Variants in the *Prostate-Specific Antigen* (*PSA*) Gene and Prostate Cancer Risk, Survival, and Circulating PSA

Gianluca Severi,[1,2] Vanessa M. Hayes,[4] Petra Neufing,[5] Emma J.D. Padilla,[4] Wayne D. Tilley,[5,6] Sarah A. Eggleton,[4] Howard A. Morris,[6] Dallas R. English,[1,2] Melissa C. Southey,[3,7] John L. Hopper,[2] Robert L. Sutherland,[4] Peter Boyle,[7] and Graham G. Giles[1,2]

[1]Cancer Epidemiology Centre, The Cancer Council Victoria; [2]Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology and [3]Department of Pathology, University of Melbourne, Melbourne, Victoria, Australia; [4]Cancer Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, New South Wales, Australia; [5]Dame Roma Mitchell Cancer Research Laboratories, University of Adelaide; [6]Hanson Institute, Adelaide, South Australia, Australia; and [7]IARC, Lyons, France

Abstract

An A to G substitution, rs925013, in the promoter of the *prostate-specific antigen gene* (*PSA*) was recently found to be associated with promoter activity and circulating PSA levels. The objective of this study was to test the associations between rs925013 and another A to G substitution, rs266882, in the *PSA* gene with prostate cancer risk using a population-based case-control study of 821 prostate cancer cases and 734 controls carried out in Perth and Melbourne, Australia. The study focused on young (i.e., <70 years) and aggressive cases (i.e., well-differentiated tumors were excluded). Cases in the Melbourne arm of the study (N = 638) were followed up prospectively for an average period of 8.2 years and deaths from prostate cancer ascertained through record linkage to study the possible association between genetic variants and disease-specific survival. PSA-circulating levels were measured in controls to test the association with the genetic variants using a cross-sectional design. Linear regression of log PSA levels, unconditional logistic regression, Cox regression, and haplotype analyses were undertaken. For rs925013, the *G* allele was associated with an increased risk of prostate cancer [odds ratio, 1.4; 95% confidence interval (95% CI), 1.1-1.7; P = 0.001], and the hazard ratio for survival for cases homozygous for the *G* allele compared with cases homozygous for the *A* allele was increased but not statistically significant (hazard ratio, 2.3; 95% CI, 1-5.6; P = 0.06). For rs266882, there was no association with overall prostate cancer risk and survival (all P > 0.1). Men homozygous or heterozygous for the G/G (rs925013/rs266882) haplotype were at higher risk of prostate cancer than men homozygous for the A/A haplotype (odds ratio, 1.3; 95% CI, 1.1-1.7; P = 0.009). Adjusted geometric means of circulating PSA levels in controls were similar in men with zero, one, and two copies of the *G* allele in rs266882 (1.2, 1.1, and 1.3 ng/mL, respectively; all $P \geq 0.2$) and rs925013 (1.1, 1.2, and 1.5 ng/mL, respectively; all P > 0.1). For rs925013, our study provides good evidence of association with prostate cancer risk, marginal evidence of association with survival, and little evidence of detectable association with circulating PSA levels in controls. We found no evidence of an independent association between rs266882 and any of the outcomes. The genotypes and haplotypes studied might be associated with the *PSA* gene function or be in linkage disequilibrium with other unmeasured and functional variants in the *PSA* or other genes. (Cancer Epidemiol Biomarkers Prev 2006;15(6):1142–7)

Introduction

The *prostate-specific antigen* (*PSA*) gene, also known as *KLK3*, is located on the long arm of chromosome 19. This gene encodes for PSA, a widely used diagnostic marker for prostate cancer. PSA is a serine protease enzyme produced by the epithelial cells of the prostate and is present in very high concentrations in the seminal fluid. PSA inhibits the coagulation of the semen and enters the serum through leakage into the extracellular fluid of the normal prostate (1). However, during tumorigenesis, serum PSA levels are elevated probably due to the loss of the normal glandular architecture. PSA is therefore an effective marker of the progression of prostate cancer and a commonly used diagnostic marker. Several studies indicate mechanisms by which PSA could have itself tumor-promoting or antitumor activity, but there is no conclusive evidence to say whether PSA is simply a tumor marker or alters tumor progression (1).

The *PSA* gene contains several androgen-responsive elements, which mediate the transcriptional response of the activated androgen receptor that plays a key role in prostate cancer (1, 2). One androgen-responsive element is located in the proximal promoter at −156 to −170 bp from the transcriptional start site of the gene and contains a polymorphic locus (*rs266882*) at −158 (A to G substitution). Some studies (3-8), but not others (9-12), found that this polymorphism is associated with the development of prostate cancer or circulating PSA levels. A recent study further characterized the *PSA* gene for polymorphisms and identified several sequence variants, including one further upstream of the *PSA* promoter at position −4643 (rs925013; ref. 13). This A to G substitution was associated with increased serum PSA levels and transcriptional activity of PSA promoter constructs (13).

We tested the hypothesis that the two single-nucleotide polymorphisms in the *PSA* gene, rs925013 and rs266882, are associated with circulating levels of PSA, prostate cancer risk, and the risk of dying of the disease using blood samples collected during a large population-based case-control study of prostate cancer.

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Note: Current address for P. Neufing: Department of Immunology, Flinders Medical Centre, Adelaide, South Australia, Australia.

Requests for reprints: Graham G. Giles, Cancer Epidemiology Center, Cancer Control Research Institute, Anti-Cancer Council of Victoria, 100 Drummond Street, Carlton, Victoria 3053, Australia. Phone: 61-3-9635-5155; Fax: 61-3-9635-5330.
E-mail: graham.giles@cancervic.org.au

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Materials and Methods

Subjects. Subjects were participants in the Melbourne and Perth arms of the Risk Factors for Prostate Cancer study, an Australian population-based case-control study of prostate cancer conducted between 1994 and 1998 and described in detail elsewhere (14, 15). The study focus was the prevention of prostate cancers likely to contribute to premature mortality and, consequently, recruitment focused on tumors diagnosed at an early age and of more aggressive morphology. To this end, tumors that were well differentiated and those with Gleason scores <5 were excluded. Eligible cases with histopathologically confirmed adenocarcinoma of the prostate diagnosed before age of 70 years were ascertained from the Cancer Registries of Victoria and Western Australia. Random samples of 100%, 50%, and 25%, respectively, of the cases diagnosed in the age groups <60, 60 to 64, and 65 to 69 years were asked to participate in the study. Eligible controls were randomly selected from males on the State Electoral Rolls (registration to vote is compulsory for adult Australian citizens) and were frequency matched to the expected age distribution of the prostate cancer cases in a ratio of one control per case. A total of 1,047 cases and 1,058 controls participated in the study (65% and 50%, respectively, of those eligible; ref. 16). A face-to-face interview was conducted using structured questionnaires to obtain information on potential risk factors, including age, history of prostate cancer in first-degree relatives, country of birth, lifestyle (including diet), and other potential risk factors for prostate cancer. Tumor stage (stage I-IV according to the American Joint Committee on Cancer; ref. 17) and grade (moderate, Gleason 5-7 or moderately differentiated; high, Gleason 8-10 or poorly differentiated) was recorded from histopathology reports.

Informed consent was obtained from all study participants. Blood samples were available from 831 cases (79% of participants) and 738 controls (70%). A detailed description of participant characteristics has been published (18). Vital status as of December 31, 2004 and cause of death were determined for the 640 cases in the Melbourne arm of the study by linking these cases to the Victorian Registry of Births, Deaths, and Marriages. During an average follow-up of 8.2 years, 68 cases (11%) were found to have died from prostate cancer.

Genotyping of the *PSA* Gene Polymorphisms. Genomic DNA was extracted from whole blood and genotyped blind to case-control status. The rs266882 polymorphism was genotyped using PCR-based denaturing gradient gel electrophoresis and the Ingeny phorU-2 denaturing gradient gel electrophoresis system (Ingeny International, Goes, the Netherlands; http://www.ingeny.com). The following primer pair, forward (5′-GTGCATCCAGGGTGATCTAGTA-3′) and reverse (5′-CTGCTGGAGGCTGGACAAC-3′), was used to amplify the 141-base fragment. To prevent complete strand dissociation during electrophoresis in a 9% polyacrylamide gel containing a 40% to 80% urea and formamide, denaturing gradient, a 40-base GC-clamp was added to the 5′-end of the forward primer. Conditions of amplification, thermal cycling, and gel conditions are available on request. The rs266882 AG genotype was identified as four denaturing gradient gel electrophoresis bands (two homoduplex and two heteroduplex), whereas the AA and GG genotypes were identified as a single upper and lower homoduplex band, respectively (Fig. 1). A random selection of 143 samples (9%) were regenotyped with a concordance rate of 100%. The rs925013 was genotyped using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Compact Sequenom MassArray system, Sequenom, San Diego, CA). Amplification was done using the following primer pair, forward (5′-ACGTTGGATGATAGAGTCAAGAGGGTACAG-3′) and reverse (5′-ACGTTGGATGTTGACCCTCTCTTTTAGGGC-3′), whereas the extension reaction was done using the extended primer (5′-TTTCTGACCTCCACCATGA-3′) and 1× ACT termination mix (ddATP, ddCTP, ddTTP, and dGTP). Conditions for amplification and the homogenous mass extend reaction are available on request. Purified extended products were dispensed onto a SpectroCHIP and genoptyped using SpectroTYPER on the MassArray. A random selection of 659 samples (42%) were regenotyped with a concordance of 99.4%.

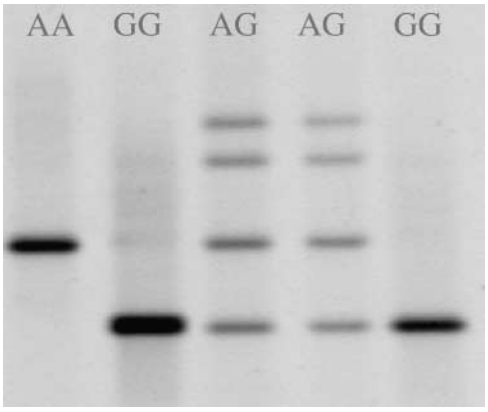

Figure 1. Denaturing gradient gel electrophoresis banding patterns for the rs266882 polymorphism. The AG genotype is depicted by four bands (two homoduplex and two heteroduplex), whereas the AA and GG genotypes are depicted by a single upper and lower homoduplex band, respectively.

Measurement of Plasma Levels of PSA in Controls. Plasma was separated by centrifugation of 10 mL fresh blood (collected in tubes containing EDTA) at 2,000 × *g* for 15 minutes and stored in 0.5 mL aliquots at −70°C. Levels of PSA in plasma were measured in the laboratory of one of the authors (H.M.) using a commercially available Microparticle Enzyme Immunoassay (AxSYM analyzer, Abbott Laboratories, Abbott Park, Illinois) with a coefficient of variation of 9.5% at 0.4 ng/mL.

Statistical Methods. Estimates of allele frequencies and tests of deviation from Hardy-Weinberg equilibrium were carried out using standard procedures based on asymptotic likelihood theory (19). Linkage disequilibrium between the two variants was assessed by using Lewontin's D′ (20), and tests for significance were based on asymptotic likelihood theory. Fisher's exact test was used to test for independence between the single-nucleotide polymorphisms and categorized risk factors [i.e., age (<55, 55-64, and 65-69), country of birth (Australia and others), family history of prostate cancer (affected first-degree relatives and no affected relatives), and tumor stage (stage I-IV) and grade (moderate and high)]. Tests for association between genotypes and the various outcome of interest (i.e., prostate cancer risk, disease-specific survival, and circulating levels of PSA) were done under codominant, dominant, and recessive models. Case-control analyses were conducted using unconditional logistic regression (21), and odds ratio (OR) estimates and their 95% confidence intervals (95% CI) were derived under likelihood theory. Adjustment for country of birth, age, history of smoking, history of prostate cancer in first-degree relatives (family history), body mass index, and alcohol consumption did not materially change the OR estimates from the logistic models. Polytomous logistic regression models were used to estimate ORs by tumor stage (dependent variable with three categories: 0, 1, and 2 for controls, stage I-II tumors, and stage III-IV tumors, respectively) and grade (dependent variable with three categories: 0, 1, and 2 for controls, moderate-grade, and high-grade tumors, respectively).

Survival analysis of prostate cancer cases in the Melbourne arm of the study was used to test the possible effect of genotypes on the risk of dying from prostate cancer. For this purpose Cox regression models were used to estimate the hazard ratios (HR) (22), adjusted for tumor grade and stage, country of birth, age, history of smoking, and family history. Factors were considered confounders and therefore included in the Cox models if they changed the HRs of any of the genetic variants by at least 5%. Further adjustment for body mass index and alcohol consumption did not materially change the HR estimates.

Serum PSA levels were highly skewed, so we used linear regression of the $\log_{10}$-transformed levels of PSA in controls to test the possible association between genotypes and circulating PSA. The linear regression models were adjusted for age and laboratory assay and were first fitted using all the controls and then refitted excluding those with PSA levels of >9 ng/mL as in Cramer et al. (13). Results are presented as adjusted back-transformed means (i.e., geometric means) and their corresponding 95% CI derived from the fitted regression models. These statistical analyses were done using Stata/SE 8.2 (Stata Corporation, College Station, TX).

Estimates of haplotype frequency and tests of association between haplotypes and prostate cancer risk and serum PSA levels were done using the suite of routines HaploStats 1.2.1 (http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm) run from the statistical program R 2.1.1 (http://www.r-project.org). These routines use the expectation maximization algorithm to compute maximum likelihood estimates of haplotype probabilities from genetic markers of unknown linkage phase and posterior probabilities that are then incorporated in regression models for binary or normal outcomes to test for association.

The likelihood ratio test was used to test nested hypotheses and the Wald test to assess statistical significance of individual variables. All tests were two sided. Following convention, nominal statistical significance was based on $P < 0.05$. No attempt was made to adjust for multiple comparisons.

Results

Genotyping of both variants failed in only ~1% of the samples leaving 821 cases and 734 controls with at least one of the two variants determined (812 cases and 713 controls with both variants determined). Only five cases (<1%) and seven controls (~1%) were not of Caucasian origin, with the great majority of subjects (98.5%) being born in Australia, British Isles, or Western Europe. Half of the cases were aged between 55 and 64 years (428, 52%), and 111 cases (14%) were aged <55 years. Two hundred and fifty-two cases (31%) had a stage III or stage IV tumor, and 219 cases (27%) were poorly differentiated or had a Gleason score >8.

The distribution of the genotypes was consistent with Hardy-Weinberg equilibrium for both loci in cases and controls and in cases and controls combined (all $P > 0.1$). The two loci were in strong linkage disequilibrium, and D′ was virtually identical in cases and controls ($D' = 0.89$ for cases and controls combined; $P < 0.001$). There was no evidence of association between either genotype and age, country of birth, and family history of prostate cancer (all $P > 0.06$).

Genotypes and Prostate Cancer Risk. For the rs925013 variant, the frequency of the *G* allele was 18% in controls and 23% in cases, and this allele was associated with increased risk of prostate cancer ($P = 0.006$ and 0.001 from the codominant and dominant models, respectively). The ORs for men heterozygous and homozygous for the *G* allele relative to men homozygous for the *A* allele were 1.4 (95% CI, 1.1-1.7; $P = 0.003$) and 1.5 (95% CI, 0.9-2.4; $P = 0.1$), respectively (codominant model). The prevalence of the *G* allele was similar in stage III to IV and stage I to II tumors (42% and 40%, respectively; $P = 0.5$), and the ORs did not differ significantly by tumor stage (all $P > 0.4$). The prevalence of the *G* allele in moderate-grade tumors was higher than in high-grade tumors (43% and 34%, respectively; $P = 0.02$), and we found marginal evidence that ORs for moderate-grade tumors, ranging from 1.3 to 1.6, were higher than ORs for high-grade tumors ($P = 0.04$, 0.02, and 0.2 for the codominant, dominant, and recessive models, respectively).

For the rs266882 variant, the frequency of the *G* allele was 48% in controls and 50% in cases, and there was little evidence of an association with prostate cancer risk from the codominant, dominant, and recessive models (ORs range, 1-1.2; all $P \geq 0.1$; Table 1). The prevalence of the *G* allele in stage III to IV tumors was higher than in stage I to II tumors (81% and 72%, respectively; $P = 0.006$). The ORs for stage III to IV tumors were higher than unity (OR, 1.3; 95% CI, 0.9-1.9; $P = 0.1$ and OR, 1.9; 95% CI, 1.3-3; $P = 0.002$ from the codominant model; OR, 1.5; 95% CI, 1.1-2.2; $P = 0.02$ from the dominant model; and OR, 1.6; 95% CI, 1.2-2.2; $P = 0.004$ from the recessive model) and significantly higher (all $P < 0.005$) than the ORs for stage I to II tumors that ranged from 0.9 to 1 (all $P > 0.3$). The prevalence of the *G* allele was similar in moderate- and

Table 1. *PSA* gene polymorphisms in controls and prostate cancer cases

	Controls, $N = 734$* (%)	Cases, $N = 819$* (%)	OR† (95% CI)	P‡
rs925013				
AA	495 (67)	488 (60)	Reference	0.006
AG	208 (28)	286 (35)	1.4 (1.1-1.7)	
GG	31 (4)	45 (5)	1.5 (0.9-2.4)	
AA	495 (67)	488 (60)	Reference	0.001
AG, GG	239 (32)	331 (40)	1.4 (1.1-1.7)	
AA, AG	703 (96)	774 (95)	Reference	0.2
GG	31 (4)	45 (5)	1.3 (0.8-2.1)	
rs266882				
AA	191 (27)	210 (26)	Reference	0.3
AG	366 (51)	400 (49)	1.0 (0.8-0.3)	
GG	156 (22)	204 (25)	1.2 (0.9-1.6)	
AA	191 (27)	210 (26)	Reference	0.7
AG, GG	522 (73)	604 (74)	1.1 (0.8-1.3)	
AA, AG	557 (78)	610 (75)	Reference	0.1
GG	156 (22)	204 (25)	1.2 (0.9-1.6)	

*Number of subjects with at least one of the two variants measured.
†ORs and 95% CIs from unconditional logistic regression analysis.
‡Test for association between genotype and prostate cancer risk (likelihood ratio test).

Table 2. *PSA* gene polymorphisms in controls and prostate cancer cases. Estimated frequency of haplotypes and association with prostate cancer risk

Haplotype	Frequency* (%)		Effect	P ‡
Rs925013/rs266882	Controls, N = 734	Cases, N = 819	OR † (95% CI)	
Global score statistic				0.02
Additive§				
A/A	52	49	Reference	
A/G	30	28	1 (0.8-1.2)	0.9
G/A	1	1	1.7 (0.7-3.8)	0.2
G/G	18	22	1.3 (1.1-1.6)	0.008
Dominant‖				
A/A	52	49	Reference	
A/G	30	28	0.9 (0.7-1.1)	0.4
G/A	1	1	1.5 (0.7-3.6)	0.3
G/G	18	22	1.3 (1.1-1.7)	0.009
Recessive¶				
A/A	52	49	Reference	
A/G	30	28	1.1 (0.7-1.5)	0.8
G/A	1	1	NA	NA
G/G	18	22	1.3 (0.8-2.2)	0.2

*Estimated haplotype probabilities estimated from genotype data using the haplo.cc function of the HaploStats library in R.
†ORs obtained using recursively the estimated posterior probabilities of pairs of haplotypes per subjects as weights in the logistic model (haplo.cc function of the HaploStats library in R).
‡The P for the global score statistic corresponds to the test for overall association between haplotypes and prostate cancer. The other Ps based on the score statistics (Schaid et al., 2002) correspond to the test for association between the specific haplotype and prostate cancer risk.
§In the additive model, it is assumed a linear effect of the number of copies of the haplotype (0, 1, and 2). Therefore, ORs estimate the risk for men carrying one copy of the haplotype relative to the risk for men homozygous for the reference haplotype (A/A). The ORs for men carrying two copies of the haplotype can be obtained by applying the following formula: $\exp[2 \times \log(OR_1)]$, where OR_1, the OR for men carrying one copy of the haplotype, is reported in the table.
‖In the dominant model, the ORs estimate the risk for men heterozygous or homozygous for the haplotype relative to the risk for men homozygous for the reference haplotype (A/A).
¶In the recessive model, the ORs estimate the risk for men homozygous relative to the risk for men heterozygous (for the same haplotype) or homozygous for the reference haplotype (A/A).

high-grade tumors (74% and 75%, respectively; P = 0.7; data not shown), and the ORs did not differ significantly by tumor grade (all $P > 0.6$).

Haplotypes and Prostate Cancer Risk. The most common haplotype was A/A (rs925013/rs266882), and this was carried by 50% of men (Table 2). The second most prevalent haplotype was A/G (29%) followed by G/G (18% in controls and 22% in cases) and G/A (1%).

There was evidence of an overall association between haplotype distribution and case-control status (P from global score statistic = 0.02). For included haplotypes, the A/G haplotype was not associated with prostate cancer risk (ORs range, 0.9-1.1; all $P \geq 0.4$ from the additive, dominant, and recessive models, respectively). The G/G haplotype was associated with an increased risk of prostate cancer (P = 0.008 and 0.009 from the additive and dominant models). From the additive model, the ORs for men carrying one or two copies of the G/G haplotype compared with men homozygous for the A/A haplotype were 1.3 (95% CI, 1.1-1.6; P = 0.008) and 1.7 (95% CI, 1.1-2.4; P = 0.008), respectively. The G/A haplotype was too rare to estimate its association with prostate cancer risk.

Risk of Dying From Prostate Cancer. Although the overall tests for association between the rs925013 variant and disease-specific survival were not statistically significant (all $P \geq 0.1$) from the codominant model, the HRs for cases heterozygous and homozygous for the *G* allele compared with cases homozygous for the *A* allele were 1.2 (95% CI, 0.7-2.1; P = 0.4) and 2.3 (95% CI, 1-5.6; P = 0.06), respectively. The rs266882 variant was not associated with disease-specific survival of prostate cancer cases (HRs range, 0.9-1.2; all $P \geq 0.5$; Table 3).

Circulating PSA Levels, Genotypes, and Haplotypes. For the rs925013 variant, adjusted geometric means increased from 1.1 (95% CI, 1-1.2) and 1.2 (95% CI, 1-1.4) to 1.5 ng/mL (95% CI, 1.1-2) for men with zero, one, and two copies of the *G* allele, respectively, but the tests for association between PSA levels and genotype were all not significant ($P > 0.1$). The exclusion of controls with PSA levels of >9 ng/mL (N = 6) slightly decreased the difference in circulating PSA by rs925013 genotype. We found no evidence that circulating PSA levels (Fig. 2) were associated with the rs266882 variants, with adjusted geometric means being 1.2 (95% CI, 1.1-1.4), 1.1 (95% CI, 1-1.2), and 1.3 ng/mL (95% CI, 1.1-1.5), respectively for men with zero, one, and two copies of the *G* allele (all Ps ≥ 0.2 from the codominant, dominant, and recessive models).

Circulating PSA levels were virtually identical in men carrying different haplotypes but for men carrying the G/G haplotype (rs925013/rs266882) that had slightly higher levels than men homozygous for the A/A haplotype. None of the estimates, however, were statistically significant (all $P > 0.7$ from the additive, dominant, and recessive models).

Discussion

For the rs925013 variant, our study provides good evidence that the *G* allele is associated with a 40% increased risk of developing prostate cancer. The increased risk of prostate cancer in carriers of the *G* allele in rs925013 was not confined to early- or advanced-stage tumors, but it was more evident in moderate-grade than in high-grade tumors. This study also found marginal evidence that prostate cancer cases homozygous for the *G* allele have a 2-fold increased risk of dying from the disease than cases carrying other genotypes. Little evidence of association with circulating PSA levels was found in this study. For the rs266882 variant, this study found no evidence of association with the risk of prostate cancer and the risk of dying from the disease. The *G* allele was associated with an increased risk of locally advanced and distant-stage tumors (stage III-IV) but not of early-stage tumors (stage I-II). No evidence of association with circulating PSA levels was found. Haplotype analysis showed that the risk of developing prostate cancer for men carrying the G/G haplotype (rs925013/rs266882) is >30% than the risk for men carrying two copies of the A/A haplotype.

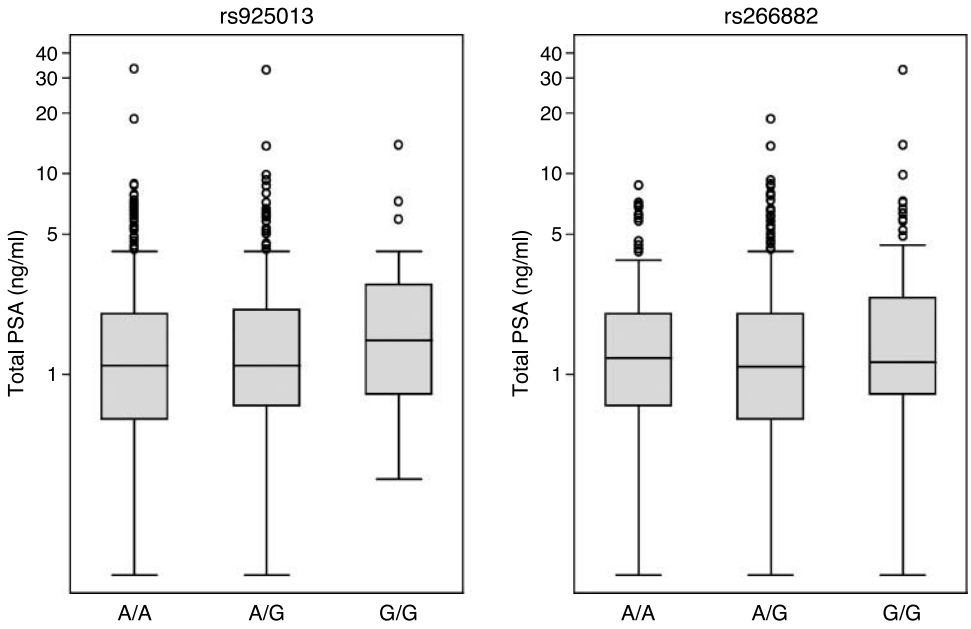

Figure 2. Circulating levels of PSA by genotype (*PSA* gene) in 733 controls.

The main strength of this study is the large number of cases and controls. With 1,553 participants, this is by far the largest study on the association between genetic polymorphisms in the *PSA* gene and prostate cancer with almost the same number of participants as all the previous studies combined. This is also the first study that followed up cases to test the possible influence of the variants in the *PSA* gene on survival and the largest among the studies that measured circulating PSA levels in controls to look for clues of the potential biological effects of the *PSA* gene variants. In addition, haplotype analysis, done using statistical techniques to derive haplotypes from genotype data, provided additional information to the standard genotype analysis, although the added information was relatively limited because the two variants were in linkage disequilibrium. The inclusion of other variants from the series identified by Cramer et al. would have added little information to the haplotype analysis because these variants were all in strong linkage disequilibrium (13).

One weakness of the study is the limited power of the survival analysis, with 68 deaths due to prostate cancer during the follow-up period. Another weakness was that we could not adjust the survival analysis for PSA levels at diagnosis because we had incomplete information on PSA levels at diagnosis for the cases. This adjustment would have allowed us to test whether the increased HRs for cases homozygous for the *G* allele in rs925013 were due to increased PSA levels at diagnosis. Although we found little evidence of an association between circulating PSA levels and genotypes in controls, we cannot exclude an association between genotypes and PSA levels at diagnosis for cases. A case-control study originally reported that men with longer CAG repeats in the androgen receptor gene and who were also carriers of the *G* allele in rs266882 of the *PSA* gene had lower levels of serum PSA (4). We could not test this hypothesis because we did not measure the number of CAG repeats in the androgen receptor gene, but this finding was not replicated in other studies (6, 9). Although

Table 3. HRs by genotype for the prostate cancer cases in the Melbourne arm of the Risk Factors for Prostate Cancer study

	Cases, *N* = 638*	Deaths from prostate cancer, *N* = 68 (%)	HR† (95% CI)	*P*‡
PSA gene (rs925013)				
AA	381	39 (10)	Reference	0.2
AG	217	23 (11)	1.2 (0.7-2.1)	
GG	40	6 (15)	2.3 (1-5.6)	
AA	381	39 (10)	Reference	0.2
AG, GG	257	29 (11)	1.4 (0.8-2.3)	
AA, AG	598	62 (10)	Reference	0.1
GG	40	6 (15)	2.1 (0.9-5)	
PSA gene (rs266882)				
AA	164	14 (9)	Reference	0.8
AG	309	32 (10)	1.0 (0.5-1.9)	
GG	161	21 (13)	1.2 (0.6-2.4)	
AA	164	14 (9)	Reference	0.9
AG, GG	470	53 (11)	1.1 (0.6-2)	
AA, AG	473	46 (10)	Reference	0.5
GG	161	21 (13)	1.2 (0.7-2.1)	

*Two cases were excluded because grade or tumor stage was not available.
†HRs from Cox models where the event of interest was death from prostate cancer. Cases that did not die from prostate cancer were censored at death from other causes or at the end of follow-up (December 31, 2004). The Cox models were adjusted for age at diagnosis, country of birth, family history of prostate cancer (presence of at least one first-degree relative diagnosed of prostate cancer), history of smoking (never, former, and current smoker), and tumor stage (stage I-II, III, and IV) and grade (Gleason score 5-7 or moderately differentiated and Gleason score 8-10 or poorly differentiated).
‡Statistical significance for association between genotype and disease-specific survival from likelihood ratio test.

we think it unlikely that genotypes were different in respondents and nonrespondents, limited response rates is another weakness of our study.

Although we cannot rule out the role of chance, there are three plausible explanations for the increased risk of prostate cancer for carriers of the *G* allele in rs925013 and for the possible increased risk of dying of the disease in cases homozygous for the same allele. The first is that rs925013 plays a role in the development of prostate cancer and in its progression from latent to more aggressive forms, independently of circulating PSA levels. The results of the case-control comparison by tumor stage and grade, however, do not provide evidence of a higher prevalence of the *G* allele in rs925013 in men with stage III to IV or high-grade tumors and do not explain the results from the survival analysis. The second explanation is that the genetic variant is associated with higher levels of PSA, and, therefore, the association between variant and prostate cancer is only a diagnostic artifact. Our lack of association between rs925013 and circulating PSA levels in controls is evidence against this explanation. However, our results are in contrast with the study by Cramer et al. who showed increased promoter activity and increased PSA levels in carriers of the *G* allele (13). Third, the associations we found may reflect associations with other genetic variants in linkage disequilibrium with rs925013. Cramer et al., for example, showed that other polymorphisms in strong linkage disequilibrium with rs266882 and rs925013 are associated with variation in circulating levels of PSA (13). The two variants might also be in linkage disequilibrium with variants in other genes involved in the pathway to prostate cancer.

Our results are more consistent with the first explanation. Carriers of the *G* allele in rs925013 would have an increased risk of prostate cancer not because they have higher levels of PSA but for some other reason. Future studies will have to test this hypothesis through a prospective design or by systematically collecting PSA levels at diagnosis before treatment in a large series of prostate cancer cases. A longer follow-up of these cases in the present study will be necessary to confirm the possible association between rs925013 and survival.

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