsed since the diagnosis or their treatment status.

Blood samples were collected in a 6 ml BD Vacutainer tube with EDTA (Becton-Dickinson). Demographic data of clinical importance were collected, and the database was prepared. PSA levels were analyzed only for the PCa and BPH groups.

DNA extraction protocol. Each blood sample was centrifuged at 3,857 g for 10 min at room temperature. From the buffy coat, DNA extraction was performed using a previously reported method with TSNT lysis buffer (composed of 1% Triton, 1% sodium dodecyl sulfate, 100 mM NaCl, 10 mM Tris-HCl pH 8.0 and 1 mM EDTA) and followed by a phenol-chloroform extraction step (27,28). The DNA was precipitated from aqueous phase with ethanol and quantified using NanoDrop 1000 (Thermo Fisher Scientific, Inc.) and its quality was verified through absorbance ratios (260/280 and 260/230 nm) and agarose gel electrophoresis.

Genotyping. Genotyping assays were performed using the commercial TaqMan SNP Genotyping Assays probes C__27532228_20 and C___2362601_10 (Thermo Fisher Scientific, Inc.) for the A49T (rs9282858) and V89L (rs523349) gene variants of the *SRD5A2* gene (NM_000348.3), respectively.

DNA samples were processed using a StepOnePlus™ Real-Time PCR system (Thermo Fisher Scientific, Inc.). Each PCR reaction was performed using 5 μl SensiFAST™ Hi-Rox Genotyping kit (Bioline; Meridian Bioscience, Inc.), 0.5 μl probe, 5 μl nuclease-free water, and 3 μl DNA (300 ng).

The amplification program used was the following: Pre-PCR read 60˚C/30 sec, holding stage 95˚C/10 min, cycling stage: i) 95˚C/15 sec; ii) 60˚C/1 min, and post-PCR read 60˚C/30 sec. The results were analyzed using the StepOne™ software v.2.2.2, (Thermo Fisher Scientific, Inc.).

Statistical analysis. Gene analysis was conducted using the Golden Helix SNP & Variation Suite 8.8.3 program (Golden Helix, Inc.). The DNA variants were analyzed for deviation from the Hardy-Weinberg Equilibrium (HWE) using the Fisher's exact test (P<0.05 was considered to indicate a statistically significant difference and HW disequilibrium). The genetic association study was performed using the dominant and recessive gene models in order to assess odds ratios (ORs),
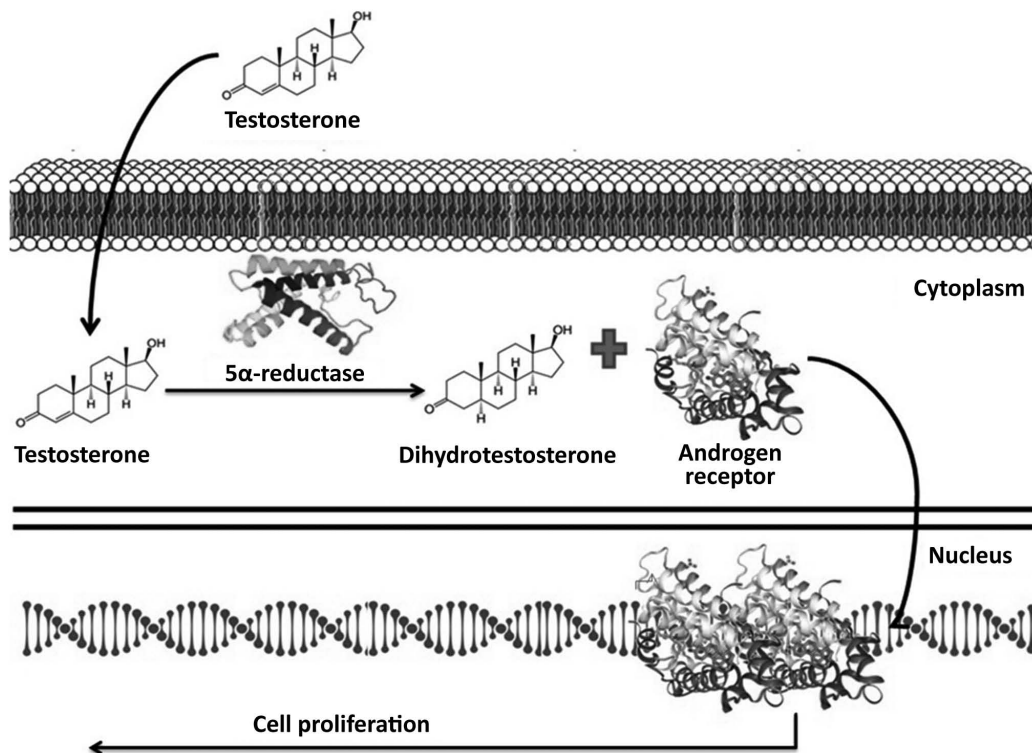
Figure 1. Androgen receptor signaling axis. Testosterone enters prostate cells, where it is reduced to DHT by the action of the steroid 5α-reductase 2. Binding of DHT to the AR generates conformational changes that enable the dimerization of the AR and the subsequent binding to the androgen-response elements of androgen-responsive genes promoting cell proliferation. DHT, dihydrotestosterone; AR, androgen receptor.

95% confidence intervals (CIs), Bonferroni P-values and false discovery rates (FDRs) (23). The dominant model considered the analyzed phenotypes of Ala/Ala+Ala/Thr vs. Thr/Thr for the rs9282858 and Val/Val+Val/Leu vs. Leu/Leu for the rs523349 variants. On the other hand, the recessive genetic model considered the analyzed phenotypes Ala/Ala vs. Ala/Thr+Thr/Thr for the rs9282858 and Val/Val vs. Val/Leu+Leu/Leu for the rs523349 variants (29).

For the regression association study, a stepwise linear regression model (qthelp://org.sphinx.svsmanual.8.8.3/doc/svsmanual/ftParts/logistic_regression.html) with recoded genotypes with the additive gene model (DD=2, Dd=1, dd=0) was used. False discovery rate correction (FDR) was calculated to exclude spurious associations (qthelp://org.sphinx.svsmanual.8.8.3/doc/svsmanual/ftParts/general_statistics.html).

Results

Clinical characteristics of cases and controls enrolled in the present study. The present study included 201 participants classified as patients with PCa (n=101) and non-PCa subjects, including males without prostate abnormalities (n=60) and subjects with BPH (n=40). For patients with PCa, the median age (IQR) was 70 (range, 64.5-75) years. Thirty percent of patients had a history of prostate pathology, while the median prostate-specific antigen (PSA) value was 20.4 (IQR, 9.65-62.82); 20.8% had type 2 diabetes mellitus (T2DM), and body mass index (BMI) calculations for this group found that 33.7% were a normal weight, 40.8% were overweight, and 22.4% had some degree of obesity. Gleason Grading was calculated according to the recommendations of the International

Society of Urological Pathology (ISUP) (30). A total of 62% of patients were classified as Gleason Grade Group 5, as they had tumors with Gleason scores of 9 and 10. The majority of patients (67%) received androgen deprivation therapy (ADT), predominantly bicalutamide alone or in combination with other drugs, such as goserelin and leuprolide. Table I shows the detailed clinical variables of the patients with PCa.

For the non-PCa subjects, the median age (IQR) was 58.8 (range, 48-67) years; 42.4% had a history of prostate pathology, while the median PSA value for subjects with BPH was 8.55 ng/ml (IQR, 5.12-17.71); 21% had T2DM, and the BMI calculations for this group found that 23.1% were a normal weight, 44.9% were overweight, and the remaining 32.1% had some degree of obesity.

Table II summarizes the relevant data of the patients with PCa and non-PCa subjects enrolled in the present study. Notably, an association was identified between PCa and biomass exposure (P=0.012; OR=2.89; CI=1.21-6.88) and tobacco use (P=0.028; OR=1.88; CI=1.07-3.31), compared with controls.

Genotyping. Table III shows the HWE analysis for PCa cases and controls, as well as the genotype frequencies. The A49T variant was out of HWE equilibrium in the population analyzed, unlike the V89L variant, which was maintained in HWE.

Statistical analysis. The results of the association analysis were categorized according to the method used. In the present study, dominant and recessive gene models were used to perform analysis. No association was identified in any of the conditions

Table I. Clinical characteristics of the enrolled patients with prostate cancer.

Clinical characteristics	Prostate cancer cases, n (%)
ISUP grade group	
Group 1	2 (2.0)
Group 2	13 (13.3)
Group 3	13 (13.3)
Group 4	9 (9.2)
Group 5	61 (62.2)
Extracapsular invasion	63 (62.4)
Neurovascular invasion	57 (56.4)
Recurrence	7 (6.9)
Metastasis	21 (20.8)
Castration-resistance	14 (13.9)
Androgen deprivation therapy	
Bicalutamide	22 (32.8)
Bicalutamide + leuprolide	9 (13.4)
Bicalutamide + goserelin	11 (16.4)
Bicalutamide + orchiecthomy	12 (17.9)
Orchiectomy	6 (8.9)
Other[b]	7 (10.4)

[a]Group 1, Gleason Score <6; Group 2, Gleason Score=7(3+4); Group 3, Gleason Score=7(4+3); Group 4, Gleason Score=8; Group 5, Gleason Score=9 and 10. [b]Other treatments: Bicalutamide + leuprolide + orchiectomy, goserelin + leuprolide, zoledronic acid + leuprolide, goserelin, orchiectomy + docetaxel, and leuprolide + goserelin + ketoconazole + prednisolone. ISUP, International Society of Urological Pathology.

of the models or in the development of PCa (A49T variant) or in conferring a protective effect (V89L variant). Table IV presents the results obtained for the variants A49T and V89L after performing a statistical analysis using the dominance and recessiveness gene models, as well as the OR and 95% CI range.

Clinical and genetic features association. The analysis between clinical and genetic features was conducted using V89L genotypes. No association was identified between genotyping and clinical variables, including PSA, ISUP Grade Group, or a history BPH. However, an association was identified between rs523349 and biomass exposure (P=0.013; OR=3.17; CI=1.23-8.17 for the G risk allele, and OR=0.32, CI=0.12-0.81 for the C protective allele) using the dominant gene model (https://doi.org/10.5281/zenodo.3932702). There was an association between V89L and patients with metastasis (Val/Val vs. Leu/Leu+Val/Leu; P=0.048; OR=0.390; CI=0.142-1.073; Table V).

Discussion

Previous studies that have used the Mexican population as a subject of study have reported an association between certain variants in the *SDR5A2* gene and diseases, including pseudo hermaphroditism and hypospadias (31-34), but not PCa. The effect of the two analyzed variants is different according to the ethnic group of study. For example, homozygous subjects possessing the V89L variant (Val/Val) may have a protective effect if its origin is from Asia, but subjects with this same phenotype may have an increased risk of developing PCa. Cancer is a complex set of diseases in which different risk factors serve a crucial role in its development, where the genetic background is only one of them.

Logistic regression is an essential tool used in many clinical applications, including in clinical prediction models (35), patient screening (36), and for the developing and validation of novel diagnostic models (37). The clinical importance of genotyping the genetic variants analyzed in the present study lies in predicting the behaviour of the metabolic AR pathway. Logistic regression may then be applied in clinical prediction models, to develop and validate novel diagnostic models, and to assess and predict the success of steroid 5 alpha-reductase 2 inhibitors (38).

In the present study, the participants enrolled were males with diagnosed PCa, males with other urological diseases, or men without any apparent urological condition that serve as healthy controls. PCa is more frequently diagnosed in the fifth decade of life; therefore, the statistical difference between the ages of PCa vs. non-PCa subjects in the present study was expected. The analysis of specific clinical variables revealed that PCa was associated with certain risk factors, including tobacco use and biomass exposure. It was found that the median PSA value derived from the PCa cases (20.4 ng/ml) was significantly higher than that for the non-PCa subjects (8.5 ng/ml) included in the present study (P<0.001), which is consistent with the results of previous studies (39,40). It was reported that 125/973 (12.8%) of participants had PSA values >4 ng/ml and 55 (44%) were diagnosed with PCa in a previous screening study conducted by part of our research group in the Northeast Mexican population (41).

A limitation of our work was the lack of availability of PSA values in healthy controls. This limitation is due to the fact that all participants were recruited as a convenience sample by the Urology Department and that non-PCa cases were men with BPH (n=40) or persons classified as men without prostate abnormalities (n=60). The association of the gene variants analyzed in this work was made by comparing PCa vs. total non-PCa subjects.

No association was identified between T2DM and prostate cancer development. However, the study subjects were only classified as diabetic or not, without accounting for their glucose levels or medications. A recent meta-analysis of 733 articles identifying 17 cohort studies that included 274,677 male patients suggested that diabetes may result in a poorer prognosis for males with PCa, but was not associated with PCa development (42). Another independent study reported an increased risk of mortality with PCa in diabetics, but not an association between diabetes and the incidence of prostate cancer (43). This may be explained by the comorbidities associated with T2DM, including atherosclerosis and renal failure, as patients with T2DM and other comorbidities may have a poorer response to treatment. Two meta-analyses reported a relative low risk of develop PCa in patients with

Table II. Demographic characteristics of patients with PCa (n=101) and control subjects (n=100).

Demographic characteristic	PCa cases	Non-PCa cases	P-value	OR (95% CI)
Age, median years (IQR)	70 (64.5-75.0)	58.8 (48-67)	9.22×10^{-15}	1.13 (1.08-1.17)
PSA, median ng/ml (IQR)	20.4 (9.65-62.82)	8.55 (5.12-17.71)	8.29×10^{-20}	1.11 (1.07-1.16)
BMI, median kg/m²	27.02 (±4.33)	27.84 (±5.52)	0.271	NS
BMI <18.5 (% underweight)	3.1	0.0	NA	NS
BMI 18.5-24.9, %	33.7	23.1	0.612	NS
BMI 25-29.9, %	40.8	44.9	0.226	NS
BMI >30, %	22.4	32.1	0.486	NS
Type 2 diabetes mellitus, %	20.8	21.0	0.732	NS
Arterial hypertension, %	38.6	33.0	0.694	NS
Alcohol intake, %	57.4	64.0	0.182	NS
Smoking habit, %	60.4	43.0	0.028	1.88 (1.07-3.31)
Biomass exposure, %	20.8	8.0	0.012	2.89 (1.21-6.88)
Family history of prostate cancer, %	18.8	12.0	0.224	NS
Family history of other cancers, %	33.7	29.0	0.603	NS

PCa, prostate cancer; OR, odds ratio; CI, confidence interval; IQR, interquartile range; PSA, prostate-specific antigen; BMI, body mass index; NA, not available; NS, not significant. P<0.05 indicates a statistically significant difference.

Table III. Hardy-Weinberg equilibrium and genotype frequencies of A49T (rs9282858) and V89L (rs523349) variants in cases and controls.

Variant	Reference alleles	Fisher's HWE P-value		Genotype frequency	
		Cases	Controls	Cases (%)	Controls (%)
A49T (rs9282858)	[C/T]	1.90E-06	0.005	T|T: 0.030 (3)	T|T: 0.010 (1)
				C|C: 0.970 (98)	C|C: 0.990 (97)
V89L (rs523349)	[C/G]	0.678	0.999	C|C: 0.376 (38)	C|C: 0.448 (44)
				C|G: 0.455 (46)	C|G: 0.439 (43)
				G|G: 0.168 (17)	G|G: 0.112 (11)

G, guanine; T, thymine, C, cytosine.

Table IV. Association analysis of A49T and V89L variants.

Variant	Genetic model	χ^2 FDR	OR (95% CI)		Allele frequency	
					Cases	Controls
A49T (rs9282858)	Dominant	0.327	T, 2.97 (0.30-29.05)	C, 0.34 (0.03-3.29)	T, 0.03	T, 0.01
	Recessive	0.327	T, 2.97 (0.30-29.05)	C, 0.34 (0.03-3.29)	C, 0.97	C, 0.99
V89L (rs523349)	Dominant	0.594	G, 1.35 (0.77-2.38)	C, 0.74 (0.42-1.30)	G, 0.396	G, 0.332
	Recessive	0.511	G, 1.60 (0.71-3.62)	C, 0.62 (0.28-1.41)	C, 0.604	C, 0.668

FDR, false discovery rate; OR, odds ratio; CI, confidence interval; G, guanine, T, thymine; C, cytosine.

T2DM (44,45). Other studies have presented controversial results regarding this comorbidity (46-48). Previously, our research team identified an increased risk of developing PCa with Gleason Scores >8 in patients with high glucose levels (49).

Additionally, a significant difference (P=0.028) was reported between tobacco use in PCa patients and non-PCa subjects (60.4% vs. 43%, respectively). Several previous studies have reported tobacco use as a risk factor for developing PCa (50-52). Smokers have an increased risk of

Table V. Analysis of rs523349 (V89L) genotypes and clinical features.

Genotype	PSA		Grade Gleason Group ISUP classification[a]			Metastasis		Benign Prostatic Hyperplasia	
	Median ng/ml	P-value	≤3	≥4	P-value	%	P-value	%	P-value
VV	48.46	0.684	14.28	23.47	0.071	3.96	0.492	20.60	0.282
VL	47.53		33.67	12.24		13.86		18.50	
LL	53.22		14.28	2.04		2.97		3.30	
VV+VL	51.07	0.655	26.50	57.10	0.125	17.82	0.766	39.10	0.3040
LL	47.53		2.04	14.30		2.97		3.30	
VV	51.75	0.134	14.30	47.90	0.125	3.96	0.048	20.60	0.635
LL+VL	48.46		14.30	23.50		16.83		21.70	

[a]Group 1, Gleason score <6; Group 2, Gleason score=7 (3+4); Group 3, Gleason score=7 (4+3); Group 4, Gleason score=8; Group 5, Gleason score=9 and 10. PSA, prostate-specific antigen; ISUP, International Society of Urological Pathology; VV, val/val; VL, val/leu; LL, leu/leu.

developing certain types of cancer, including lung cancer. However, it has been recognized that tobacco use may contribute toward the development of urological cancer, including prostate, bladder, ureters and kidney (50-53). This could be because chemical compounds released when tobacco is burned are distributed from the lung blood vessels to other tissues of the body.

When tobacco is burned, it produces carcinogenic compounds, mainly polycyclic aromatic hydrocarbons (PAHs) and nicotine-derived nitrosamines, including N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). In brief, tumorigenesis is caused by the formation of DNA adducts. These adducts generate mutations in key cell maintenance genes; the NNNs and NNKs may bind to acetylcholine receptors and promote events, including proliferation, growth, survival and cell migration (54). Furthermore, PAHs may bind to aryl hydrocarbon receptors (AHRs), which activate CYP1A1 and CYP1B1; the CYPs add an epoxide group to the PAHs, and these PHAs-epoxide complexes may bind to DNA to form adducts, which are crucial for tumorigenesis (55).

Notably, there was a significant difference between biomass exposure in patients with PCa and that in non-PCa subjects (20.8 vs. 8%; P=0.012; OR=2.89; CI=1.21-6.88). The Mexican population (mainly its Northern population) has a tradition for cooking grilled foods and performing other activities, such as working for petrochemical, steel and construction industries, that generate particles of sizes of <10 μm, or being exposed to xenobiotic compounds, including polycyclic aromatic hydrocarbons (PAHs) (56). In the present study, the participants were surveyed to see with what frequency they cook their foods using wood. A previous study demonstrated how the exposure to this biomass is associated with the development of different types of cancer, primarily lung cancer (57). The mechanism by which the biomass compounds promote cancer development is unclear, but we hypothesize that it is associated with PAHs, primarily benzo(a)pyrene. Benzo(a)pyrene serves a role in the formation of DNA adducts (58), specifically in the mutations involving the nucleotide change from G to T in the codons 157, 158, 248, and 273 of the *TP53* gene,

with catastrophic consequences according to the IARC *TP53* Database (R20, July 2019) (59). The two risk factors identified in the present study associated with benzo(a)pyrene were tobacco use and biomass exposure. The risk factor that was statistically significant following FDR correction was biomass exposure. Stepwise regression was a useful tool to identify this risk factor.

There are few studies on biomass exposure and PCa development, the most important of which is the Cancer Prevention Studio-II (CPS-II) of the American Cancer Society (60); however, the authors assessed risk factors for lung cancer, so the association with PCa was not investigated.

The A49T variant is associated with an increase in the activity of the 5-alpha reductase 2 enzyme, which converts testosterone to dihydrotestosterone (DHT). This more potent metabolite binds to the AR and results in the subsequent activation of target genes that promote cell proliferation, inhibition of apoptotic signals, and PSA overexpression.

A meta-analysis published in 2011 by Li *et al* (19) reported a significantly higher risk of developing stage III/IV PCa in homozygous variant subjects carrying the A49T variant (Thr/Thr allele), using a recessive gene model (P=0.0001; OR=2.13; CI=1.44-3.15) (19). In the present study, no association was identified between *SRD5A2* gene variants and PCa. The results demonstrated that 3% of subjects were carriers of the A49T (Thr/Thr allele) variant and it was not associated with PCa development (P=0.327). A limitation of the results of the present study is the HW disequilibrium, possibly due to the low frequency of this allele or the lack of heterozygous individuals in the sampled population (61,62). Future studies should include an increased number of analyzed samples to verify these results.

Other ethnic groups, Ecuadorian, African American and Latin, had an association with the development of PCa and the A49T variant (Thr/Thr allele) (16,63,64). By contrast, no association between this allele and PCa was identified in analyzes performed on Hispanic and Brazilian populations (65,66). Table VI shows these previously reported genotyping studies and their association with PCa development.

Table VI. Clinical effect of A49T (rs9282858) variant in other populations.

Authors, year	Population	Effect	Genotype(s)	Refs.
Makridakis *et al*, 1999	African-American	Associated to cancer development	Thr/Thr	(16)
Ribeiro *et al*, 2002	Brazilian	Not associated with cancer development	Thr/Thr	(65)
Pearce *et al*, 2008	Hispanic/African-American	Not associated with cancer development	Ala/Thr	(66)
Paz-y-Miño *et al*, 2009	Ecuadorian	Associated risk of prostate cancer	Thr/Thr vs. Ala/Thr	(63)
Fang *et al*, 2017	Latino	Associated with cancer development	Thr/Thr	(64)

Although patients harboring the A49T variant (Thr/Thr allele) were expected to have high PSA levels, the patients in the present study with the Thr/Thr genotype had PSA values of 5.23, 8.00, and 19.98 ng/ml, possibly due to the tumor stage at the time of diagnosis. A more significant number of patients would be required to verify if there is any correlation between these two variables and PCa development in the Mexican-mestizo population.

As for the V89L variant, the homozygous allele variant Leu/Leu causes a decrease in the catalytic activity of steroid 5 alpha-reductase 2, while the homozygous wild-type Val/Val genotype has been associated with PCa development and higher Gleason stages.

In 1992, Batista *et al* (5) measured steroid 5-alphare-ductase 2 activity indirectly by quantifying testosterone metabolites in Afro-American and Asian individuals, finding differences attributed to enzyme activity levels; however, they did not take into account the genotypes of the analyzed subjects (8). This is due to the Leu/Leu allele, and a study in the Asian population reported this result with a protective effect by decreasing the risk of developing PCa (9).

In the present study, patients with this allele may be developing PCa due to other independent metabolic AR pathways, including damage repair DNA genes (67), *PTEN* (68) or *TP53* (69). By contrast, the allele Leu/Leu was associated with cancer development in genotyping studies of European American (70) and Hispanic populations (71). Notably, this allele has been associated with PCa with Gleason scores >8 in the French population (72) and metastasis (73).

The present study identified a decreased statistical trend with regards to metastatic PCa when analyzing carriers of alleles Leu/Leu+Val/Leu vs. Val/Val (P=0.048; OR=0.390; CI=0.142-1.073). To the best of our knowledge, there are no reports that directly determined the cause of this association, but we hypothesized that it may be due to the differential gene expression associated with metastasis in PCa, including *EGR2, EGR3, MTA1, MYBL2* (74), *SYNPO2, EGR3, RDX, FOXM1* (75), *KLF6, MMP9* or *WNT5A* (76). Additionally, when a naïve PCa is treated with ADT, its evolution to a metastatic state may be due to other transcriptional factors, including *GATA2 or FOXA1* (77), fusion mutation *TMPRSS2-ERG* (78,79) or AR variants (AR-V7) (80,81).

SRD5A2, CYP17A1, and *CYP19A1* are involved in the steroid metabolic (GO:0008202) and steroid biosynthetic processes (GO:0006694). In *Mus musculus*, benzo(a)pyrene has been associated with the Protein-Protein interaction

Network oxidation-reduction process (GO:0055114; Permanent link: http://bit.ly/2jIbOER) (82). The genes involved in this network participate in the cytochrome P450-mediated oxidation (*Cyp17a1, Cyp19a1, Cyp1a2, Cyp21a1, Cyp3a11, Cyp3a13, Cyp3a16, Cyp3a25* and *Cyp3a41a*), and the reduction of oestrogens and androgens (*Srd5a2, Akr1d1, Hsd17b1* and *Hsd3b4*).

The Val/Val allele is more common in white Hispanic/non-Hispanic and Ecuadorian populations and it has been associated with PCa development; in the Mexican-mestizo population investigated in the present study, this association was not found. It has been seen that this allele may contribute toward worsening clinical prognosis associated with the overall survival, as was described in a previous study when comparing the different genetic variants and lower AR activity (83).

Table VII summarizes other genotyping studies of V89L and its potential associations with PCa development, metastasis, Gleason score, or protective effects against PCa (9,63,70-73,84).

The analysis performed in the present study demonstrated that there was no association between the *SRD5A2* rs523349 genotypes and Gleason scores ≥8; however, there was a decreased tendency between patients with metastasis and rs523349 genotypes (Val/Val vs. Leu/Leu+Val/Leu; P=0.048; OR=0.390; CI=0.142-1.073).

The clinical prognosis of the cohort of patients with PCa included in the present study was associated with 5-alpha reductase 2 variants. However, there are two main limitations: i) The present study included patients with localized and metastatic disease without discriminating the time of evolution, and ii) the type of ADT at the time of enrolment. The first-line treatment of ADT in clinical practice is the administration of bicalutamide, as the public medical care does not include new generation treatments in Mexico, including enzalutamide or abiraterone acetate.

Finally, regarding benign prostate diseases, a meta-analysis undertaken in 2017 by Zeng *et al* (85) found a risk of developing BPH in individuals carrying the A49T variant (OR=2.75; CI=1.32-5.69), but this was not statistically significance (P=0.373). By contrast, the results of studies concerning the V89L variant and its association with the development of BPH and PSA changes have been contradictory (86,87). A previous study reported that the Val/Leu+Leu/Leu genotype (P=0.047; OR=1.62; CI=1.00-2.61) was associated with the development of this benign condition, but not with the development of PCa (88). However, in another study, none of these genotypes were

Table VII. Clinical effect of V89L (rs523349) variant in other populations.

Author, year	Population	Effect	Genotype(s)	Refs.
Makridakis *et al*, 1997	Asian	Protective against cancer	Leu/Leu	(9)
Söderström *et al*, 2002	Caucasian	Associated with metastasis	Leu/Leu	(73)
Loukola *et al*, 2004	European-Americans	Associated with cancer development	Leu/Leu vs. Val/Leu[a]	(70)
Salam *et al*, 2005	Hispanic	Associated with cancer development	Leu/Leu	(71)
Cussenot *et al*, 2007	French	Gleason Scores >8	Leu/Leu	(72)
Torkko *et al*, 2008	Non-Hispanic White	Associated with cancer development	Val/Val[b]	(84)
	Hispanic White	Associated with cancer development	Val/Val[c]	
Paz-y-Miño *et al*, 2009	Ecuadorian	Associated with cancer development	Val/Val	(63)

[a]Analysis of genotyping results showed a HW disequilibrium; [b]results combined with variants in *VDR* gene; [c]results combined with variants in *CDX2* gene.

associated with the development of BPH (85). In the future, a longitudinal analysis of Mexican patients with this type of benign disease should be performed to describe the frequency of the different genetic variants and to determine if they have a role in the prognosis of PCa as potential biomarkers for personalization of pharmacological treatments using 5 alpha-reductase enzyme inhibitors (87).

In conclusion, we identified the allelic frequencies of both the A49T and V89L variants of the steroid 5 alpha-reductase 2 gene in the Mexican population. No association was identified between either of the variants and the development of PCa, but no increased risk of developing PCa was identified due to lifestyle factors, including the exposure to biomass and tobacco use. Furthermore, an association between V89L and biomass exposure was identified using the dominant gene model. Additionally, there was an association between V89L and patients with metastasis.

To the best of our knowledge, the present study was the first to screen the Mexican population for these variants, and one of the focussing on the Latino population. For these ethnic groups, molecular characterization of PCa is required to improve the understanding of this disease, and to determine if the results of molecular characterization studies may serve a role in the prognosis of PCa or as potential biomarkers for the personalization of pharmacological treatments.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JRDB conducted the experiments, acquired, analyzed and interpreted the data; and drafted the manuscript. JRDB, HLGB, JFYL, LSGG and CNSD designed the study, conducted the experiments, analyzed, interpreted the data, and critically revised the manuscript. CNSD and AMRE made substantial contributions to the conception of the study, and drafted, and critically revised the manuscript. DHB, AMGB, GVM, MAOM and LSGG, as clinicians, selected the patients, performed the biopsies to obtain the samples, collected the clinical information from medical records, and contributed toward the design of the study. DAH and BSG assisted in technical support during the experimental work and were involved in the acquisition, analysis and interpretation of data. RGG and MNM, as pathologists, conducted the histopathological diagnoses of the patients. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The protocol was approved by the Ethics and Research Committee of the School of Medicine (Universidad Autónoma de Nuevo León, Mexico; UR16-00007). Prior to blood extraction, written informed consent was obtained from the eligible participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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