Null Results in Brief

PTEN Polymorphism (IVS4) Is Not Associated with Risk of Prostate Cancer

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Introduction

Germline polymorphisms in several genes important to the pathogenesis of prostate cancer have been associated with an increased incidence of disease. A recently identified tumor suppressor gene, *PTEN/MMAC1/TEP1*, may also have an important role in prostate carcinogenesis and progression. Previous reports have demonstrated frequent loss of PTEN expression in localized prostate cancer specimens, which have been associated with poor prognostic clinical features (1, 2). In breast cancer patients with a positive family history of disease, there was no increased association with *PTEN* mutations. However, a frequent polymorphism, referred to as IVS4, located at 210+109ins5, in which an ACTAA insertion occurs 109 bp downstream of exon 4 in intron 4, was associated with a lower mean age of diagnosis (3). Therefore, we performed a case-control study nested within the Physicians' Health Study to determine whether this particular polymorphism was associated with risk of prostate cancer or with clinical features of aggressive disease.

Materials and Methods

Study Population. Subjects for this analysis were drawn from among the 14,916 male participants who were cancer-free at baseline from the Physicians' Health Study, a randomized double-blinded, placebo-controlled trial of aspirin and β-carotene (4). Study characteristics including enrollment, blood collection and processing, stage classification, clinical follow-up, and documentation of prostate cancer were described previously (5).

Laboratory Analysis of PTEN Polymorphism. Genomic DNA was extracted from archival blood as reported previously (5). Twenty ng of sample DNA was added to the PCR reaction mixture that consisted of 0.8 μM primers (5′ GGG GGT GAT AAC AGT ATC TA 3′) and (5′ CTT TAT GCA ATA CTT TTT CCT A 3′), 2.5 mM $MgCl_2$, and 2.25 units Qiagen Taq polymerase in a final reaction volume of 22 μl. Cycling conditions were 25 cycles of 95.0°C for 1 min, 55.0°C for 1 min, and 72.0°C for 1 min. The PCR product was digested with 20 units AflII (New England Biolabs, Beverly, MA) and separated on a 2% agarose gel stained with ethidium bromide to visualize the bands. Sequencing was performed on an initial sampling of PCR products in order to confirm the appropriate genotyping. Genotyping was successfully completed in >99% of the samples tested. A total of 600 cases and 803 controls were analyzed.

Statistical Analysis. The Hardy-Weinberg equilibrium was tested by a goodness of fit χ^2 test to compare the observed *versus* the expected genotype frequencies within cases and controls. We used contingency tables and calculated odds ratios and 95% CI[2] to assess whether the PTEN polymorphism was related to prostate cancer risk. Age-adjusted odds ratios were used to estimate the RR, with unconditional logistic regression analysis. We stratified the analysis further according to Gleason grade, clinical stage, age at diagnosis, and risk of disease progression, whereby high risk was defined as either high grade (Gleason ≥7) or advanced stage (C or D), whereas low risk cases were both low grade and early stage. All *P*s are two-sided; all analyses were performed with SAS (6).

Results

In this study, the overall allelic frequency of the IVS4 polymorphism was 33.68%, consistent with previous reports (3). Homozygotes for the polymorphism constituted 11.2% of controls and 9.5% of cases, and the distribution of the polymorphism fit well to the Hardy-Weinberg equilibrium. As illustrated in Table 1, prostate cancer patients homozygous for the polymorphism exhibited no statistically significant association with prostate cancer grade or stage compared with controls. Regardless of whether cases were separated by Gleason score (<7 *versus* ≥7), clinical stage (A/B *versus* C/D), or both, there was no clear statistically significant correlation with homozygotes for the IVS4 polymorphism.

Because a relation between age of diagnosis and incidence of the IVS4 polymorphism was observed in breast cancer, we investigated whether the same was true in our prostate cancer population. The median age at diagnosis in our population was 69 years, so statistical comparisons were made between cases diagnosed above and below this cutoff. Interestingly, men <69 years of age who were homozygous for the IVS4 polymorphism had a statistically significant lower incidence of high grade/stage prostate cancer with a RR of 0.27 (95% CI, 0.11–0.70). Heterozygotes displayed similar rates of low grade/stage and high grade/stage disease as did men who were homozygous wild type. To evaluate further whether men with advanced prostate cancer were more likely to harbor the IVS4 *PTEN* polymorphism, we genotyped 90 men with hormone-refractory,

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[2] The abbreviations used are: CI, confidence interval; RR, relative risk.

Table 1 Distribution of the PTEN polymorphism and the relative risk of prostate cancer

PTEN Genotype	No insertion	Heterozygous	Insertion
Overall			
No. cases (%)	262 (43.7)	281 (46.8)	57 (9.5)
No. controls (%)	346 (43.1)	367 (45.7)	90 (11.2)
RR (95% CI)	1.00 (reference)	1.01 (0.81–1.27)	0.86 (0.59–1.24)
Low-risk PCa			
No. cases/controls	133/176	119/174	33/41
RR (95% CI)	1.00 (reference)	0.91 (0.66–1.26)	1.09 (0.65–1.82)
High-risk PCa			
No. cases/controls	112/148	136/177	19/43
RR (95% CI)	1.00 (reference)	1.01 (0.72–1.41)	0.59 (0.32–1.07)
Gleason <7			
No. cases/controls	123/163	116/171	29/37
RR (95% CI)	1.00 (reference)	0.90 (0.65–1.26)	1.07 (0.62–1.84)
Gleason ≥7			
No. cases/controls	62/88	86/94	11/26
RR (95% CI)	1.00 (reference)	1.27 (0.81–1.97)	0.59 (0.27–1.29)
Stage AB			
No. cases/controls	186/245	193/247	44/56
RR (95% CI)	1.00 (reference)	1.03 (0.79–1.35)	1.07 (0.69–1.66)
Stage CD			
No. cases/controls	76/101	88/120	13/34
RR (95% CI)	1.00 (reference)	0.98 (0.65–1.47)	0.51 (0.25–1.03)

metastatic prostate cancer. The median age of diagnosis of this population was 65.5 years. Genotyping revealed that the overall allelic frequency of the IVS4 polymorphism was 61 of 180 (33.89%), with 9 of 90 (10%) homozygotes, suggesting no greater frequency in men who develop metastatic prostate cancer compared with controls.

Conclusions

In summary, this study demonstrates no clear association between the incidence of the *PTEN* polymorphism IVS4 and risk of prostate cancer overall or of aggressive disease in particular. However, this study addresses the association of only one *PTEN* polymorphism. Other *PTEN* polymorphisms could be associated with the incidence of prostate cancer and may warrant additional investigations in the future.

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