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Association of Prostate-Specific Antigen Promoter Genotype with Clinical and Histopathologic Features of Prostate Cancer

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Abstract

The serum test for the secreted protease prostate-specific antigen (PSA) is the most widely used screening tool for prostate cancer. The PSA gene contains multiple functional and nonfunctional single nucleotide polymorphisms (SNP) in its promoter. We showed previously that the rs925013 G/A SNP, but not the rs266882 G/A SNP, was significantly associated with serum PSA in healthy men. In this study, we evaluated the association of the PSA promoter genotype with clinical data in a cohort of 1,224 men with prostate cancer. Previous work with a subset of this cohort has shown that percent high-grade (Gleason grades 4 and 5) cancer was the strongest predictor of biochemical recurrence (PSA relapse). We found a statistically significant association ($P < 0.05$) of the rs925013 SNP with several clinical and histomorphologic variables. The G allele was associated with higher serum PSA at diagnosis, higher percent Gleason grade 3 cancer, and lower percent high-grade and Gleason grade 4 cancer. The rs266882 SNP was modestly associated with PSA at diagnosis in a dominant model but was not associated with cancer grade. Neither SNP was associated with biochemical recurrence. The statistically significant predictors of biochemical recurrence were tumor location in the peripheral zone [odds ratio (OR), 10.71; 95% confidence interval (95% CI), 3.15-36.49], presence of any Gleason grade 4/5 cancer (OR, 4.26; 95% CI, 1.30-14.00), presence of any intraductal cancer (OR, 1.03; 95% CI, 1.00-1.04), and serum PSA at diagnosis (OR, 2.04; 95% CI, 1.50-2.77).

Introduction

Prostate cancer is the most common noncutaneous solid malignancy in U.S. men and the second leading cause of cancer death in this group (1). Numerous studies have attempted to define clinical and histologic variables that can be used as prognostic markers to predict

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which patients are likely to present with disease progression (2). The factors that have been studied include Gleason score, preoperative serum prostate-specific antigen (PSA), preoperative PSA doubling time, lymph node involvement, capsular penetration, positive surgical margins, seminal vesicle invasion, DNA ploidy in biopsy sample, and tumor location in the peripheral zone. Using morphometric measurements of radical prostatectomy specimens step-sectioned at 3-mm intervals combined with long-term follow-up, Stamey et al. have shown that percent cancer of Gleason grade 4/5, tumor volume, serum PSA, and prostate weight are the primary predictors of failure after radical prostatectomy (3). In addition, positive lymph nodes, location of tumor in the peripheral zone (versus transition zone), capsular penetration greater than 1 cm, seminal vesicle invasion, and positive surgical margins have been associated with biochemical failure (4-7). However, many of these factors are interrelated, making it difficult to distinguish between cause and effect. Using multivariate logistic regression, Stamey et al. showed that percent of cancer of Gleason grade 4/5 is the most significant independent predictor for biochemical relapse (3).

PSA is an androgen-regulated serine protease produced by secretory epithelial cells lining the lumen of normal prostatic glands and the majority of prostate cancers (8-10). The prostate is the major source of PSA in men (8, 10-12). Because of its prostate specificity, PSA has become the most widely used marker for prostate cancer screening and response to therapeutic intervention. In addition to the use of PSA as a serum marker for prostate cancer, much previous work has focused on the role of PSA as a growth factor protease (13-17). The resulting data have been somewhat contradictory in that some effects of PSA, such as proteolysis of insulin-like growth factor binding protein-3 (15, 16) and parathyroid hormone-related protein (13), are predicted to promote growth (18), whereas other effects of PSA, such as cleavage of latent transforming growth factor-β(19) and fibroblast growth factor (20, 21), might inhibit the growth of prostate cancer cells.

Evidence for a functional role of PSA in prostate cancer development has relied on genetic association studies using single nucleotide polymorphisms (SNP) in the PSA gene. The most widely used SNP for these studies is located in an androgen response element positioned -158 nucleotides 5′ to the transcription start site (G/A rs266882; ref. 22). Several case-control studies have evaluated the rs266882 SNP for its association with prostate cancer risk, with some finding increased risk (23-28) and others finding no risk effect (29-33). Work by us has failed to find a functional effect of this SNP on PSA promoter transcriptional activity or with an association of the SNP with circulating PSA levels (29, 32). Further work by us has identified a major haplotype in the upstream enhancer region of the PSA gene that is significantly associated with circulating levels of PSA in men with no detectable prostate cancer (34). This haplotype is composed of multiple SNPs in linkage disequilibrium, including several that we showed affected PSA promoter activity *in vitro*. Notably, the G allele of the G/A rs925013 SNP located at nucleotide -4,643 was significantly associated with 15% to 30% greater circulating PSA levels. Recent work by another group has shown significant association of the rs925013 SNP with prostate cancer risk in a case-control study population from Australia (30).

We hypothesized that genetic determinants of PSA gene expression are associated with prostate cancer disease aggressiveness. In this study, we tested the association of the

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rs266882 and rs925013 SNPs with clinical and histomorphologic variables, including percent of Gleason grade 3, 4, or 5 cancer and serum PSA at diagnosis, in 1,224 men with prostate cancer.

Materials and Methods

Study Subjects and Baseline Clinicopathologic Data

Supplementary Tables S1 and S2 present the clinical and pathologic data associated with this study population of 1,224 men who underwent radical prostatectomy at Stanford University Medical Center between 1983 and 1998. No other form of primary therapy was done on these men. There is no information on race in this cohort. Historically, during this period, greater than 95% of men who underwent prostatectomy at Stanford University were Caucasian. This study received institutional review board approval at both institutions.

Surgically removed prostates have been subjected to a comprehensive histopathologic review according to the method described previously (35). Briefly, each specimen was fixed in formalin, serially blocked at 3-mm intervals, and embedded in paraffin. A 5-μm-thick section was cut from each block and stained with H&E for histologic assessment. The cancer volume was calculated by tracing the exact tumor outline on each slide, determining the area of tumor at each level of section with a digitizing pad, summing the tumor areas at different levels multiplied by the section thickness, and correcting the volume for tissue shrinkage during processing. Tumor grade was determined according to the Gleason system (36). The Stanford-modified Gleason scale was used to estimate the proportion of the largest cancer in each case that was poorly differentiated (grades 4 and 5) or well differentiated (grades 1-3; ref. 37). The percentage of each cancer occupied by Gleason grades 4 and 5 (percent Gleason grade 4/5) was estimated by a single pathologist (J.E. McNeal). This measurement of tumor grade was shown to be the strongest predictor of PSA failure in a study of 372 men who represent a subset of the men included in the present study (3).

The linear extent of any full-thickness capsular penetration into periprostatic fat was quantified in centimeters parallel to the prostatic capsule. Any positive margins were likewise identified and quantified in centimeters of extent. Percent seminal vesicle invasion was estimated on a single coronal section.

The median follow-up is 62 months (interquartile range, 38-88 months), with 277 months the maximum follow-up. Biochemical (PSA) failure is used here as a longitudinal outcome measure. For most subjects, test results are available approximately every 6 months, although the length of follow-up and test intervals varies. Biochemical failure is defined as two consecutive PSA values above a cutoff point of 0.07 ng/mL for PSA measured by the sensitive Tosoh method and 0.2 ng/mL for measurements by less sensitive methods. When a subject experienced PSA failure, time to failure is calculated as the number of months between the date of surgery and the first of the two consecutive PSA values that exceeded the cutoff point. Subjects whose serum PSA level never drops to below the detectable level were classified as experiencing PSA failure at time 0. In our study group, 187 subjects experienced biochemical failure.

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DNA Isolation from Paraffin-Embedded Tissue

DNA was extracted from formalin-fixed, paraffin-embedded seminal vesicle tissues not invaded by prostate cancer as described previously (38) with minor modification.

Genotyping of PSA Promoter SNPs

The Sequenom platform was used to perform SNP genotyping. All of the procedures followed a standard Sequenom protocol. Briefly, PCR was done in a total volume of 5 μL with 10 ng genomic DNA, 2.5 mmol/L $MgCl_2$, 0.1 μL HotStarTaq polymerase (Qiagen), 200 μmol/L deoxynucleotriphosphates, and 200 nmol/L primers. The PCR cycling condition was as follows: 95°C for 15 min followed by 45 cycles of 95°C for 20 s, 50°C for 30 s, and 72°C for 1 min with final extension of 72°C for 3 min. The Homogeneous MassExtend reactions were done in a total volume of 9 μL with 50 μmol/L deoxynucleotriphosphates/dideoxynucleotriphosphates each, 0.063 μ/μL Thermo Sequenase (both from Sequenom), and 600 nmol/L extension primers. The PCR cycling condition was as follows: 94°C for 2 min followed by 99 cycles of 94°C for 5 s, 52°C for 5 s, and 72°C for 5 s. After cleaning up the Homogeneous MassExtend reaction products with the SpectroCLEAN, the products were spotted to SpectroCHIP using SpectroPOINT and then scanned through the SpectroREADER. Genotypes were automatically recorded by SpectroTyper 3.1 software.

The primers were designed using SpectroDESIGNER (Sequenom). All of the PCR primers have 10-base oligo tag sequences. For the rs266882, the first PCR primer was ACGTTGGATGCACACCCAGAGCTGTGGAAG, the second PCR primer was ACGTTGGATGGTG-CATCCAGGGTGATCTAG, and the extension primer was ATTGCAGAACAGCAAGT. For the rs925013, the first PCR primer was ACGTTGGATGATAGAGTCAA-GAGGGTACAG, the second PCR primer was ACGTTG-GATGTTGACCCTCTCTTTTAGGGC, and the extension primer was TTCTGACCTCCACCATG. Two CEPH-positive control DNAs and two water negative controls were arranged randomly in each 96-well DNA plate.

Statistical Analysis

Summary statistics (median, mean, range, and SD) were calculated for continuous variables, including age, prostate weight, percent of grade 3 tumor, percent of grade 4 tumor, percent of grade 5 tumor, percent of high-grade tumor (Gleason grades 4 and 5), and percent of intraductal tumor. Number and percentage of individuals who could be categorized into lymph node positive or negative, cure or recurrent, location (peripheral zone or transition zone), or seminal vesicle invasion negative or positive were also calculated. Associations of sequence variants with categorical variables were tested among cases using a χ^2 test of $2 \times N$ table. A Fisher's exact test was done when at least one of the cell counts was smaller than 5. A Kruskal-Wallis test is used for the general association when we have more than 2 values (three genotypes without assuming the genetic model), and a Wilcoxon test was used when we have two values to be tested (dominant model). PSA levels were logarithm transformed to improve assumption of normality. We tested the effect of prostate cancer risk associated genotypes of rs266882 and rs925013 on PSA levels using a 2 *df* general model and dominant model using regression analysis.

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Multivariate analysis was done to test the association between sequence variants and disease recurrence using logistic regression, adjusting for other clinical and histomorphologic phenotypes that included age at diagnosis, lymph node invasion, location, seminal vesicle invasion, percent of intraductal tumor, margin status (positive or negative), capsule penetration (positive or negative), and serum PSA (ln transformed). The above analysis was carried out using SAS (version 9.1; The SAS Institute). All reported *P* values are based on a two-sided test and an α level of 0.05 was considered statistically significant.

Results

We used the Sequenom Mass Array system to evaluate the genotype of the PSA promoter at the rs266882 and rs925013 SNPs in our study population. Two hundred thirty-eight and 239 of the 1,224 cases were not evaluable for the rs266882 and rs925013 SNPs, respectively (Table 1). One hundred fifty of these were due to unavailability of the tissue blocks for DNA isolation. There was no bias in disease aggressiveness among the samples with no tissue blocks available compared with those with tissue blocks available (data not shown). Of those samples with DNA extracted, 92% gave informative genotypes. The rs266882 SNP was present in the population with a roughly equal distribution of the A and G alleles, 49% and 51%, respectively (Table 1). The rs925013 SNP was present in the population with A and G distributions of 77.5% and 22.5%, respectively (Table 1). These distributions are consistent with other reports (22, 34, 39) and were in Hardy-Weinberg equilibrium.

Associations of the rs266882 SNP with the continuous variables of percent Gleason grades of cancer, prostate weight, and tumor volume were not statistically significant (Table 2). In contrast, the rs925013 SNP was statistically significantly associated with percent of high-grade (4 + 5) cancer, percent grade 3 cancer, and percent grade 4 cancer (Table 2). The rs925013 SNP was not significantly associated with percent grade 5 cancer, prostate weight, or tumor volume. For the significant associations of the rs925013 SNP, the less frequent G allele was associated with a higher median percent of grade 3 (more well-differentiated) cancer, whereas the A allele was associated with a higher median percent of high-grade (poorly differentiated) cancer.

Table 3 shows the associations of both SNPs with the categorical variables of lymph node invasion, presence of intraductal cancer, location of the tumor, and presence of seminal vesicle invasion. The only significant association was between the rs266882 SNP and lymph node invasion. The A allele was associated with less lymph node invasion.

Our previous study of healthy men not previously diagnosed with prostate cancer showed a significant association of the G allele of the rs925013 SNP with higher serum PSA levels (34). The rs266882 SNP was not significantly associated with serum PSA in that study. In this study, we tested the association of these two SNPs with serum PSA at the time of diagnosis (Table 4). Similar to our previous study, we found no significant association of the rs266882 SNP with serum PSA. Use of a dominant genetic model provided a modest statistically significant association of the A allele with lower PSA ($P = 0.03$). In concordance with our previous findings, we found a highly statistically significant association of the G allele of the rs295013 SNP with higher serum PSA, either with ($P =$

0.0008) or without ($P = 0.003$) the assumption of a dominant genetic model. Recessive models were also fit to the PSA values but did not provide additional benefit (data not shown).

In our study population, we have a minimum of 5-year follow-up in the majority of patients (990), with 187 experiencing biochemical failure (recurrence). We did multivariate analysis to test the association of multiple factors with disease recurrence. Neither the rs2662882 SNP nor the rs925013 SNP was associated with disease recurrence (Table 5). Presence of tumor in the peripheral zone [odds ratio (OR), 10.71; $P = 0.0001$], presence of any grade 4/5 tumor (OR, 4.26; $P = 0.02$), presence of intraductal cancer (OR, 1.03; $P = 0.005$), and PSA at diagnosis (OR, 2.04; $P < 0.0001$) were statistically significantly associated with disease recurrence. Kaplan-Meir survival analysis also revealed no statistical association of either SNP with disease recurrence (data not shown).

Discussion

In this study, we showed that the G allele of the rs925013 SNP in the PSA gene is associated with a higher percentage of well-differentiated Gleason grade 3 cancer in the prostates of men treated by radical prostatectomy. This allele is also associated with a higher serum PSA at diagnosis. These data are significant because they are the first to support a link between a functional SNP in the PSA gene and prostate cancer aggressiveness. These data also support our previous findings that showed an association of the rs925013 SNP with circulating PSA levels (34). However, this study extends those findings to PSA levels at diagnosis in men with prostate cancer. However, the data on disease aggressiveness and PSA at diagnosis are somewhat contradictory in that the G allele of the rs925013 SNP is associated with both higher percentage Gleason grade 3 (good prognosis) and higher serum PSA at diagnosis (poor prognosis). The root cause of this discrepancy is unknown and will likely require further mechanistic studies to resolve. A subset of this study population has been used previously to assess predictive markers of disease recurrence (3). In this study, we extended this approach to the entire population and found significant predictive value of tumor in the peripheral zone, presence of any grade 4/5 cancer, lymph node involvement, and serum PSA. We did not find association of either of the PSA SNPs evaluated with disease recurrence. However, if the PSA rs925013 SNP affects disease recurrence through affecting PSA levels and Gleason grade, then adjusting for these, as we did in our model, would be predicted to generate the null hypothesis. Additionally, the analysis of the rs925013 SNP was likely underpowered given the low prevalence of the G allele (22.5%) in this population. Previous work with a subset of this cohort showed that percent Gleason grade 4/5 cancer was predictive of disease recurrence (3). Our findings of significant association of the A allele of the rs925013 SNP with percent high-grade cancer suggests that a larger study may reveal association of this SNP with disease recurrence.

We did not find significant association of the rs2662882 SNP with any clinical variable, except lymph node invasion. This SNP was associated with PSA at diagnosis only when a dominant model was used. The isolated association of this SNP with lymph node invasion may indicate an effect of this SNP on invasion independent of the other variables. Alternatively, the observation may be the result of a statistical artifact. Results from

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previous studies with this SNP in case-controlled studies have been variable. In our hands, *in vitro* promoter assays failed to show a functional effect of this SNP on PSA promoter strength despite its presence in an androgen response element important for PSA gene expression (29). Our results were confirmed by one group (40), but a recent report suggests that the rs266882 SNP may alter androgen receptor affinity (28). Additional studies to interrogate the role of this SNP in PSA gene function may be warranted.

Previous studies have suggested a functional role for PSA in the etiology of prostate cancer based on analysis of biological targets of PSA proteolytic activity. Notably, these biological targets have included insulin-like growth factor binding protein-3 (15, 16), latent transforming growth factor-β(19), parathyroid hormone-related protein (13), and fibroblast growth factor (20, 21), all of which may regulate the growth and differentiation of the prostate cell and/or its neighbors. Direct biological evidence for the relevance of these or other targets of PSA is lacking. Whether PSA promotes, inhibits, or plays no role in prostate cancer growth, differentiation, or aggressiveness is unresolved.

Our use of a SNP in the PSA gene that affects the transcriptional strength of the PSA promoter combined with a well-defined study population offered us a unique ability to link PSA gene expression with prostate cancer aggressiveness. Our data suggest that increased PSA expression at the cellular level is protective against aggressive disease. One other study has used the rs925013 SNP in a prostate cancer case-control design to assess the association of this SNP with the presence or absence of prostate cancer (30). In that study, the G allele was associated with an increased risk of prostate cancer (OR, 1.4) but not with differences in serum PSA in controls or with disease recurrence. Differences between the present study and the study of Severi et al. could be due to differences in study design; the study of Severi et al. was powered to assess associations between cases and controls, whereas the present study assessed cases only and was designed to associate genotype with disease aggressiveness. The differences could also be due to differential functions of PSA in tumor development and tumor aggressiveness.

The association of the G allele of the rs925013 SNP with prostate cancer cases in the study of Severi et al. could also be a result of screening bias. In our previous work and in the present study, we have found a significant association of the G allele of this SNP with higher serum PSA levels. A PSA screening test on men with G at rs925013 is more likely to present above the threshold of suspicion; therefore, these men are more likely to be selected for further screening methods such as needle biopsy, resulting in a higher likelihood of cancer being detected. Men in this situation may have cancer detected at an earlier stage because of more aggressive screening. Resolving the root of these differences will require further investigation in additional study populations that are powered to detect both cancer development and its aggressiveness and perhaps in a study population in which cancer was detected before the PSA screening era.

In conclusion, we found a significant association of PSA promoter genotype with prostate cancer Gleason grade and PSA at diagnosis. Future work should focus on evaluation of the PSA promoter in additional study populations that can simultaneously evaluate the role of

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PSA in the development of prostate cancer and tumor aggressiveness with appropriate statistical power.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Genotype distribution

SNP	Unevaluable (*n*)	AA	AG	GG
rs266882	238	244 (24.75)	482 (48.88)	260 (26.37)
rs925013	239	600 (60.91)	326 (33.10)	59 (5.99)

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Table 2

Association tests for continuous phenotypes

	rs266882				rs925013			
	Genotype	*n*	Median (IQR)	*P**	Genotype	*n*	Median (IQR)	*P**
High grade (% grade 4/5)	GG	258	17.5 (5-50)	0.77	AA	596	15 (2-40)	0.03
	AG	478	20 (5-40)		AG	322	20 (5-40)	
	AA	242	15 (2-50)		GG	59	10 (2-30)	
Gleason grade 3 (%)	GG	258	80 (40-95)	0.74	AA	594	80 (40-95)	0.03
	AG	476	80 (50-95)		AG	321	80 (40-95)	
	AA	241	80 (40-98)		GG	59	90 (70-98)	
Gleason grade 4 (%)	GG	258	15 (5-40)	0.8	AA	594	15 (2-40)	0.04
	AG	477	15 (5-40)		AG	321	20 (5-45)	
	AA	240	15 (2-40)		GG	59	10 (2-25)	
Gleason grade 5 (%)	GG	255	0 (0-0)	0.86	AA	594	0 (0-0)	0.35
	AG	474	0 (0-0)		AG	317	0 (0-0)	
	AA	241	0 (0-0)		GG	58	0 (0-0)	
Prostate weight	GG	257	48 (38-62)	0.58	AA	595	46	0.29
	AG	478	42 (38-58)		AG	322	47	
	AA	242	46 (38-58)		GG	59	50	
Tumor volume	GG	258	2.6 (1.4-5.3)	0.86	AA	596	2.4 (1.3-4.8)	0.16
	AG	477	2.6 (1.3-5.0)		AG	321	2.7 (1.5-5.3)	
	AA	242	2.6 (1.3-4.9)		GG	59	2.9 (1.1-5.6)	

NOTE: IQR, interquartile range, which describes the range between the 25th and the 75th percentiles.

* *P* values based on Kruskal-Wallis test. The Wilcoxon rank-sum test was used when assuming genetic models.

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Table 3

Association of PSA genotype with categorical phenotypes

	rs266882				rs925013			
	Genotype	**Node(−)**		**Node(+)**	**Genotype**	**Node(−)**		**Node(+)**
Lymph node invasion	GG	234 (25.55)		22 (38.60)	AA	558 (60.85)		34 (61.82)
	AG	458 (50.00)		18 (31.58)	AG	303 (33.04)		18 (32.73)
	AA	224 (24.45)		17 (29.82)	GG	56 (6.11)		3 (5.45)
	P^*			0.02	$P^{\dagger}$			0.98
	Genotype	Cure		Recur	Genotype	Cure		Recur
Disease status	GG	212 (25.73)		44 (28.95)	AA	503 (61.04)		92 (60.93)
	AG	407 (49.39)		72 (47.37)	AG	271 (32.89)		51 (33.77)
	AA	205 (24.88)		36 (23.68)	GG	50 (6.07)		8 (5.30)
	P^*			0.71	P^*			0.92
	Genotype	0		>0	Genotype	0		>0
Intraductal[‡]	GG	206 (25.72)		52 (30.06)	AA	491 (61.30)		104 (60.47)
	AG	391 (48.81)		84 (48.55)	AG	259 (32.33)		60 (34.88)
	AA	204 (25.47)		37 (21.39)	GG	51 (6.37)		8 (4.65)
	P^*			0.37	P^*			0.61
	Genotype	Peripheral zone		Transition zone	Genotype	Peripheral zone		Transition zone
Location	GG	204 (25.76)		41 (29.83)	AA	488 (61.85)		83 (59.71)
	AG	389 (49.12)		62 (45.26)	AG	254 (32.19)		48 (34.53)
	AA	199 (25.13)		34 (24.82)	GG	47 (5.96)		8 (5.76)
	P^*			0.57	P^*			0.86
	Genotype	*	+	−	Genotype	*	+	−
Seminal vesicle invasion	GG	13 (25.49)	18 (33.96)	227 (26.00)	AA	38 (71.70)	26 (50.00)	531 (60.96)
	AG	24 (47.06)	24 (45.28)	430 (49.26)	AG	12 (22.64)	23 (44.23)	287 (32.95)
	AA	14 (27.45)	11 (20.75)	216 (24.74)	GG	3 (5.66)	3 (5.77)	53 (6.08)
	P^*			0.75	P^*			0.22

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NOTE: Genotype numbers: *n* (%).

[*] *P* values based on χ^2 test.

[†] P values based on Fisher's exact test.

[‡] Intraductal categorized by absence (0) or presence (>0). Treating as a continuous variable based on percent of tumor was not significant (data not shown).

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Table 4

Serum PSA and PSA promoter genotype

	Genotype	*n*	Mean* (95% confidence interval)	*P*†
rs266882	GG	253	11.25 (10.07-12.43)	0.08
	AG	473	9.68 (8.94-10.49)	
	AA	241	9.58 (8.58-10.70)	
Dominant	GG	253	11.25 (10.07-12.43)	0.03
	AG/AA	714	9.68 (9.12-10.38)	
rs925013	AA	589	9.21 (8.58-9.87)	0.003
	AG	315	11.02 (9.97-12.18)	
	GG	59	11.82 (9.49-14.88)	
Dominant	AA	589	9.21 (8.58-9.87)	0.0008
	AG/GG	374	11.13 (10.18-12.18)	

*PSA levels were log-transformed and adjusted for age and geographic region. The values presented were back-transformed.

†*P* values were obtained assuming a 2 *df* general test and dominant model, respectively.

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Table 5

OR estimates for disease recurrence

Variable	OR (95% confidence interval)	P*
rs925013 SNP (AG/GG vs AA)	0.74 (0.46-1.21)	0.23
rs266882 SNP (AG/AA vs GG)	0.95 (0.55-1.63)	0.84
Age at diagnosis	1.03 (0.99-1.06)	0.11
Lymph node (+ vs -)	0.98 (0.42-2.31)	0.97
Location (peripheral zone vs transition zone)	10.71 (3.15-36.49)	0.0001
Seminal vesicle (+ vs -)	1.69 (0.75-3.81)	0.2
Penetration (any vs 0)	1.40 (0.85-2.31)	0.07
Surgical margin (any vs 0)	1.64 (0.90-2.98)	0.11
Any grade 4/5 vs 100% grade 3	4.26 (1.30-14.00)	0.02
Intraductal (+ vs -)	1.03 (1.00-1.04)	0.005
PSA†	2.04 (1.50-2.77)	<0.0001

*Based on likelihood ratio test.

†ln transformed.