Short Communication

The Common Variant rs1447295 on Chromosome 8q24 and Prostate Cancer Risk: Results from an Australian Population-Based Case-Control Study

Gianluca Severi,[1,2] Vanessa M. Hayes,[4,5] Emma J.D. Padilla,[4] Dallas R. English,[1,2] Melissa C. Southey,[3,6] Robert L. Sutherland,[4,5] John L. Hopper,[2] 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, Darlinghurst, New South Wales, Australia; [5]Faculty of Medicine, University of New South Wales, Sydney, New South Wales, Australia; and [6]International Agency for Research on Cancer, Lyons, France

Abstract

A recent study from deCode reported an association between common variants in the region 8q24 and prostate cancer risk. The strongest association was found with the single nucleotide polymorphism rs1447295. We genotyped 821 prostate cancer cases and 732 population controls for rs1447295 to test the association between this common variant and prostate cancer risk, and examine whether this association depends on Gleason score. Our case-control study confirmed the association between rs1447295 and prostate cancer risk ($P = 0.0005$). The odds ratio (OR) for prostate cancer was 1.52 [95% confidence interval (CI), 1.20-1.93] for carriers of any A allele compared with noncarriers. The OR for Gleason score 5 to 6 prostate cancer (1.48; 95% CI, 1.13-1.95) was similar to the OR for Gleason score 7 to 10 prostate cancer (1.58; 95% CI, 1.18-2.11, P for heterogeneity = 0.7). We conclude that the A allele of rs1447295 is associated with a higher risk of prostate cancer regardless of tumor aggressiveness, suggesting that such a variant, or a variant in linkage disequilibrium with it, plays a role early in prostate carcinogenesis. (Cancer Epidemiol Biomarkers Prev 2007;16(3):610–2)

Introduction

Several lines of evidence support a role for genetic factors in the development of prostate cancer, including evidence from twin studies and studies of familial aggregation (1-3). Identifying genes associated with risk of prostate cancer is, therefore, attracting a great deal of attention but has proved difficult (4). Recently, evidence emerged from one of the deCode projects linking prostate cancer and gene(s) in the q24 region on chromosome 8 (5). This came from scanning 1,068 microsatellite markers in 871 Icelandic prostate cancer cases grouped into 323 extended families (5). In a case-control comparison from the same study, several microsatellites and single nucleotide polymorphisms in the region were found to be associated with prostate cancer risk. The variants with the strongest association were the DG8S737 ''−8'' allele and the A allele of rs1447295. The odds ratio (OR) for prostate cancer was 1.72 ($P = 1.7 \times 10^{-9}$) for carriers of any A allele of rs1447295 compared with noncarriers. This association was replicated in the same report by two other European-based case-control series from Sweden and Chicago (5).

We used our population-based case-control study from Australia to examine the association between the A allele in rs1447295 and prostate cancer risk and to determine whether the association varied by tumor aggressiveness (i.e., Gleason score).

Materials and Methods

Study Population. Details of the study are described elsewhere (6, 7). In brief, eligible cases with histopathologically confirmed adenocarcinoma of the prostate diagnosed in Melbourne and Perth, Australia in the period 1994 to 1997 were ascertained from the Cancer Registries of Victoria and Western Australia. Our recruitment was restricted to tumors diagnosed at an early age (<70 years) and of more aggressive morphology (Gleason score ≥5) as described elsewhere (6, 7). Tumor stage (stages I-IV; ref. 8), grade, differentiation, or Gleason score were recorded from histopathology reports. Eligible controls were randomly selected from males registered on the Electoral Rolls (registration to vote is compulsory), and were frequency-matched by age to cases. Information on age, history of prostate cancer in first-degree relatives, country of birth, life-style, and other potential risk factors for prostate cancer were obtained in face-to-face interviews from 1047 cases and 1058 controls that decided to participate in the study (65% and 50% respectively, of those eligible) (9). Informed consent was obtained from all study participants. Blood samples were available from 831 cases (79% of participants) and 738 controls (70%). A description of participant characteristics has been published (10).

Genotyping. Genomic DNA was buffy coat–extracted and the rs1447295 was genotyped using fluorescent-based TaqMan allelic discrimination (Applied Biosystems, Foster City, CA). A total reaction volume of 5 μL included 5 ng of

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Note: G. Severi and V.M. Hayes contributed equally to the study.

Requests for reprints: Gianluca Severi, Cancer Epidemiology Centre, The Cancer Council Victoria, 1 Rathdowne Street, Carlton, Victoria 3053, Australia. Phone: 61-39635-5412; Fax: 61-39635-5330. E-mail: gianluca.severi@cancervic.org.au

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Table 1. The rs1447295 variant in 8q24 and prostate cancer risk in the Australian Risk Factors for Prostate Cancer case-control study

	Controls (%), N = 732	Cases (%), N = 821	OR* (95% CI)	P †
Codominant model				0.002
CC	586 (80.1)	595 (72.5)	Reference	
CA	135 (18.4)	212 (25.8)	1.55 (1.21-1.97)	0.0005
AA	11 (1.5)	14 (1.7)	1.25 (0.56-2.78)	0.6
	$P_{H\text{-}W}$‡ > 0.9	$P_{H\text{-}W}$ = 0.4		
Dominant model				0.0005
CC	586 (80.1)	595 (72.5)	Reference	
Any A	146 (19.9)	226 (27.5)	1.52 (1.20-1.93)	

*ORs and 95% CIs from unadjusted unconditional logistic regression analysis. Adjustment for family history of prostate cancer, age, country of birth, body mass index, and smoking history did not materially change the OR estimates.
†Test for association between genotype and prostate cancer risk (likelihood ratio test).
‡Exact P value relative to the test for Hardy-Weinberg equilibrium.

template DNA, 2.5 μL of 2× TaqMan Universal PCR Master Mix, and 0.125 μL of 20× single nucleotide polymorphism genotyping assay mix. PCR cycling was done using an ABI Prism 7900HT sequence detection system under the following conditions: 95°C for 10 min followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. The ABI Prism 7900HT sequence detection system and the ABI sequence detection system software version 2.2 were used for genotype analysis.

Statistical Analysis. Estimates of allele frequencies and tests of deviation from Hardy-Weinberg (H-W) equilibrium were carried out using standard procedures based on asymptotic likelihood theory (11). Fisher's exact test was used to test for independence between the single nucleotide polymorphism and age (<55, 55-64, 65-69), country of birth (Australia or others), family history of prostate cancer (affected first-degree relatives or no affected relatives), and tumor stage (stage I-II, III, or IV). To maintain consistency with the report from deCode (5), Gleason score 5 to 6 tumors were grouped as moderately differentiated, whereas Gleason score 7 tumors were grouped with Gleason score 8 to 10 as poorly differentiated or undifferentiated tumors. Tests for association between genotype and prostate cancer risk were done under codominant and dominant models. Case-control analyses were conducted using unconditional logistic regression (12) to estimate ORs and their 95% confidence intervals (CI). Polytomous logistic regression models were used to estimate ORs by tumor stage, Gleason score, and age at diagnosis. Potential confounders (i.e., country of birth, age, history of smoking, history of prostate cancer in first-degree relatives, and body mass index) were included in the models if they changed the ORs by at least 5%. All statistical analyses were done using Stata/SE 8.2 (Stata Corporation, College Station, TX). We used the likelihood ratio test to assess the relative fits of nested models and the Wald test to assess statistical significance of individual variables. All tests were two-sided and nominal statistical significance was based on $P < 0.05$.

Results

Genotyping of the rs1447295 variant was successful in 99% of the samples leaving 821 cases and 732 controls for analysis. Half of the cases were aged between 55 and 64 years (432, 53%) and 111 (14%) were aged <55 years. Altogether, 252 cases (31%) had stage III or stage IV disease, and 358 (44%) tumors had a Gleason score of 7 or higher or were described as high grade.

The genotype distribution was consistent with Hardy-Weinberg equilibrium for cases, controls, and for cases and controls combined (all $P > 0.3$). There were no significant associations between genotype and country of birth, age, or family history of prostate cancer for either cases or controls (all $P \geq 0.05$).

The frequency of the A allele was 11% for controls and 15% for cases. The A allele was significantly associated with increased risk of prostate cancer (P from the likelihood ratio test = 0.002 and 0.0005 for the codominant and dominant models, respectively; Table 1). The unadjusted OR for men carrying any copy of the A allele, relative to noncarriers, was 1.52 (95% CI, 1.20-1.93). Adjustment for potential confounders did not materially change the OR. Table 2 shows that the

Table 2. The rs1447295 variant in 8q24 and prostate cancer risk by tumor stage and grade, and age at onset in the Australian Risk Factors for Prostate Cancer case-control study

	A allele carriers, no. of cases (%)	OR* (95% CI)	P †	P heterogeneity ‡
Tumor stage				0.8
Stage I-II	158 (28.0)	1.56 (1.20-2.02)	0.001	
Stage III	53 (26.9)	1.48 (1.03-2.12)	0.04	
Stage IV	13 (23.6)	1.24 (0.65-2.37)	0.5	
Tumor grade				0.7
Gleason score 5-6	125 (27.0)	1.48 (1.13-1.95)	0.005	
Gleason score 7-10	101 (28.2)	1.58 (1.18-2.11)	0.002	
Age at diagnosis (y)				0.9
<55	30 (27.0)	1.49 (0.94-2.35)	0.09	
55-64	120 (27.8)	1.54 (1.17-2.04)	0.002	
65-69	76 (27.3)	1.51 (1.10-2.08)	0.01	

NOTE: Estimates from a dominant model.
*ORs and 95% CIs from unadjusted polytomous logistic regression analysis (mlogit function in STATA 8.2). The dependent variables included four categories for stage (0 for controls, 1 for stage I-II, 2 for stage III, and 3 for stage IV tumors) and age at diagnosis (0 for controls, 1 for <55-year-olds, 2 for 55- to 64-year-olds, and 3 for 65- to 69-year-olds), and three categories for Gleason score (0 for controls, 1 for Gleason score 5-6 tumors, and 2 for Gleason 7-10 tumors). Adjustment for family history of prostate cancer, age, country of birth, body mass index, and smoking history did not materially change the OR estimates.
†Test for association between genotype and prostate cancer risk (likelihood ratio test).
‡Test for homogeneity of ORs across tumor stage, grade, and age at onset (likelihood ratio test).

proportion of carriers of the A allele was similar for stage I to II (28%), stage III (27%), and stage IV tumors (24%), for Gleason score 5 to 6 (27%) and Gleason score 7 to 10 tumors (28%), and for cases diagnosed at ages <55 years (27%), 55 to 64 years (28%), and 65 to 69 years (27%). As a result, the ORs for prostate cancer did not differ significantly by tumor stage, Gleason score, or age at diagnosis (all $P > 0.7$), and they were all between 1.24 (95% CI, 0.65-2.37, stage IV tumors) and 1.58 (95% CI, 1.18-2.11, Gleason score 7-10 tumors). The OR for Gleason score 5 to 6 tumors was 1.48 (95% CI, 1.13-1.95).

Discussion

Our study confirms an association between rs1447295 and prostate cancer and estimates that carriers of any copy of the A allele have a 52% (95% CI, 20-93%) higher risk than non-carriers, regardless of tumor aggressiveness (i.e., Gleason score). The results from our case-control study suggest that the association between rs1447295 and prostate cancer risk is unlikely to vary greatly by tumor stage or by age at diagnosis.

This study focused on early age at onset and excluded low-grade tumors. Gain in chromosome 8q24 has previously been found in clinically advanced prostate cancers (cT3 and cT4; ref. 13), whereas overrepresentation and amplification of the c-myc gene in 8q24 seems to be associated with poor prognosis (14). In the deCode report, the authors presented ORs for rs1447295 separately for Gleason score 2 to 6 and Gleason score 7 to 10 tumors. Although the difference in ORs was small, they concluded that the rs1447295 variant might have a stronger association with more aggressive forms of prostate cancer (5). Our results do not support this hypothesis and, although we cannot rule out the possibility of small differences in the ORs by Gleason score, the similarity of ORs by tumor stage and Gleason score suggests that genetic variants in 8q24 responsible for the association might have an effect early in carcinogenesis.

The allelic frequency for our control population (11%) was similar to that reported for the Swedish and Icelandic control populations in the deCode study (13% and 11%, respectively; ref. 5). For African-Americans, the allele frequency was reported to be higher (34%) than in the European-based studies, but the association with prostate cancer was weaker (OR, 1.15; $P = 0.29$; ref. 5). The study of African-Americans was relatively small and further studies are needed to confirm whether the association with prostate cancer differs by ethnicity.

Although our replication of reports regarding rs1447295 clearly shows that genetic variation in 8q24 was associated with prostate cancer risk, the region is large and the priority now is to narrow it down and find the functional variant or group of variants responsible for the association. Until this task is accomplished, the estimates of population-attributable risk such as those provided by Amundadottir and colleagues for the ''−8'' allele of DG8S737 (i.e., ~8% in populations of European ancestry and 16% in African-Americans) might be premature.

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