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Number of prostate cancer risk alleles may identify possibly ‘insignificant’ disease

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Abstract

OBJECTIVE—To determine whether the cumulative effects of five prostate cancer risk alleles (three single-nucleotide polymorphisms [SNPs] on chromosome 8Q24 and two SNPs on chromosome 17a) could help to identify possibly ‘insignificant’ disease.

MATERIALS AND METHODS—We genotyped 629 men of European ancestry who underwent radical prostatectomy at our institution between 2002 and 2007. Possibly ‘insignificant’ CaP was defined using the Ohori criteria (organ-confined, tumour volume <0.5 mL, Gleason pattern ≤4). Statistical analysis was used to compare patients with ‘insignificant’ and all other ‘significant’ cancer based upon genotype. Carrier status for the 5 SNPs were compared between patients with ‘insignificant’ disease and a separate population of 801 controls without CaP.

RESULTS—Overall, 38 (6.0%) patients with CaP met the Ohori criteria for ‘insignificant’ disease. Men with ‘significant’ cancer had a greater frequency of any of the five risk alleles than either patients with ‘insignificant’ disease or controls. None of the individual alleles genotyped on chromosomes 8 or 17 distinguished between ‘significant’ and ‘insignificant’ CaP. However, carriers of two or more risk alleles were more likely to have ‘significant’ disease.

CONCLUSIONS—Although no single risk allele distinguished ‘insignificant’ CaP, ‘insignificant’ disease was nearly three times as likely among carriers of ≤ one risk allele. Future studies are needed to further elucidate the cumulative relationship between CaP risk alleles and CaP aggressiveness.

Keywords

8q24; 17q; genetics; Ohori criteria; insignificant prostate cancer

INTRODUCTION

Chromosomal alleles located along 8q24 and 17q have been well validated as prostate cancer (CaP) risk loci. The initial susceptibility locus along 8q24, referred to as region 1 (128.54–128.62 Mb) [1], was first identified by a genome-wide linkage analysis of Icelandic families [2]. A subsequent admixture study among African-American men confirmed that these loci, as well as other alleles nearby, contributed to an increased risk of CaP [3]. Since then, several fine-mapping and additional genome-wide analyses have not only confirmed these results but also identified two additional regions along 8q24 (region 2, 128.12–128.28

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CONFLICT OF INTEREST None declared.

Mb; and region 3, 128.47–128.54 Mb) [1,4,5]. Interestingly, none of these genomic areas contain known coding regions. Subsequent analyses in men of European descent identified two CaP risk alleles along chromosome 17q [6]. One lies in a non-coding region at 17q24.3 and the other in the second intron of the *TCF2/HNF1 β* gene at 17q12.

A recent case–control study of Swedish patients confirmed that single-nucleotide polymorphisms (SNPs) from the three different regions of 8q24 and the two SNPs from 17q were significantly and independently associated with CaP risk. Moreover, the combined effects of these five alleles on CaP risk appeared to be cumulative and, together with family history, were associated with a ≈ 9.5 -fold increase in CaP risk [7]. In addition, it has been estimated that the five SNPs can explain more than 45% of CaP cases in the population [8,9]. The association between these five significant loci and CaP risk has now been confirmed in multiple populations [8,9].

There is conflicting evidence concerning the relationship between these five risk alleles and CaP aggressiveness. Some studies have shown an association with an increased tumour grade and other adverse features, in addition to the development of early-onset disease [2-4,10-15]; other studies have shown no significant relationship between these alleles and features of CaP [8,16,17].

Despite the recent demonstration of an improvement in survival with PSA screening in the European Randomized Study of Screening for Prostate Cancer [18], there is considerable concern regarding diagnosis and treatment of tumours that may never progress, i.e. clinically insignificant tumours. In the future, it is possible that risk alleles will be incorporated into CaP screening protocols or used to identify men at high risk of developing the disease. Due to their conflicting relationship with CaP aggressiveness, it is unclear whether they would increase or decrease the diagnosis of potentially ‘insignificant’ CaP [19]. As yet, no study has compared the frequency of possibly ‘insignificant’ CaP between carriers and non-carriers of the CaP risk alleles. The purpose of the present study was to determine the relationship between these genotypes and pathologically ‘insignificant’ disease in a large surgical population.

MATERIALS AND METHODS

Our study cohort included 629 men of European ancestry who underwent radical prostatectomy at Northwestern Memorial Hospital from 2002 to 2007. Of these patients, 90% were treated by a single surgeon (W.J.C.) and the rest by other urologists from the Northwestern University Specialized Program of Research Excellence (SPORE) group. In addition, we identified 801 men of European ancestry with no evidence of CaP to serve as a control population. All controls had a PSA level < 2.5 ng/mL, normal DRE and no prior history of a prostate biopsy. Of these men, 247 were recruited as healthy control subjects for genetic studies at Northwestern University and the University of Chicago, as previously described [2]. An additional 554 healthy volunteers of European ancestry were recruited as part of a CaP screening programme conducted in April 2007 [20]. The study received institutional review board approval, and all participants provided informed consent.

Patient demographics, biopsy and prostatectomy findings were documented for all patients with CaP. Organ-confined disease was defined as a tumour confined to the prostate with negative surgical margins (stage pT2RO). Men with extraprostatic tumour extension ($> pT3$), positive surgical margins (R1), seminal vesicle invasion or lymph node metastases (N1) were categorized as having non-organ-confined disease. ‘Insignificant’ tumours were defined using the criteria by Goto *et al.* [21]: organ-confined, tumour volume < 0.5 mL, and no primary or secondary Gleason pattern 4 or 5 in the biopsy or prostatectomy specimens.

DNA samples were isolated from whole blood, and genotypes were available for all participants, as previously described [2,6]. In particular, we assayed for the presence of the A allele of rs1447295 (region 1), the A allele of rs16901979 (region 2), and the G allele of rs6983267 (region 3). Genotypes were also determined for the T allele of rs4430796 on 17q12 and the G allele of rs1859962 on 17q24. Tests for Hardy–Weinberg equilibrium were performed for each SNP separately among case subjects and control subjects with the use of Fisher's exact test.

For the purposes of analysis, participants were categorized based upon their allele frequency of the five aforementioned CaP susceptibility alleles. Carrier status was defined assuming a best-fit dominant model. Patients with CaP were further classified as having 'insignificant' or 'significant' disease based on the histological features. Chi-squared, Fisher's exact tests and ANOVA were used to compare clinico-pathological features, including the proportion of patients with 'insignificant' disease, between carriers and non-carriers of the CaP risk alleles. The Cochran–Armitage trend test was used to compare allele frequency among patients with 'insignificant' disease, those with 'significant' disease and healthy volunteer controls. In addition, logistic regression was used to examine whether genotype predicted 'insignificant' disease. Receiver operating characteristic (ROC) analysis was also performed to evaluate the prediction of 'insignificant' disease using PSA and genotype. SAS 9.2 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

RESULTS

In all, 629 patients underwent radical prostatectomy and had genotype information available for all five CaP susceptibility alleles along 8q24 and 17q. The mean age at diagnosis of patients with CaP was 58.7 years, and 29.8% of patients reported at least one first-degree relative with CaP. The clinical stage was $\leq$ T1c in 71.8% of patients, T2 in 27.7%, and T3a in 0.5%. The median PSA at diagnosis was 5.0 ng/mL. In the radical prostatectomy specimen, 504 patients (80.1%) had organ-confined disease with negative (cancer-free) surgical margins. Positive (cancerous) surgical margins, extracapsular tumour extension, seminal vesicle invasion and lymph node metastases were present in 121 (19.2%), 119 (18.9%), 29 (4.6%), and five (0.8%) patients, respectively. The prostatectomy Gleason score in these patients was $\leq$ 6 in 331 (52.7%), 7 in 248 (39.5%), and 8–10 in 49 (7.8%) patients. As a comparison group, 801 healthy volunteer controls with a mean age of 58.1 years and a median PSA level of 0.8 ng/mL were also genotyped.

Overall, 38 (6.0%) patients met the Ohori pathological criteria for 'insignificant' disease. Table 1 compares the clinical features between these 38 patients and the remainder with 'significant' CaP. Men with 'insignificant' cancer tended to be younger ($P = 0.08$) and had a significantly lower PSA at diagnosis ($P < 0.0001$), whereas other clinical characteristics were similar between the groups.

Genotypes of the five CaP risk alleles were determined for all cases and controls, and each SNP followed Hardy-Weinberg equilibrium. Table 2 shows the allele frequency of the five CaP susceptibility alleles in the study population. Patients with either 'insignificant' or 'significant' CaP had a higher frequency than controls of all five CaP risk alleles. Specifically, analysis for trend showed that the frequency of some risk allele significantly increased as a function of disease status (e.g. controls $<$ 'insignificant' $<$ 'significant'). However, pairwise comparisons showed no statistical difference in allele frequencies for each individual risk allele between men with 'insignificant' and 'significant' CaP (data not shown).

Since no individual risk allele appeared to distinguish 'insignificant' disease accurately, we also determined whether the number of risk alleles had a cumulative relationship with CaP aggressiveness (Table 3). A higher proportion of patients with possibly 'insignificant' cancer were carriers of $\leq$ one risk allele than was the case in men with 'significant' tumours (15.8 vs 6.8%, $P = 0.05$). Furthermore, men with 'insignificant' cancers had 2.6-fold increased odds of having $\leq$ one allele (95% CI, 1.02–6.54). Although it did not reach statistical significance, patients with 'significant' CaP were more likely to carry two to three alleles (77.2 vs 73.7%) and four to five alleles (16.1 vs 10.5%) than patients with 'insignificant' disease. Interestingly, patients with 'insignificant' disease had a similar likelihood of carrying $\leq$ one allele to the controls (15.8 vs. 13.5%, $P = 0.63$). In addition, the overall frequency of carrying any specific number of alleles was statistically indistinguishable between patients with 'insignificant' disease and controls (data not shown).

Because PSA and the number of risk alleles were associated with the likelihood of 'insignificant' CaP, these variables were combined in ROC analysis for the prediction of 'insignificant' cancer (Fig. 1). The resultant area under the curve (AUC) was 0.703, suggesting that PSA and the number of risk alleles had reasonable discrimination for the presence of possibly 'insignificant' disease. Interestingly, although there was a trend towards an increasing number of the five alleles and higher Gleason grade, multivariate analyses in this relatively small population of men of European ancestry revealed no significant relationships between an increasing number of alleles and aggressive disease (e.g. Gleason score ≥ 8 , positive lymph nodes and/or PSA >20 ng/mL; data not shown).

DISCUSSION

Susceptibility to CaP has a clear genetic component, as suggested by the approximately twofold increased risk among men with a family history of the disease [22]. However, until recently, no reproducible risk allele had been identified that could account for a significant proportion of CaP cases. Within the past few years, studies have shown that SNPs located in three different regions of chromosome 8q24, along with two regions on chromosome 17q, are highly associated with CaP risk [2,6,14].

Despite uncertainty about the mechanism by which these alleles exert their effects, recent evidence suggests they might act in a cumulative fashion [7,9]. The present study supports a possible cumulative relationship not only with CaP risk, but also with the likelihood of pathologically 'significant' disease. Specifically, we found that patients carrying $\leq$ one of these risk alleles were significantly more likely to have CaP that met previously published criteria for 'insignificant' disease. Furthermore, patients with 'insignificant' tumours were carriers of similar numbers of risk alleles as controls. Interestingly, although these five risk alleles may be useful in predicting incidental or low-risk disease, they do not appear to predict aggressive disease in our population. Taken together, these findings suggest that, although individual alleles were not useful to predict CaP significance, there might be a cumulative relationship with aggressiveness.

Since the initial description of CaP genetic risk alleles, there has been a large effort directed towards determining any association between the risk alleles and aggressive clinico-pathological features. While our study is the first to document an association with 'insignificant' disease, the results of previous studies have supported the relationship between the risk alleles and aggressive clinical and pathological characteristics. There has been an increased frequency of the risk alleles observed in patients with younger age, familial CaP, increased clinical and pathological Gleason grade and advanced stage disease [2,4,10-17,20,23-26]. However, it is important to note that many other recent studies, some

of which involved relatively large cohorts of patients, have failed to detect an association with aggressive disease [8,16,27]. These discrepancies in outcomes may be related to small sample size, genetic variability in different populations, interpretation of allele carrier status (i.e. best-fit genetic model) and inconsistent criteria used to identify aggressive CaP. Additionally, these findings may be due to the fact that most of the CaP risk alleles were initially discovered by comparing all types of CaP cases with controls. Therefore, future studies that identify risk alleles that are present at higher frequencies in patients with aggressive than in those with non-aggressive CaP may be required to verify these results. Some recent reports have begun to elucidate alleles that specifically identify advanced disease along both chromosomes 8 and 17 [13,28].

It should be noted that carrier status was not the only predictor of 'insignificant' disease in this cohort. Patients who had a lower preoperative PSA were also statistically more likely to have 'insignificant' disease. On ROC analysis, a combined model including PSA and the number of risk alleles on chromosomes 8 and 17 had an AUC of 0.703. It should be noted that other genetic factors may also influence the risk of clinically 'significant' disease. For example, more than 30 different alleles have now been identified with documented associations to CaP risk [16]. Because data were not available for most of these alleles in this patient cohort, their relationship with CaP aggressiveness remains to be determined.

It has previously been shown that current clinical staging techniques are often inaccurate for the prediction of potentially indolent disease. This is particularly relevant in terms of the appropriate patient selection for active surveillance. Correspondingly, some patients who are offered active surveillance are ultimately found to have aggressive pathological features on final analysis [29]. Therefore, additional variables that could help predict the likelihood of 'insignificant' disease are urgently needed. Because carriers of $\leq$ one CaP allele were more likely to meet the Ohori criteria for possibly 'insignificant' disease at radical prostatectomy, additional study of these alleles among men managed with active surveillance protocols is warranted.

Our study has several limitations. First, only a small proportion of patients in our series met the criteria for 'insignificant' CaP, and therefore our statistical power was limited. In addition, the criteria we used for potentially 'insignificant' disease, although commonly cited in the literature, can misclassify some individuals. Also, all CaP patients in the present study underwent radical prostatectomy; therefore, it is unknown what their outcomes would have been in the absence of definitive therapy. This also represents a selection bias. Another potential confounder is that the control population may have contained patients who had undiagnosed CaP, despite having a negative DRE and a PSA level <2.5 ng/mL. Unfortunately, this is an inherent limitation of many studies as large groups of elderly men with BPH only on autopsy are difficult to identify. In addition, as discussed, new CaP susceptibility alleles continue to be identified, and it is possible that additional alleles will provide a more robust association with CaP features. Accordingly, further studies are warranted to evaluate the cumulative relationship between a larger number of risk alleles and potentially 'insignificant' CaP. In general, the mechanisms underlying CaP pathogenesis are complex and multifactorial. In the future, a greater understanding of CaP biology may allow us to further individualize risk assessment, diagnosis and therapy through the incorporation of genetic information.

In conclusion, genetic markers have the potential to identify men at greater risk for developing CaP. This is the first study to suggest that carriers of two or more of five risk alleles on chromosomes 8 and 17 might be more likely to have 'significant' tumours than carriers of $\leq$ one allele. Larger studies are warranted to confirm these results and examine whether CaP susceptibility alleles can aid in future prognostication.

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Abbreviations

AUC	area under the curve
CaP	prostate cancer
ROC	receiver operating characteristic
SNP	single-nucleotide polymorphism

FIG. 1. Receiver operating characteristic (ROC) analysis for the prediction of 'insignificant' prostate cancer using PSA and the number of risk alleles (defined by carrier status).

TABLE 1

Comparison of the clinical characteristics of patients who met pathological criteria for 'significant' and 'insignificant' prostate cancer (CaP)

	'Significant' CaP	'Insignificant' CaP	<i>P</i> value
Mean age $\pm$ SD, years	58.8 $\pm$ 6.9	56.8 $\pm$ 7.0	0.08
Mean body mass index $\pm$ SD, kg/m ²	27.2 $\pm$ 3.6	26.4 $\pm$ 3.4	0.15
+ family history (%)	153 (29.5%)	10 (27.8%)	0.83
Median PSA, ng/mL	5.1	3.7	<0.001

TABLE 2

Frequency of five significant prostate cancer (CaP) susceptibility alleles

SNP	Chromosomal region	Associated allele	'Significant' CaP (n = 591), %	'Insignificant' CaP (n = 38), %	Controls (n = 801), %	P value for trend
rs1447295	8q24 (region1)	A	12.4	13.2	10.1	0.06
rs16901979	8q24 (region2)	A	5.7	5.3	3.4	0.004
rs6983267	8q24 (region3)	G	54.1	51.3	50.6	0.07
rs4430796	17q12	T	57.0	56.6	50.8	0.001
rs1859962	17q24	G	52.8	50.0	48.9	0.04

SNP, single-nucleotide polymorphism. The frequency of each risk allele was determined in patients with 'insignificant' disease, those with 'significant' disease and healthy volunteer controls. There was a higher frequency of every allele in patients with CaP than in controls.

TABLE 3

Comparison of the number of 8q24 and 17q risk alleles between patients with 'significant' and 'insignificant' prostate cancer (CaP)

Carrier of CaP allele	'Significant' disease, %	'Insignificant' disease, %	<i>P</i> value
Carrier 0 or 1	6.8	15.8	0.05
Carrier 2 or 3	77.2	73.7	0.12
Carrier 4 or 5	16.1	10.5	0.36
Carrier ≥ 2	93.2	84.2	0.05