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Positive association between vascular endothelial growth factor (VEGF) -2578 C/A variant and prostate cancer

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Abstract.

BACKGROUND: Vascular endothelial growth factor (VEGF) gene is an important angiogenesis regulator related to cancer development and progression. We evaluated the association between -2578 C/A (rs699947) VEGF polymorphism and PCa in Mexican subjects, to contribute to knowledge of VEGF role in genetic epidemiology of prostate cancer (PCa).

OBJECTIVE: The aim of this study was to evaluate the association between -2578 C/A VEGF variant and PCa in Mexican population.

METHODS: A total of 249 men (77 PCa cases and 172 controls) from the Northwestern region of Mexico were screened for the -2578 C/A VEGF variant. The polymorphism was determined by polymerase chain reaction-based restriction analysis.

RESULTS: Genotype frequencies for C/C, C/A, and A/A, were 0.48, 0.49, 0.03 for cases and 0.41, 0.45, 0.14 for controls respectively. Genotype A/A of -2578 VEGF variant reduces the risk of PCa in an 84% among studied population (Odds Ratio 0.16; 95% CI: 0.04–0.71, $P = 0.007$). C/C carriers showed an increased PCa risk of 6.1 times among the study population.

CONCLUSIONS: Inheritance of -2578 A/A genotype of VEGF gene may modify PCa susceptibility risk in Mexican population.

Keywords: Prostate cancer, VEGF, polymorphism, genetic susceptibility

1. Background

Prostate cancer (PCa) is a complex neoplastic disorder in which interaction between genetic and non-genetic factors contributes to disease initiation and progression [1–4]. Biological aggressiveness of PCa is closely linked to its capacity to spread and metastasize, but for a tumor to acquire such characteristics it should induce and sustain a supply of new vessels (angiogen-

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esis) to provide the oxygen and nutrients necessary for relentless growth and expansion of tumor mass [5, 6]. Mechanisms such as production of pro-angiogenic molecules, activation of the endothelium, the capillary wall degradation, and migration of endothelial cells are essential in the angiogenesis processes and therefore in the tumor progression [6]. The vascular endothelial growth factor (VEGF) is the most important member of a family of ligands with 40% to 80% of homology (VEGF-A, B, C, D, and placental growth factor respectively), is also a hypoxia-induced gene, and has a potent vascular permeability function [5,7]. VEGF-A (also called VEGF) induces endothelial cell division, locomotion/migration, survival, and endothelial progenitor cells mobilization from the bone marrow to sites of active angiogenesis [7,8]. VEGF is expressed by several tumors at higher levels than when it appears in normal tissues, and its over-expression suggests unfavorable prognosis [8–10]. In prostate tumors, VEGF expression is markedly increased in patients with histologically advanced PCa and it correlates with the growth and spread of tumor cells via activation of the blood and lymphatic vasculature [10]. VEGF over-expression has also been related to prostate tumor aggressiveness and it could be a useful tumor marker for early diagnosis as well as a predictor factor for recurrence and metastasis [10,11]. Some single nucleotide polymorphisms (SNP) in VEGF gene may affect VEGF protein concentrations influencing the angiogenesis process and may be related to inter individual variations in tumor risk, progression, and resistance to treatment [9]. The genotype C/C of the SNP rs699947 in the -2578 nucleotide position of the VEGF promoter, has been associated with higher levels of VEGF in blood mononuclear cells [12]. This polymorphism correlates with disease stages in which angiogenesis plays a critical role [9,13–15]. Despite the high prevalence of aggressive prostate tumors in Mexico [16], there are no association reports between -2578 C/A VEGF polymorphism and risk of PCa. To contribute with the assessment of genetic susceptibility of PCa, in this study we evaluate the association between -2578 C/A VEGF variant and PCa in Mexican population.

2. Materials and methods

2.1. Biological samples

We performed a retrospective case-control study in which all the subjects with available DNA sample on the bio-repository for research of PCa risk factors from Biochemistry and Molecular Medicine Department of Medical School from Universidad Autonoma de Nuevo Leon, Mexico, were included. All the DNA samples were obtained from peripheral blood of subjects who underwent transrectal biopsy (TRB) following confirmed clinical criteria [serum prostate specific antigen (PSA) value $\geqslant$ 4 ng/mL or abnormal digital rectal examination (DRE)], or transurethral resection of the prostate (TURP) [17]. The Institutional Board of Review approval was obtained (ID number: B107-001/CB-ACS/UAZ 002/2013) and so was the informed consent from participant subjects. A total of 249 DNA samples were available, 77 of them were subjects with histopathologic diagnosis of PCa (cases group) and 172 controls were DNAs from subjects who underwent a TRB or TURP, but had no pathological evidence of PCa; controls with TURP procedure additionally had a previous negative histological diagnosis for PCa by TRB in their clinical records. For most of the participants, the PSA values were determined before TRB or TURP procedures. All the participants were born in the Northwestern area of Mexico, which includes the states of Zacatecas, San Luis Potosi, Tamaulipas, Coahuila, and Nuevo Leon.

2.2. SNPs selection

The -2578 C/A polymorphism of VEGF gene was selected considering some premises: 1) the high prevalence of aggressive tumors reported in a previous Mexican early PCa screening PSA and DRE-based [16], 2) tumor aggressiveness is related with angiogenesis, while a high VEGF expression in cancer is considered as an unfavorable prognosis marker, 3) in addition to their association with cancer in other populations, -2578 C/A VEGF polymorphism has an impact in the mRNA level, and finally, 4) -2578 VEGF A/A genotype frequency was reported in a 10% among Northwestern Mexican population [13]; therefore, their potential association with PCa could have an impact in an important proportion of Mexican men.

2.3. Genotyping

The allelic discrimination of -2578 C/A VEGF variant (rs699947) was performed by polymerase chain reaction coupled to restriction fragments of length polymorphisms (PCR-RFLP) according to protocol, primer, reagents, and cycling conditions, reported by Garza-Veloz et al. [13]. The PCR-RFLP products

Table 1
General characteristics of population studied

Characteristic	Controls ($n = 172$)	PCa ($n = 77$)	P value
Age (median years)	69	70	0.163
$\leqslant 70$	101	41	
>70	71	36	
†Family history of cancers			
Yes	41	20	0.699
No	131	57	
§PSA (median ng/ml)	7.2	21.6	< 0.001*
0.1–4.0	39	11	
4.1–8.0	39	2	
8.1–10	18	6	
>10	39	55	
Gleason score			
< 7	–	15	
$\geqslant 7$	–	62	

†Two patients of control group had no family history of cancer data; §PSA information was not available for 37 controls and three cases respectively; *Statistical significance.

Table 2
Genetic inherency models

Model	Genotype	Controls $n = 172$	PCa group $n = 77$	OR (95% CI)	P value
Codominant	C/C	70 (40.7%)	37 (48%)	1	
	C/A	78 (45.4%)	38 (49.4%)	0.92 (0.53–1.61)	0.774
	A/A	24 (13.9%)	2 (2.6%)	0.16 (0.04–0.70)	0.007*
Dominant	C/C	70 (40.7%)	37 (48%)	1	0.279
	C/A-A/A	102 (59.3%)	40 (52%)	0.74 (0.43–1.27)	
Recessive	C/C-C/A	148 (86%)	75 (97.4%)	1	0.007*
	A/A	24 (13.9%)	2 (2.6%)	0.16 (0.04–0.71)	
Overdominant	C/C-A/A	94 (54.6%)	39 (50.6%)	1	0.558
	C/A	78 (45.4%)	38 (49.4%)	1.17 (0.69–2.01)	
Log-additive	–	–	–	0.63 (0.41–0.98)	0.087

*Statistical significance.

(C/C: 285 bp; C/A: 285 bp, 206 bp, 79 bp; A/A: 206 bp, 79 bp, respectively) were electrophoresed through 3.0% agarose gels, and visualized by ethidium bromide staining. For each PCR-RFLP round, we included six known genotyped samples as quality controls of the procedures.

2.4. Statistical analysis

Comparative analysis of age data and PSA values between study groups was carried out using Man-Whitney U test according to the distribution of data. Comparisons for categorical variables including family history of cancer, allele or genotype frequencies, and Hardy Weinberg equilibrium, were performed using chi-square and/or Fisher's exact test. To select the inheritance model that best fitted our data, the criteria of Akaike information criterion (AIC) and P values were considered. In this modeling, the co-dominant, dominant, recessive, over-dominant, and additive inheritance models were tested (Table 2). Odds ratio (OR) was calculated for positive associations. Statistical and genetic analyzes were performed in SigmaPlot v.12 and SNP Stats [18] software respectively. P values $<$ 0.05 were considered to be statistically significant.

3. Results

The number of subjects included in the protocol was 249 (77 cases and 172 controls); all of them were born in the Northwestern area of Mexico. General characteristics of study population are shown in Table 1. There were no differences between age and family history of cancer between the study groups ($P > 0.05$). Histological diagnosis of the controls included chronic prostatitis (60%), benign prostatic hyperplasia (BPH) with secondary chronic prostatitis (22%), benign prostatic tissue (8.7%), and atrophy (2%). Chronic prostatitis was found in association to benign prostatic tissue in

36.2% of the samples, followed by 38.1% atrophic tissue, and finally accompanied by prostatic infarct in 2%. 80.5% of PCa cases had a Gleason score above or equal to 7. PSA values ranged between 0.16 ng/ml and 2573 ng/ml in cases; and between 0.62 ng/ml and 54.8 ng/ml in controls ($P < 0.001$).

Table 2 summarizes genotype frequencies, the results of inherited models evaluated, and the association tests between the SNP studied and PCa. There were no Hardy-Weinberg unbalances ($P = 0.74$). The best model that fitted the obtained data was the recessive model, with an AIC value of 302.9 ($P = 0.0025$), followed by the codominant model with an AIC of 304.8 ($P = 0.01$), respectively. Genotype frequencies for C/C, C/A, and A/A, were 0.48, 0.49, 0.03 for cases and 0.41, 0.45, 0.14 for controls, respectively. A positive association was found between A/A genotype and PCa in the recessive model (OR = 0.16; 95% CI: 0.04–0.71, $P = 0.007$). The positive association between A/A genotype and PCa was consistent in the codominant model when C/C or C/A genotypes were considered as reference (OR = 0.16; 95% CI: 0.03–0.70, $P = 0.007$). Accordingly, the A/A genotype had a protector effect for PCa in around 84% of the subjects studied. To calculate the risk for PCa for men with no VEGF -2578 A/A genotype, we considered the homozygous A/A as reference. In this approximation, the carriers of C/C or C/A genotype increased their odds for PCa by 6.1 times (95% CI: 1.4–26.42, $P = 0.007$).

To evaluate if the risk of PCa observed for C/C carriers was consistent with other histologic diagnosis subgroups, the controls were subclassified as subjects with benign prostatic tissue, inflammatory no-BPH tissue (included chronic prostatitis with secondary acute prostatitis, and/or benign prostatic tissue without additional BPH tissue), and BPH (all with secondary chronic prostatitis) respectively. In all comparisons, the C/C carriers showed increased odds for PCa compared with A/A carriers. The risk of PCa was of 9.2 when the benign prostatic tissue group was considered as control (95% CI: 1.2–67.4, $P = 0.039$), 6.44 for the comparison with the inflammatory no-BPH tissue group (95% CI: 1.39–29.8, $P = 0.008$), and 9.3 times using the BPH group as control (95% CI: 1.64–52.1, $P = 0.009$). Considering the benign prostatic tissue group as a reference we evaluated the association between A/A genotype and the risk of having an unnecessary biopsy. For the comparison, all pathological diagnoses including cancer were grouped. The A/A genotype did not show a significant effect on the rate of unnecessary biopsying in the studied population ($P = 0.519$).

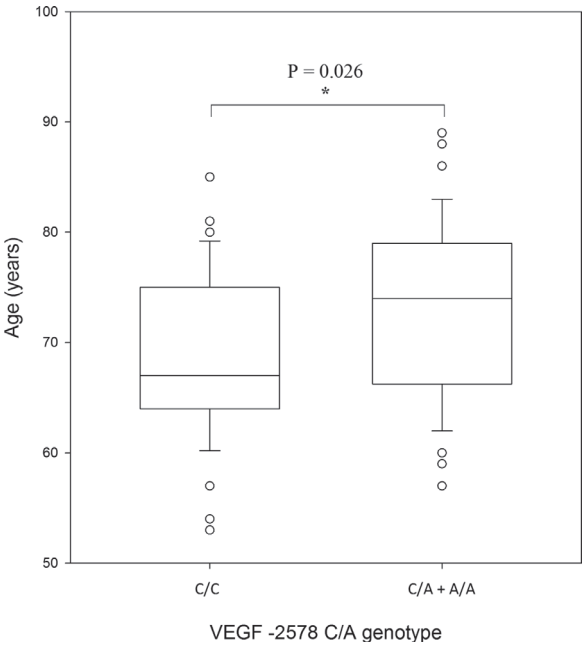

Fig. 1. Association between VEGF -2578 C/A genotype and age of PCa onset.

In order to test the association between the -2578 C/C homozygous genotype and PCa onset, cases were sub-classified according to the C/C, and C/A-A/A genotypes. VEGF -2578 C/C subjects showed an onset of disease of around 4.1 years earlier (Fig. 1) than subjects with other genotypes ($P = 0.026$).

PSA values showed no genotype-dependent variation for both, the recessive and co-dominant models ($P > 0.05$). Likewise, an association between genotype C/C and aggressiveness of the tumor (reflected as Gleason score) was not found ($P > 0.05$); however, it is important to mention that in this test only the two A/A cases were used in the comparison; this may have statistical implications. The subjects with A/A genotype corresponded to PCa cases moderately differentiated according to their Gleason's score which had values of 6 (3+3), and 7 (4+3), respectively. They had no family history of PCa and their PSA values were 10.1 and 13.8 ng/ml respectively.

Allele distribution analysis showed a minor allele frequency of 0.37 for cases and 0.27 for controls, respectively. The observed PCa protector effect of the A/A genotype was also reflected in the allele frequencies. When compared with C carriers, subjects with at least one A allele showed a diminished OR for PCa (OR = 0.65, 95% CI: 0.43–0.98, $P = 0.04$). There were no differences between alleles and tumor aggressiveness and/or PSA levels ($P > 0.05$).

4. Discussion

VEGF family plays prominent roles in several human diseases including cancer. VEGF is the major angiogenic cytokine mediator; therefore, it is crucial for tumor development and metastasis [8]. In addition to macrophages and cancer infiltrating T cells [19], VEGF is expressed by several human solid tumors including PCa [20–27]. VEGF over-expression in prostate tumors has been correlated with increased tumor microvessel density [28], disease progression, lymph node metastasis, resistance to treatment, and poor survival [10]. The -2578 C/A VEGF genetic variant has been associated with high levels of VEGF [12] therefore, it may influence the angiogenesis process, and/or it may be related to inter-individual variation for prostate tumors development. We evaluate the association between the -2578 C/A VEGF polymorphism and PCa in Mexican men. We report that this SNP may modify the risk of PCa up to 84%, supporting its involvement in prostate carcinogenesis. Despite that in Mexico there are no studies of -2578 C/A polymorphism and PCa, the frequency of -2578 A/A genotype has been reported in a range of 11–12% in Northwestern Mexican women [13] and Mexican ancestry participants of the HapMap International Project, respectively [29]. These data suggest that the low frequency of A/A carriers in the cases group observed in our study was directly related with their status of disease. Worldwide, there are two previous case-control studies evaluating the relationship between -2578 C/A VEGF SNP and PCa risk in U.S. and Austrian men, respectively [7,30]. Conversely to our findings, negative results for this association were reported in both protocols, reflecting the influence of inter-population genetic variability.

The -2578 C/A VEGF variant has been evaluated in several types of cancers with interesting results; for example, in Caucasian breast cancer patients, the A/A genotype was associated with increased breast cancer risk [31]. This genotype has also been associated with larger tumor sizes, poor differentiation, and advanced stage of disease in hepatocellular carcinoma [32]. Tunisian -2578 C allele carriers showed neoplastic progression and more aggressive forms of nasopharyngeal carcinoma [15]. Renal cell carcinoma patients carrying the A allele presented less lymph node metastases and a better survival rate than patients with the C/C genotype [33]. Conversely to positive reports, VEGF -2578 C/A was not associated to risk of gastric cancer or cutaneous malignant melanoma [34, 35]. In addition to differences in ethnic background between populations, the contrasting reports (including ours) might indicate that the involvement of VEGF -2578 C/A polymorphism in carcinogenesis may vary according to the kind of tumor and disease progression.

A possible mechanism by which this functionally relevant SNP affects the risk for some tumors may be related to the fact that the -2578 C/A SNP lies within a potential GATA-2 binding site and the C allele may favor the activation of the VEGF promoter by the GATA-2 transcription factor, a *cis*-acting element reported as a critical factor for tumor growth and angiogenesis [15]. The -2578 C/C genotype may therefore affect positively VEGF levels, enhancing angiogenesis and further expansion of tumor cells. Conversely, the -2578 A allele may influence the transcriptional activity by reducing the binding specificity of this motif. Our results are in agreement with this mechanism because -2578 C/C genotype conferred an increased risk of PCa up to 6.1 times in the study population, while the VEGF -2578 A/A genotype was associated to a protective effect for the disease (up to an 84%), and this behavior was constant for A allele carriers. In the same sense, despite that age is one of the most recognized risk factors for PCa, in our study PCa cases carriers of A allele, showed an onset of disease of around 4.1 years later than subjects with C/C genotype. Our findings are not unexpected and support the hypothesis of VEGF as a basic mediator of tumor angiogenesis [36]. In our study, an association between -2578 C/C genotype and inflammatory processes and/or aggressiveness of the tumor was not found, but it is possible that the small number of subjects with A/A genotype with PCa and benign prostatic tissue diagnosis may induce bias in these association tests.

Although this study included only one VEGF SNP, it showed that a common variant of this gene may be a valuable marker for predicting susceptibility to PCa and may provide direct evidence of the involvement of angiogenic genetic determinants in the PCa. Further studies on the effect of other VEGF polymorphisms, as well as the analyses of combined effects of additional genetic risk factors associated to angiogenesis pathway, are necessary for a better understanding of their role in determining risk and disease progression in PCa.

5. Conclusion

Inheritance of genotype -2578 A/A of VEGF gene may modify the PCa susceptibility in Mexican popula-

tion, providing a protective effect for the disease up to 84%.

List of abbreviations

VEGF:	Vascular endothelial growth factor.
PCa:	Prostate cancer.
SNP:	Single nucleotide polymorphism.
TRB:	Transrectal biopsy.
PSA:	Prostate specific antigen.
DRE:	Digital rectal examination.
TURP:	Transurethral resection.
OR:	Odds ratio.

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Conflict of interest

Authors declare that they have no competing interests.

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