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Methylation of the *RARB* Gene Increases Prostate Cancer Risk in Black Americans

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Abstract

Purpose—Gene promoter hypermethylation may be useful as a biomarker for cancer risk in histopathologically benign prostate specimens.

Materials and Methods—We performed a nested case-control study of gene promoter methylation status for 5 genes (*APC, RARB, CCND2, RASSF1* and *MGMT*) measured in benign biopsy specimens from 511 prostate cancer case-control pairs. We estimated the overall and race stratified risk of subsequent prostate cancer associated with methylation status.

Results—On race stratified analysis *RARB* methylation was associated with a higher cancer risk in black American men (OR 2.18, 95% CI 1.39–3.44). *APC* methylation was associated with an increased risk of high grade tumors (OR 2.43, 95% CI 1.20–4.90), which was higher in black than in white men (OR 3.21 vs 2.04). In cases *RARB* and *APC* gene methylation in benign prostate samples persisted in matched malignant specimens. In black cases the combined risk associated with *RARB* and *APC* methylation (OR 3.04, 95% CI 1.44–6.42) was greater than the individual risk of each gene and significantly different from that in white cases (OR 1.14, 95% CI 0.56–2.30).

Conclusions—*RARB* gene methylation in histopathologically benign prostate samples was associated with a statistically significant increased risk of subsequent prostate cancer in black men. Methylation data on additional genes may improve risk stratification and clinical decision making algorithms for cancer screening and diagnosis.

Keywords

prostate; prostatic neoplasms; risk; DNA methylation; African Americans

Prostate carcinogenesis is associated with the silencing of tumor suppressor genes via promoter hypermethylation.[1–3] For example, *GSTP1* is almost universally methylated in prostate cancer and considered a highly specific marker for cancer in prostate tissue.[4,5] Other genes are methylated less frequently but show signs of hypermethylation earlier in prostate carcinogenesis, making them potential markers of early cancer detection.[3]

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Gene promoter methylation can also occur in prostate nonneoplastic cells before malignant transformation.[6] For instance, *RARB* methylation in histopathologically negative tissue predicted prostate cancer in followup biopsies done within 24 months with 84% sensitivity.[7] In a study of prostatectomy specimens *RARB* was hypermethylated in 72% of benign prostate adjacent to tumor.[8] Similarly, in ex vivo core biopsies taken 1 to 4 mm from prostate cancer foci 4 of 11 specimens showed methylation in 1 or more of the tumor suppressor genes *APC*, *RARB* and *RASSF1*.[9] Methylation of these genes in the preneoplastic state is consistent with a field defect that presages cancer in the human prostate and suggests the possibility of a biomarker for early detection before overt carcinogen-esis.[10,11]

Because DNA methylation is a common, consistent event that may develop early in carcinogenesis, it is an attractive biomarker for prostate cancer detection. Specific methylation events have also been linked to disease outcomes,[12,13] suggesting that methylation biomarkers can be used to target treatment interventions toward the cases that would most benefit. A methylation based risk profile would also benefit the majority of men with negative prostate biopsies who are still considered at high risk due to increased PSA.[14] Since many of these men have clinically indolent disease, aggressive clinical intervention would only decrease quality of life and not disease related mortality.[15]

Black American men experience a significantly higher incidence of prostate cancer and a more aggressive prostate cancer presentation.[16] They are more likely to die of the disease[17] and may have a higher incidence of cancer after negative biopsy than white men.[18] Studies suggest that gene promoter methylation patterns in the prostate may differ by race.[19,20] Characterizing the tumor suppressor genes inactivated by hypermethylation and the timeline of when such changes occur may improve early prostate cancer detection and targeted disease management, which could have a significant impact on these racial disparities.

To determine the significance of aberrant gene promoter methylation in early prostate carcinogenesis, we performed a matched case-control study nested in a large historical cohort of men with benign prostate tissue specimens to test whether gene promoter methylation in benign tissue is associated with incident prostate cancer and tumor aggressiveness, and whether these associations are race specific.

Methods

Study Sample

After obtaining approval from the Henry Ford Health System institutional review board, we identified a historical cohort of 6,692 men with a benign prostate specimen collected by needle core biopsy or TURP between January 1990 and December 2002. A nested case-control sample was drawn from this cohort based on study eligibility criteria, including PSA recorded within a year of cohort entry and no history of a prostate cancer diagnosis. Cohort entry date was defined as the date that the initial benign prostate specimen was harvested. Case diagnosis date was the date of the first cancer positive tissue specimen or the date that a clinician first reported a clinical diagnosis of prostate cancer. Patients diagnosed with prostate cancer less than 1 year from the cohort entry date were ineligible for study. We identified 808 potentially eligible cases diagnosed with prostate cancer before July 2007.

We used incidence density sampling to select controls with replacement from all cohort members at risk at the time of case occurrence. The reference date for controls was equal to the time between the case enrollment and diagnosis dates added to the control enrollment date. Matching criteria included age at cohort entry (±2 years), cohort entry date (±2 years), race (black or white) and specimen type (biopsy or TURP). We matched 802 of 808

potentially eligible cases. Further review decreased the final analytical sample to 574 case-control pairs.[21] In the current study we ran methylation assays on the first 526 pairs enrolled and measured methylation levels for at least 1 gene in 511 of these pairs.

DNA Isolation and Methylation Specific PCR

Methylation studies were performed using DNA from benign prostate tissue acquired from needle biopsies and TURP, and from tumor specimens acquired from prostatectomy tissue. Sections (5 μ) of paraffin embedded prostate tissue were placed individually on untreated, uncharged glass microscope slides, air dried and placed in a 60C oven for 45 minutes to remove excess paraffin. Heated slides were immersed in xylene overnight, washed for 3 minutes in 100%, 95% and 80% ethanol, and air dried. All specimens were processed in 50 μl microdissection buffer composed of tris, KCl, $MgCl_2$, Tween® 20 and water, followed by overnight digestion with Proteinase K (Sigma-Aldrich®) at 55C.

Extracted DNA was bisulfite treated using the EZ DNA Methylation-Gold™ Kit to convert unmethylated cytosines into uracils, while leaving methylated cytosines unchanged. Nested methylation specific PCR was used due to its ability to amplify degraded DNA associated with formalin fixation and long-term storage in paraffin.[22] In addition, stage I primers were multiplexed to maximize DNA recovered from tissue (less than 200 ng for most biopsies). Stage I primers recognize bisulfite modified templates but do not discriminate methylated from unmethylated alleles. Thus, the stage I PCR product was diluted and subjected to repeat PCR (stage II) with primers specific to a methylated template located around the transcriptional start site, where methylation (when present) strongly correlates with gene silencing. To ensure that only methylated alleles were amplified, stage II PCR reactions were performed at annealing temperatures greater than the melting temperature of stage I primers. Sensitivity was approximately 1/500 even with the nested methylation specific PCR approach due to tissue degradation from formalin fixation and storage in paraffin. The supplementary table (http://jurology.com/) lists primer sequences. Supplementary figure 1 (http://jurology.com/) shows the gene region queried.

Statistical Analysis

We performed conditional logistic regression analysis to estimate prostate cancer incidence ORs during followup. Individual matching was controlled for age, race and specimen type. Models that assessed associations between the prostate cancer incidence and gene promoter methylation for each of the 5 study genes were tested with and without adjusting for PSA and the presence of HGPIN. Stratified models were compared using a conditional logistic regression model that included interaction terms with the stratified variable. We performed sensitivity analysis of the effect of shorter followup on risk estimates to determine whether cases diagnosed soonest (those with the highest probability of misclassification at cohort entry) may have had biased results.

Results

Demographics and Clinical Characteristics

The average age of cases was 65.9 years at cohort entry and 41% of the cases were black (table 1). Approximately 50% of the cases were diagnosed between 1 and 4 years after cohort entry. The remaining cases were diagnosed between 4 and 15 years after cohort entry. Cases had significantly higher PSA at cohort entry and higher PSA velocity between the time of cohort entry and diagnosis. They underwent an average of 2 more PSA tests during this period than controls. Of the cases 40% and 52% showed stage 1 and 2 tumors, respectively, and 30% had advanced tumor grade, defined as Gleason score 8 or greater, or 7 with a grade 4 primary tumor.

Associations

Gene promoter methylation and prostate cancer—Of the 5 genes assayed increased methylation of *RARB* had the strongest association with prostate cancer (OR 1.76, 95% CI 1.29–2.40, table 2). Adjusting for PSA at cohort entry and the presence of HGPIN moderately decreased this association (OR 1.59, 95% CI 1.15–2.21). When stratified by race, the association between *RARB* methylation and prostate cancer was significant only in black men (OR 2.18, 95% CI 1.39–3.44). The other significant race specific association was the adjusted risk estimate for methylation of the *MGMT* gene in white men (OR 0.50, 95% CI 0.27–0.95).

To determine whether the association of prostate cancer with methylation status was modified by matched time variables or disease aggressiveness, we stratified analyses by the interval between cohort entry and diagnosis or end of followup in controls as well as by age and tumor grade (table 3). When stratified by time to diagnosis, the highest ORs were observed for *RARB* and *APC* methylation in black men diagnosed 4 or more years after cohort entry. In this group the methylation of either gene increased the risk estimate more than twofold (*RARB* methylation OR 2.53, 95% CI 1.30–4.91 and *APC* methylation OR 2.45, 95% CI 1.08–5.54). When stratified by age, we observed an increased risk estimate only for *APC* methylation in black men 65 years old or older (OR 2.08, 95% CI 0.97–4.46). Interestingly, age stratification also revealed an inverse association for *MGMT* methylation in older white men (OR 0.27, 95% CI 0.09–0.82). When cases were stratified by tumor grade (low vs high), the highest risk estimates were observed in black men. *APC* methylation was associated with high grade tumors (OR 3.21, 95% CI 1.06–9.75), while *RARB* methylation was more prominently associated with an increased risk of low grade tumors (OR 2.54, 95% CI 1.37–4.74).

Because cases with the shortest time to diagnosis had the highest probability of misclassification at cohort entry, we also performed sensitivity analysis to determine whether missed diagnoses at cohort entry may have biased results (supplementary fig. 2, http://jurology.com/). Pairs were removed sequentially from the sample, starting with the pair with the shortest followup. We then repeatedly re-estimated the OR to determine the effect of the earliest diagnosed cases on risk estimates. Results suggested that misclassifying controls at cohort entry would not bias results away from the null.

Clinical risk factors and gene methylation—Since HGPIN and increased PSA are independently associated with an increased prostate cancer risk, we tested for associations between these 2 factors and gene promoter methylation status (table 4). The presence of HGPIN was significantly higher in benign prostate with *RARB* methylation independent of race ($p < 0.0001$). However, no other significant associations were found between HGPIN or PSA and methylation status. To determine whether HGPIN or increased PSA significantly modified the prostate cancer risk associated with methylation, we next tested a series of interaction models. We found no significant interaction of HGPIN and PSA with any of the 5 study genes.

However, when the case sample was restricted to high grade tumors, the estimated OR of the interaction between PSA and *APC* methylation was statistically significant and consistent across race groups ($p = 0.01$). In a logistic regression model the OR of high grade prostate cancer associated with *APC* methylation increased from 0.63 (95% CI 0.19–2.16) at PSA 2 ng/ml to 3.67 (95% CI 1.50–9.01) at PSA 6 ng/ml. The risk estimate for low grade cancer was not affected by PSA level (fig. 1).

Gene Methylation

Joint effects of APC and RARB—Because *APC* and *RARB* were frequently methylated and associated with prostate cancer, we examined whether methylation of these genes had joint effects on prostate cancer risk estimates (table 5). In the full sample little additional cancer risk was associated with *APC* methylation when the *RARB* gene was also methylated. However, in black men *APC* methylation appeared to increase the risk associated with *RARB* methylation, particularly after adjusting for PSA and the presence of HGPIN (OR 3.04, 95% CI 1.44–6.42). This was not true in white men, in whom the joint OR *of RARB* and *APC* methylation was only slightly increased (OR 1.14), which differed significantly from that in black men (p = 0.01).

Correlation in benign and malignant tissue—The association of *RARB* and *APC* methylation in benign tissue with incident prostate cancer suggests a generalized field defect that should be retained in the primary tumor. Therefore, in the 58 cases with available prostatectomy specimens we compared the methylation profile of all 5 genes in benign vs prostatectomy tissues (table 6). These cases were evenly distributed between black and white men. Cohort entry PSA, followup duration and the percent of methylation were comparable to those of the larger case sample. Prostatectomy cases were slightly younger (mean ± SD age 60.2 ±6.1 years) and had tumors of more advanced stage (15.4%) and grade (39.7%). As expected, the overall prevalence of methylation increased dramatically from the benign to malignant state for 4 of the 5 study genes with the percent methylation of only the *MGMT* gene lower in prostate cancer than in benign specimens (14.6% vs 4.2%). Figure 2 shows the change in methylation status from the benign to the malignant state in paired benign-tumor prostate specimens for the 3 genes most frequently methylated in benign prostate (*APC*, *RARB* and *RASSF1*). The *APC* gene was most likely to stay methylated in tumor when it was methylated in the benign prostate (100% to 93% methylated), or change to the methylated state when it was unmethylated in the benign prostate (0% to 70% methylated).

Discussion

RARB gene methylation in benign prostate biopsy tissue was associated with an increased risk of subsequent cancer. Moreover, this increased risk associated with *RARB* methylation alone or in the presence of *APC* methylation was only statistically significant in black men.

Racial differences in methylation patterns and prostate cancer were noted previously. Woodson et al found a higher frequency of *CD44* methylation in black than in white men.[20] Another study showed that *GSTP1* methylation was more strongly related to prostate cancer in black than in white or Asian men.[23] More recently, Kwabi-Addo et al observed significant differences between black and white men in the benign prostate tissue methylation levels of several genes that were highly methylated in prostate tumor tissue, including *RARB*.[19]

We found that men with *RARB* methylation in benign prostate were at increased risk for subsequent prostate cancer, which persisted almost 10 years after the initial benign biopsy. This increased risk was more than double in black men. This is consistent with the findings of Troyer et al, who reported that methylated *RARB* was the most sensitive marker for a subsequent diagnosis of prostate cancer within 24 months of an initial negative biopsy.[7] However, *RARB* was also highly methylated in tissue from black controls. While Troyer et al implicitly assumed that cancer was present at the initial negative biopsy, we excluded cases diagnosed within a year of cohort entry to enrich for incident prostate cancer. Given the age range of our cohort and the high prevalence of undiagnosed prostate cancer in older men,[24] synchronous prostate cancer was likely missed in some men in our cohort due to

biopsy sampling error. However, considering the high rate of negative initial prostate biopsies among all prostate biopsies performed[25] and the fact that *RARB* methylation likely indicates a prostate cancer field defect,[8,9] our findings are generalizable to men with histopathologically benign biopsy findings.

Similar to *RARB*, *APC* methylation likely indicates a generalized prostate cancer field defect but *APC* is not as highly methylated in benign tissue adjacent to tumors.[8,9] While *APC* methylation in benign prostate biopsy with HGPIN is strongly associated with a later cancer diagnosis,[26] we found that HGPIN more strongly correlated with methylation in *RARB* than in *APC*. In our sample there was a slightly higher percent of HGPIN in men with *APC* methylation, which became more pronounced and approached statistical significance in white patients after stratifying by race (table 4). While there were similarities between the *APC* DNA sequences probed in in our study and that by Trock et al, they did not report the racial distribution of their sample.[26] In our study *APC* methylation was more strongly associated with high grade tumors. This finding is consistent with several reports that *APC* methylation is an independent predictor of poor prostate cancer prognosis.[12,13] Interestingly, in our series the association of *APC* methylation with high grade tumors increased with increasing PSA, suggesting that combining PSA with *APC* methylation status could have the potential to better stratify patient risk.

Our study has several limitations. The recovery of small amounts of DNA from biopsy specimens limited our analysis to 5 genes and DNA degradation limited our ability to amplify specific gene promoters with methylated or unmethylated stage II primers. This led to a relatively high rate of missing methylation data. Of all genes analyzed data on any 1 specific gene was missing in 7% to 16% of individuals, resulting in the removal of 11% to 21% of pairs from analysis due to the matched study design. Missing data were not related to case-control status or associated with potential confounders, such as HGPIN or PSA. Because our study was observational, cohort members were not uniformly followed after cohort entry. Cases underwent significantly more PSA tests between cohort entry and diagnosis than controls. The frequency of PSA tests in our study sample was greater than current screening recommendations even in controls but adjusting for the number of PSA tests did not substantively change our results.

Approximately 1 million prostate biopsies are performed annually in the United States, of which 700,000 are negative for cancer.[14] The concept of using a measurement of epigenetic field defects to detect early stage prostate cancer is becoming more accepted as a potential clinical modality to decrease the high rate of unnecessary prostate biopsies and treatment.[10] Methylation risk profiles may also assist physicians in selecting patients who would benefit most from aggressive followup, while sparing others unnecessary procedures and distress. This study is a step toward that goal. The race specific nature of our findings also highlights the need for additional molecular studies in under studied populations to better understand potential biological differences in prostate cancer progression. In conclusion, if measuring gene methylation status in prostate cancer is to have clinical usefulness, we must better understand methylation in early carcinogenesis and its implications in populations of different racial and ethnic backgrounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and Acronyms

HGPIN	high grade prostatic intraepithelial neoplasia
PCR	polymerase chain reaction
PSA	prostate specific antigen
TURP	transurethral prostate resection

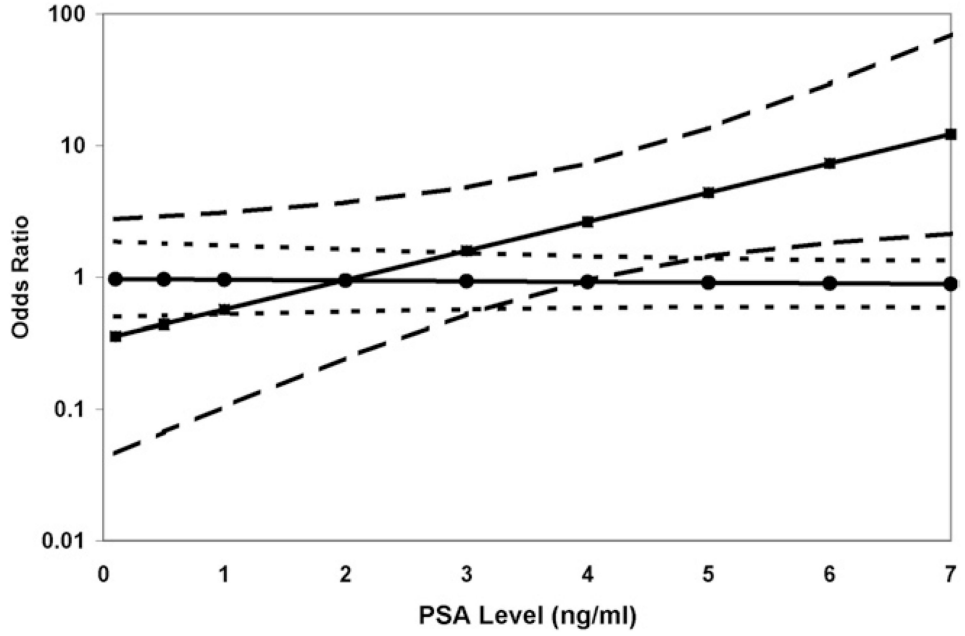

Figure 1.

Logistic regression model estimate of ORs of low (circles) and high (squares) grade prostate cancer associated with positive *APC* methylation status by PSA at cohort enrollment. Dashed lines represent 95% CI of risk estimates.

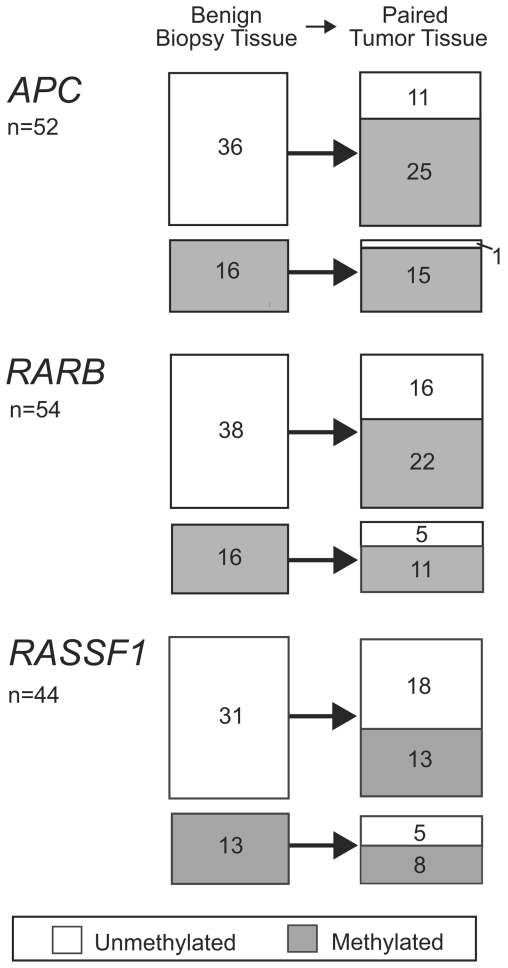

Figure 2.

Gene promoter methylation status of *APC*, *RARB* and *RASSF1* genes in case paired benign and tumor specimens. In *APC* and *RARB* genes, for which biopsy tissue methylation was associated with prostate cancer risk, unmethylated benign tissue frequently became methylated in tumor tissue. Benign tissue methylation tended to predict tumor tissue methylation.

Table 1
Demographic and clinical characteristics of 511 case-control pairs

	Controls	Cases
No. race (%):*		
White	300 (59)	300 (59)
Black	211 (41)	211 (41)
Mean ± SD age at cohort entry*	65.9 ± 7.5	65.9 ± 7.5
Median cohort entry date*	1/95	11/94
Median followup (yrs)*	4.0	4.0
No. benign specimen (%):		
Needle core biopsy*	481 (94)	481 (94)
TURP	30 (6)	30 (6)
Mean ± SD PSA:†		
Cohort entry (ng/ml)	5.7 ± 5.7	7.8 ± 7.2
No. tests cohort entry-diagnosis	6.7 ± 4.7	8.7 ± 5.3
Velocity (ng/ml/yr)	0.003 ± 2.1	7.3 ± 97.7
No. tumor stage (%):	—	
1		202 (40)
2		264 (52)
3		37 (7)
4		6 (1)
No. Gleason grade (%):	—	
6 or Less		228 (49)
7 (3 + 4)		97 (21)
7 (4 + 3)		49 (10)
8–10		95 (20)

*Matched variables

†p <0.001

Table 2

Gene promoter methylation status and prostate cancer

	% Methylated		Crude Model		Adjusted Model*	
Gene (No. pairs)	Controls	Cases	OR (95% CI)	p Value	OR (95% CI)	p Value
Overall:						
MGMT (414)	11.8	8.5	0.68 (0.43–1.08)	0.106	0.69 (0.42–1.13)	0.139
APC (453)	22.1	24.5	1.15 (0.84–1.58)	0.378	1.12 (0.80–1.56)	0.502
RARB (442)	21.9	32.6	1.76 (1.29–2.40)	<0.001	1.59 (1.15–2.21)	0.005
CCND2 (404)	8.7	8.4	0.96 (0.56–1.65)	0.891	0.97 (0.55–1.69)	0.908
RASSF1 (426)	22.8	19.2	0.80 (0.57–1.12)	0.194	0.82 (0.57–1.17)	0.280
Black:						
MGMT (166)	9.0	8.4	0.93 (0.44–1.98)	0.847	1.14 (0.51–2.51)	0.754
APC (180)	24.4	30.0	1.36 (0.83–2.21)	0.220	1.37 (0.83–2.26)	0.224
RARB (184)	25.0	42.4	2.18 (1.39–3.44)	<0.001	2.09 (1.30–3.36)	0.002
CCND2 (160)	5.6	10.0	2.00 (0.81–4.95)	0.134	1.85 (0.73–4.67)	0.192
RASSF1 (165)	20.6	16.4	0.74 (0.42–1.32)	0.309	0.85 (0.47–1.56)	0.611
White:						
MGMT (248)	13.7	8.5	0.57 (0.31–1.03)	0.061	0.50 (0.27–0.95)	0.034
APC (273)	20.5	20.9	1.02 (0.67–1.55)	0.916	0.95 (0.61–1.47)	0.804
RARB (258)	19.8	25.6	1.43 (0.93–2.20)	0.106	1.24 (0.78–1.96)	0.356
CCND2 (244)	10.7	7.4	0.60 (0.29–1.23)	0.162	0.62 (0.30–1.32)	0.215
RASSF1 (261)	24.1	21.1	0.83 (0.54–1.27)	0.389	0.80 (0.51–1.26)	0.336

*Adjusted for PSA and HGPIN at baseline.

Table 3

Gene promoter methylation status by matching factors and case characteristics

	Overall		Black		White	
Gene	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value**
1–4-Yr followup:						
MGMT	0.66 (0.32–1.38)	0.270	0.90 (0.32–2.51)	0.836	0.44 (0.15–1.29)	0.134
APC	0.85 (0.53–1.38)	0.515	0.83 (0.42–1.65)	0.597	0.93 (0.47–1.86)	0.841
RARB	1.74 (1.06–2.87)	0.029	1.89 (0.93–3.84)	0.078	1.68 (0.83–3.41)	0.147
CCND2	0.77 (0.34–1.75)	0.528	2.14 (0.55–8.40)	0.275	0.36 (0.11–1.25)	0.107
RASSF1	0.72 (0.42–1.24)	0.239	0.44 (0.17–1.15)	0.093	0.97 (0.49–1.92)	0.938
4–15-Yr followup:						
MGMT	0.71 (0.36–1.38)	0.312	1.44 (0.39–5.31)	0.580	0.55 (0.25–1.23)	0.148
APC	1.47 (0.91–2.37)	0.119	2.45 (1.08–5.54)	0.032	1.13 (0.61–2.11)	0.698
RARB	1.66 (1.05–2.60)	0.029	2.53 (1.30–4.91)	0.006	1.06 (0.56–2.03)	0.856
CCND2	1.19 (0.54–2.61)	0.658	1.46 (0.41–5.26)	0.562	1.05 (0.38–2.90)	0.920
RASSF1	0.90 (0.55–1.47)	0.679	1.42 (0.61–3.32)	0.414	0.73 (0.39–1.34)	0.304
Age less than 65:						
MGMT	0.82 (0.40–1.65)	0.572	0.80 (0.21–2.97)	0.736	0.83 (0.36–1.91)	0.665
APC	1.02 (0.62–1.65)	0.950	0.84 (0.40–1.79)	0.658	1.15 (0.61–2.20)	0.663
RARB	1.73 (1.10–2.74)	0.018	2.17 (1.15–4.10)	0.017	1.35 (0.69–2.62)	0.378
CCND2	0.46 (0.18–1.19)	0.110	0.82 (0.17–4.04)	0.807	0.32 (0.09–1.10)	0.070
RASSF1	0.88 (0.53–1.46)	0.621	0.85 (0.36–2.00)	0.713	0.89 (0.47–1.67)	0.712
Age 65 or greater:						
MGMT	0.63 (0.32–1.25)	0.188	1.35 (0.50–3.69)	0.554	0.27 (0.09–0.82)	0.020
APC	1.20 (0.75–1.92)	0.447	2.08 (0.97–4.46)	0.059	0.82 (0.43–1.54)	0.529
RARB	1.52 (0.93–2.47)	0.096	2.15 (1.00–4.60)	0.049	1.15 (0.59–2.22)	0.680
CCND2	1.29 (0.60–2.76)	0.510	2.67 (0.71–10.01)	0.144	0.83 (0.30–2.29)	0.715
RASSF1	0.80 (0.47–1.35)	0.402	1.00 (0.41–2.44)	0.998	0.73 (0.37–1.43)	0.357
Low grade tumor:						
MGMT	0.75 (0.43–1.30)	0.301	1.51 (0.56–4.07)	0.418	0.53 (0.26–1.06)	0.074
APC	0.85 (0.58–1.26)	0.419	1.05 (0.58–1.90)	0.866	0.71 (0.42–1.21)	0.210

Gene	Overall		Black		White	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
RARB	1.63 (1.09–2.42)	0.017	2.54 (1.37–4.73)	0.003	1.16 (0.67–1.99)	0.597
CCND2	1.10 (0.56–2.13)	0.788	1.55 (0.55–4.34)	0.409	0.85 (0.35–2.10)	0.730
RASSF1	0.85 (0.56–1.28)	0.436	0.91 (0.47–1.76)	0.774	0.80 (0.47–1.36)	0.412
High grade tumor:						
MGMT	0.56 (0.21–1.54)	0.262	0.65 (0.16–2.74)	0.561	0.44 (0.10–1.83)	0.258
APC	2.43 (1.20–4.90)	0.014	3.21 (1.06–9.75)	0.040	2.04 (0.80–5.18)	0.133
RARB	1.55 (0.88–2.74)	0.132	1.64 (0.76–3.52)	0.207	1.40 (0.59–3.31)	0.439
CCND2	0.73 (0.25–2.09)	0.558	3.87 (0.41–36.35)	0.237	0.29 (0.06–1.38)	0.118
RASSF1	0.73 (0.35–1.50)	0.389	0.67 (0.16–2.92)	0.597	0.85 (0.37–1.95)	0.707

*Adjusted for PSA and HGPIN at baseline.

Table 4
Gene promoter methylation status by HGPIN and PSA

	% HGPIN		Mean ± SD PSA (ng/ml)	
Methylated Gene (No. pairs)	**Pos/Neg Methylation**	**p Value**	**Pos/Neg Methylation**	**p Value**
Overall:				
MGMT (753)	6.9/9.0	0.5	6.8 ± 5.3/6.9 ± 6.9	0.9
APC (826)	10.3/7.8	0.4	6.8 ± 5.6/6.8 ± 6.9	0.9
RARB (806)	14.0/5.3	<0.0001	7.3 ± 7.2/6.7 ± 6.3	0.3
CCND2 (772)	8.6/8.0	0.9	6.5 ± 5.0/6.8 ± 6.5	0.7
RASSF1 (790)	9.8/8.5	0.6	6.2 ± 5.2/7.0 ± 6.9	0.09
Black:				
MGMT (300)	3.9/9.1	0.7	7.6 ± 6.0/7.5 ± 7.7	0.9
APC (324)	7.8/8.6	>0.99	6.9 ± 4.8/7.8 ± 8.2	0.3
RARB (322)	13.1/4.2	0.005	8.2 ± 8.2/7.2 ± 6.7	0.3
CCND2 (303)	3.7/8.0	0.7	7.3 ± 5.4/7.3 ± 7.3	>0.99
RASSF1 (303)	6.0/8.7	0.8	6.8 ± 5.8/7.9 ± 7.9	0.2
White:				
MGMT (453)	8.5/8.9	>0.99	6.3 ± 4.8/6.4 ± 6.2	0.9
APC (502)	12.5/7.3	0.1	6.7 ± 6.2/6.3 ± 5.9	0.5
RARB (484)	14.8/6.0	0.0005	6.4 ± 6.0/6.4 ± 6.1	0.97
CCND2 (469)	11.6/8.0	0.4	6.1 ± 4.7/6.4 ± 5.9	0.7
RASSF1 (487)	11.5/8.3	0.4	6.0 ± 4.9/6.4 ± 6.0	0.4

Table 5

Joint effects on prostate cancer risk of gene promoter methylation status at RARB and APC

Combined Gene Methylation Status	% Methylated		Crude Model		Adjusted Model*	
	Controls	Cases	OR (95% CI)	p Value	OR (95% CI)	p Value
Overall:						
APC−/*RARB*−	63.1	54.2	1		1	
APC+/*RARB*−	14.6	12.4	0.99 (0.65–1.53)	>0.9	0.96 (0.61–1.49)	0.8
APC−/*RARB*+	14.1	20.0	1.69 (1.14–2.51)	0.009	1.45 (0.96–2.18)	0.08
APC+/*RARB*+	8.2	13.4	1.91 (1.19–3.08)	0.008	1.85 (1.12–3.05)	0.02
Black:						
APC−/*RARB*−	59.3	42.6	1		1	
APC+/*RARB*−	16.0	11.7	1.06 (0.51–2.22)	0.9	1.00 (0.47–2.12)	>0.9
APC−/*RARB*+	15.4	25.9	2.19 (1.22–3.93)	0.009	1.88 (1.02–3.46)	0.04
APC+/*RARB*+	9.3	19.8	2.96 (1.44–6.05)	0.003	3.04 (1.44–6.42)	0.004
White:						
APC−/*RARB*−	65.7	62.0	1		1	
APC+/*RARB*−	13.6	12.8	0.99 (0.58–1.68)	>0.9	0.96 (0.55–1.69)	0.9
APC−/*RARB*+	13.2	16.1	1.34 (0.77–2.33)	0.3	1.19 (0.67–2.11)	0.6
APC+/*RARB*+	7.4	9.1	1.29 (0.67–2.49)	0.4	1.14 (0.56–2.30)	0.7

*Adjusted for PSA and HGPIN

Table 6

Methylation status in histopathologically benign and matched prostate tumor specimens in 58 cases

	Specimen	
	Benign	**Tumor**
No. race (%):		
White	31 (54)	—
Black	27 (47)	—
Cohort entry:		
Mean ± SD cohort entry age	60.2 ± 6.1	3.6 ± 2.0
Median cohort entry date	9/7/94	4/27/99
Mean ± SD PSA cohort entry-diagnosis (ng/ml)	7.7 ± 4.6	14.2 ± 12.9
% Tumor stage:	—	
2A		26
2B		40
2C		19
3A		9
3B		5
3C		2
% Gleason grade:	—	
6 or Less		47
7 (3 + 4)		14
7 (4 + 3)		12
8–10		28
% Pos methylation (No. pts):		
MGMT (53)	13	6
APC (52)	31	77
RARB (54)	30	61
CCND2 (49)	4	39
RASSF1 (44)	30	48