

## Fine-Mapping of *IL16* Gene and Prostate Cancer Risk in African Americans

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### Abstract

**Background:** Prostate cancer is the most common type of cancer among men in the United States, and its incidence and mortality rates are disproportionate among ethnic groups. Although genome-wide association studies of European descents have identified candidate loci associated with prostate cancer risk, including a variant in *IL16*, replication studies in African Americans (AA) have been inconsistent. Here we explore single-nucleotide polymorphism (SNP) variation in *IL16* in AAs and test for association with prostate cancer.

**Methods:** Association tests were conducted for 2,257 genotyped and imputed SNPs spanning *IL16* in 605 AA prostate cancer cases and controls from Washington, D.C. Eleven of them were also genotyped in a replication population of 1,093 AAs from Chicago. We tested for allelic association adjusting for age, global and local West African ancestry.

**Results:** Analyses of genotyped and imputed SNPs revealed that a cluster of *IL16* SNPs were significantly associated with prostate cancer risk. The strongest association was found at rs7175701 ( $P = 9.8 \times 10^{-8}$ ). In the Chicago population, another SNP (rs11556218) was associated with prostate cancer risk ( $P = 0.01$ ). In the pooled analysis, we identified three independent loci within *IL16* that were associated with prostate cancer risk. SNP expression quantitative trait loci analyses revealed that rs7175701 is predicted to influence the expression of *IL16* and other cancer-related genes.

**Conclusion:** Our study provides evidence that *IL16* polymorphisms play a role in prostate cancer susceptibility among AAs.

**Impact:** Our findings are significant given that there has been limited focus on the role of *IL16* genetic polymorphisms on prostate cancer risk in AAs. *Cancer Epidemiol Biomarkers Prev*; 21(11); 2059–68. ©2012 AACR.

### Introduction

Prostate cancer is the most common type of cancer among men in the United States, and its disproportionately higher rate of incidence and mortality among African Americans (AA) are very likely due to a combination of genes and environmental factors. Although genome-wide association studies (GWAS) in men of European descents have identified candidate loci associated with high prostate cancer risk (1–6), the same locus tend not to

be replicated in studies of AAs (7, 8). Moreover, GWAS have not yet produced many insights into prostate cancer disparities (9–11). For instance, a single-nucleotide polymorphism (SNP) in *IL16*, rs4072111, was associated with prostate cancer aggressiveness in European Americans (EA; 1), but a subsequent study in AAs did not replicate the result (7). Given that currently available GWAS panels vary in genome coverage across ethnic groups because of marker selection and linkage disequilibrium (LD) differences, the "exact" replication of index SNPs identified in people of European descent is often problematic in populations with lower LD (12). In populations with lower LD such as AAs, fine-mapping of SNPs by direct genotyping or by imputation combined with deep resequencing provides for high resolution assessment of variation and is useful for "local" replication (replicating associations between phenotype and variants in the same gene/locus as previously reported index SNPs), and finding causal markers (13, 14).

Recent research has shown the link between inflammatory processes and the development of cancer cells. Proinflammatory mediators, such as cytokines, chemokines, prostaglandins, and nitric oxide along with enzymes that respond to these factors are involved in cancer cell

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**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

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doi: 10.1158/1055-9965.EPI-12-0707

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proliferation and progression (15, 16). It also has been hypothesized that inflammatory processes can cause oxidative damage in prostate epithelial cell that can promote proliferative inflammatory atrophy, a precursor of prostatic intraepithelial neoplasia and prostate cancer (17–19). Cytokines are key regulator of inflammation, but overexpression of cytokines during chronic inflammation creates a microenvironment that facilitates initiation and progression of tumor cells (16, 20).

Both immune and tumor cells produce cytokines, including interleukin 16 (IL-16), a chemoattractant for immune cells with CD4 coreceptors, including T cells, monocytes, macrophages, eosinophils, and dendritic cells. Signaling of IL-16 is mediated by the CD4 coreceptor, and IL-16 activates these CD4 immune cells. As in many other organs, T cells, B lymphocytes, macrophages, and mast cells are found in prostate, and greater numbers of CD4 T cells than CD8 cells are found in inflamed benign prostatic hyperplasia (17, 21). Although specific functions of IL-16 in immune response are not well understood, IL-16 is considered as a key mediator of inflammatory response (22) and activates TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-15 (23). These cytokines are known to promote tumor cell growth (15).

Here we explore SNP variation in *IL16* in AAs and test for association with prostate cancer. Lower LD across the gene region in African-descent populations may explain the lack of replication of the *IL16* SNP in AAs. Thus, to search for associated SNPs in AAs, first, we tested association of genotyped and imputed SNPs in and around *IL16* in AA prostate cancer cases and controls from Washington, D.C. Second, we selected 11 SNPs for more

close examination and attempted to replicate the results using an independent group of AA prostate cancer cases and controls from Chicago, IL.

## Materials and Methods

### Subjects

The discovery sample set consists of 260 prostate cancer cases and 345 controls from Washington, D.C., which were a part of a recent GWAS for prostate cancer in AAs (24). These participants were recruited through the Division of Urology at Howard University Hospital in Washington, D.C. (25, 26). The replication sample set includes 543 prostate cancer cases and 550 controls recruited from 3 Chicago area hospitals (27). All the participants are unrelated self-identified AAs (Table 1).

### Genotyping

Subjects in the discovery sample set from Washington, D.C. were genotyped as a part of a large GWAS consortium for prostate cancer in AAs using the Illumina Infinium 1M-Duo bead array (24). We ranked the top 10% of SNPs (out of a total of 1,047,986 SNPs) associated with prostate cancer, and the *IL16* variant, rs7175701, was one of these top ranking SNPs. From the GWAS data, 264 SNPs in *IL16* and 500 Kb region (250 Kb up- and downstream) around *IL16* (81239219-81855109) were available for analysis.

We then selected 11 SNPs from the GWAS panel and genotyped them in the replication sample set. Seven of these 11 SNPs were chosen because of very low *P* values in the discovery data set, including rs7175701 and 6 SNPs in

**Table 1.** Subject characteristics

	Cases	Controls	<i>P</i> <sup>a</sup>
Discovery—Washington, D.C.			
Number of subjects	260	345	
Age (mean and <i>SD</i> ) <sup>b</sup>	64.3 (9.7)	58.7 (10.5)	<0.001
PSA in ng/mL (mean and <i>SD</i> ) <sup>b</sup>	48.9 (140.9)	2.6 (6.9)	
Global WAA (mean and <i>SD</i> ) <sup>b</sup>	81.2 (15.8)	79.5 (18.0)	0.26
Local 15q26.3 WAA (mean and <i>SD</i> )	80.6 (18.1)	79.6 (18.2)	0.51
Replication—Chicago, IL			
Number of subjects	543	550	
Age (mean and <i>SD</i> ) <sup>b</sup>	63.5 (7.3)	58.2 (10.4)	<0.001
PSA in ng/mL (mean and <i>SD</i> ) <sup>b</sup>	45.0 (195.4)	3.3 (4.9)	
Global WAA (mean and <i>SD</i> ) <sup>b</sup>	79.1 (15.0)	78.7 (14.7)	0.81
Pooled			
Number of subjects	803	895	
Age (mean and <i>SD</i> )	64.0 (8.9)	58.5 (10.4)	<0.001
PSA in ng/mL (mean and <i>SD</i> )	47.7 (159.1)	2.9 (6.2)	
Global WAA (mean and <i>SD</i> )	80.3 (15.5)	79.1 (16.5)	0.27

<sup>a</sup>t test *P* values.  
<sup>b</sup>Mean age, PSA levels, and % WAA is not statistically different between discovery and replication cases (*P* > 0.05).

high LD with rs7175701. Four of the 7 SNPs are predicted to affect *IL16* expression (28). In addition, we included rs4072111, rs11556218, rs11325, and rs1131445. rs4072111 was identified in the previous GWAS among EAs (1); rs11556218 is associated with colorectal and gastric cancer and nasopharyngeal carcinoma risk among Chinese (29, 30); and rs11325 and rs1131445 are in the 3' untranslated region and are predicted to affect miRNA binding (31). Genotyping of the replication samples was conducted using iPLEX Sequenom MassARRAY. All the SNPs used in the analyses had genotyping success rates of >95%.

In addition, we estimated of global West African ancestry (WAA) using a previously verified panel of 105 unlinked ancestry informative markers (AIM) in both sample sets (32). To estimate local 15q26.3 WAA, we used HapMap3 Yoruba (YRI) and Utah residents (CEPH) with Northern and Western European ancestry (CEU) data and selected 42 AIMs within 15q26.3, which were genotyped in the Washington, D.C. samples. These 15q26.3 AIMs show large frequency differences between the putative ancestral populations of AAs (YRI and CEU;  $\delta > 0.7$  and  $F_{ST} > 0.5$ ), and were weakly linked or completely unlinked in our Washington, D.C. samples ( $r^2 < 0.1$ ).

### Imputation

Variants within *IL16* and 2 Mb region (1 Mb up- and downstream) around *IL16* were imputed in our discovery sample set using IMPUTE2 and 1000 Genomes Phase I (interim) data as reference panels (33). Instead of choosing reference panels, which are proxies for putative ancestral populations, IMPUTE2 uses multipopulation reference panels and looks for shared haplotypes between reference panels and study samples regardless of their ancestry (34). In this region, 840 SNPs were genotyped in the GWAS, but after imputation, there were 32,613 variants. For the association analyses, SNPs within the *IL16* gene and 500 Kb region (250 Kb up- and downstream) around *IL16* were included after removing low-quality, poorly imputed variants [information metric  $< 0.5$ , variants with more than 10% missing genotypes, Hardy-Weinberg equilibrium (HWE)  $P \leq 0.001$ ] and rare variants [minor allele frequency (MAF)  $< 0.01$ ]. Low-frequency variants (MAF between 0.05–0.01) were included in the analyses to assess whether these low-frequency variants, which are rare or monomorphic in European populations and were not included in the Illumina genotyping array show strong association in AAs. After quality control (QC) filtering, a total of 2,257 (1,992 imputed and 264 genotyped) SNPs were included for association tests. To validate the imputation results, first, we masked 23 genotyped SNPs, including rs7175701, conducted imputation, and then tested for association. Second, we conducted a cross-validation analysis using MACH and HapMap data (35, 36; Supplementary Methods).

### Statistical analyses

For QC, departure from HWE was tested for all the genotyped SNPs in both discovery and replication sample

sets. None of the genotyped SNPs showed deviation from HWE at  $P < 0.001$ . We tested for allelic association and calculated ORs and 95% confidence intervals (CI) using logistic regression, adjusting for age at the time of diagnosis and global WAA in discovery, replication, and pooled data sets. For the analyses of the discovery sample set, we conducted additional association tests adjusting for local WAA in addition to age and global WAA. Empiric  $P$  values were obtained using adaptive permutation procedures implemented in PLINK. Heterogeneity of disease and SNP associations between the discovery and replication sample sets was tested using the  $\chi^2$  and Breslow-Day test. In the pooled data set, additional statistical tests were carried out by stratifying the subjects based on their age and prostate-specific antigen (PSA) levels at the time of diagnosis. For tests of association stratifying by age, we used the following schemes; (i) all cases and controls with age  $\geq 60$ ; (ii) cases with age  $\geq 60$  and all controls; and (iii) cases with age  $< 60$  and all controls. For the tests of association stratifying by PSA levels, we also used 3 schemes; (i) cases with PSA  $\leq 10$  ng/mL and all controls; (ii) cases with PSA  $> 10$  ng/mL and all controls; and (iii) cases with PSA  $\leq 10$  ng/mL and cases with PSA  $> 10$  ng/mL. The tests for association were conducted using PLINK (37).

Both local 15q26.3 and global WAA were estimated separately from the AIMs genotype data using the Bayesian Markov chain Monte Carlo (MCMC) method implemented in the program STRUCTURE 2.1 (38, 39). STRUCTURE 2.1 was run under the admixture model using prior population information and independent allele frequencies. We ran the MCMC method using  $K = 2$  parental populations and a burn-in length of 30,000 for 70,000 repetitions.

LD patterns were examined using Haploview (40). A LD plot of the top-ranked 30 imputed and genotyped SNPs that were strongly associated with prostate cancer was made using 1000 Genomes Phase 1 YRI data. The correlations between each of 11 selected SNPs were also calculated and visualized on LD plots. LD plots of the discovery and replication controls were compared with LD plots of YRI and European populations. 1000 Genomes Phase 1 genotype data of unrelated YRI and European [CEU, Finnish (FIN), British England and Scotland (GBR), and Toscani from Italy (TSI)] individuals were obtained from the ENIGES SPSmart SNP database (41). Genotype data from the 4 European populations were combined. Haplotype blocks were constructed using the Gabriel and colleagues' method (42).

### Results

Mean genetic ancestry estimates were similar for both local WAA on 15q26.3 (mean 80.1%) and global WAA (80.2%; Kendall's  $\tau = 0.444$ ;  $P < 0.001$ ) for our discovery sample set. There was no statistical difference in local WAA between cases and control.

The discovery sample set was a part of the prostate cancer GWAS in AAs (24), and the *IL16* variant, rs7175701,

was one of the top ranking SNPs ( $P = 5.7 \times 10^{-7}$ ) in this subgroup of AAs (Supplementary Table S1). Our association tests of the 264 SNPs in the *IL16* region in the discovery sample set revealed a cluster of 10 SNPs within the gene that were associated with prostate cancer risk (Supplementary Fig. S1). These SNPs remained significant even after permutation testing, with rs7175701 showing the strongest association ( $P_{EMP} = 1.0 \times 10^{-6}$ ).

Then, we closely examined the association of the 11 selected SNPs genotyped in the GWAS. SNP rs7175701 and 6 SNPs around it showed significant association (Table 2). When association tests were conducted adjusting for local 15q26.3 WAA in addition to age and global WAA, the overall association was stronger. rs7175701 exhibited the strongest association and the  $P$  value was lower ( $P = 9.8 \times 10^{-8}$ ). After adjustment, the second most significantly associated genotyped SNP, rs8026245, also had slightly lower  $P$  value ( $P = 2.0 \times 10^{-5}$ ). When a multivariate analysis was conducted to test the association conditioning on rs7175701, rs4616256 remained significant ( $P = 0.04$ ), suggesting that multiple independent risk causing alleles are segregating at *IL16*. This association remained significant ( $P = 0.04$ ), even after adjusting for local WAA. On the other hand, rs7175701 and 4 SNPs around it were significant, after conditioning on rs4616256. These 4 SNPs are in strong LD with rs7175701, whereas rs4616256 is weakly linked to rs7175701 ( $r^2 = 0.07$ ; Supplementary Fig. S2A).

In the combined set of imputed and genotyped SNPs (2,257), we found additional SNPs that show strong association with prostate cancer, but rs7175701 still had the lowest  $P$  value (Fig. 1 and Supplementary Table S2). The 3

top ranked SNPs are adjacent to each other, strongly linked ( $r^2 > 0.977$ ), and located in the same haplotype block (Supplementary Fig. S3). Many other SNPs are also strongly or moderately in LD with rs7175701. After conditioning on rs7175701 in logistic regression analysis, we found that many variants were not significant or considerably less significant, except for 2 (rs16972963 and rs12899457 with  $P$  values of  $6.8 \times 10^{-6}$  and  $8.0 \times 10^{-4}$ , respectively). These 2 variants are localized in a gene desert area more than 200 Kb downstream of *IL16*. The association tests of imputed and genotyped SNPs adjusting for local WAA in addition to age and global WAA remained very similar, and the top 3 most significantly associated SNPs were the same. Moreover, we conducted a cross-validation experiment using MACH and HapMap YRI and CEU data as reference panels, and the 4 top ranked SNPs were exactly the same (Supplementary Note and Supplementary Fig. S4).

Results of the analysis of the replication sample set for the 11 selected SNPs contrasted with the results of analysis of the discovery sample set (Table 3). None of the SNPs that showed strong association with prostate cancer risk in the discovery sample set were significantly associated with prostate cancer risk in the replication sample set. Instead, one missense mutation near the end of *IL16* gene (rs11556218), which was not associated with prostate cancer in the discovery sample set, was significantly associated with prostate cancer risk ( $P = 0.01$ ).

In the pooled analysis of the 11 SNPs, the 7 SNPs that were strongly associated in the discovery sample set and the SNP that was associated with prostate cancer

**Table 2.** Results of association tests for Washington, D.C. discovery samples

SNP	Position <sup>a</sup>	MA <sup>b</sup>	OR (95% CI)	Asymptotic $P$ values <sup>c</sup>	Empirical $P$ values <sup>d</sup>	Adjusting for local WAA $P$ values <sup>e</sup>	Multivariate analysis <sup>f</sup>	
							rs7175701	rs4616256
rs12907134	81525319	A	0.66 (0.50–0.87)	$3.9 \times 10^{-3}$	$4.9 \times 10^{-3}$	$1.6 \times 10^{-3}$	0.78	0.02
rs7179134	81533378	A	1.62 (1.23–2.13)	$5.3 \times 10^{-4}$	$8.1 \times 10^{-4}$	$3.2 \times 10^{-4}$	0.43	$1.4 \times 10^{-3}$
rs7180245	81535149	A	1.66 (1.26–2.18)	$2.9 \times 10^{-4}$	$3.9 \times 10^{-4}$	$1.8 \times 10^{-4}$	0.31	$8.1 \times 10^{-4}$
rs7175701	81558623	C	1.98 (1.51–2.58)	$5.7 \times 10^{-7}$	$1.0 \times 10^{-6}$	$9.8 \times 10^{-8}$	NA	$1.8 \times 10^{-5}$
rs8026245	81561001	G	1.73 (1.33–2.24)	$3.7 \times 10^{-5}$	$2.9 \times 10^{-5}$	$2.0 \times 10^{-5}$	0.96	$7.0 \times 10^{-5}$
rs11637363	81567973	C	1.55 (1.18–2.03)	$1.4 \times 10^{-3}$	$1.6 \times 10^{-3}$	$6.3 \times 10^{-4}$	0.21	0.38
rs4616256	81575754	A	1.85 (1.29–2.65)	$7.8 \times 10^{-4}$	$1.1 \times 10^{-3}$	$6.3 \times 10^{-4}$	0.04	NA
rs4072111	81578139	A	0.70 (0.39–1.25)	0.23	0.24	0.21	0.28	0.38
rs11556218	81598269	G	1.27 (0.93–1.74)	0.14	0.16	0.11	0.12	0.23
rs11325	81601340	T	1.09 (0.83–1.42)	0.53	0.67	0.36	0.63	0.90
rs1131445	81601782	C	0.85 (0.65–1.11)	0.22	0.26	0.24	0.39	0.41

<sup>a</sup>Base pair position on the chromosomal is based on NCBI build 37/hg19.

<sup>b</sup>Minor alleles.

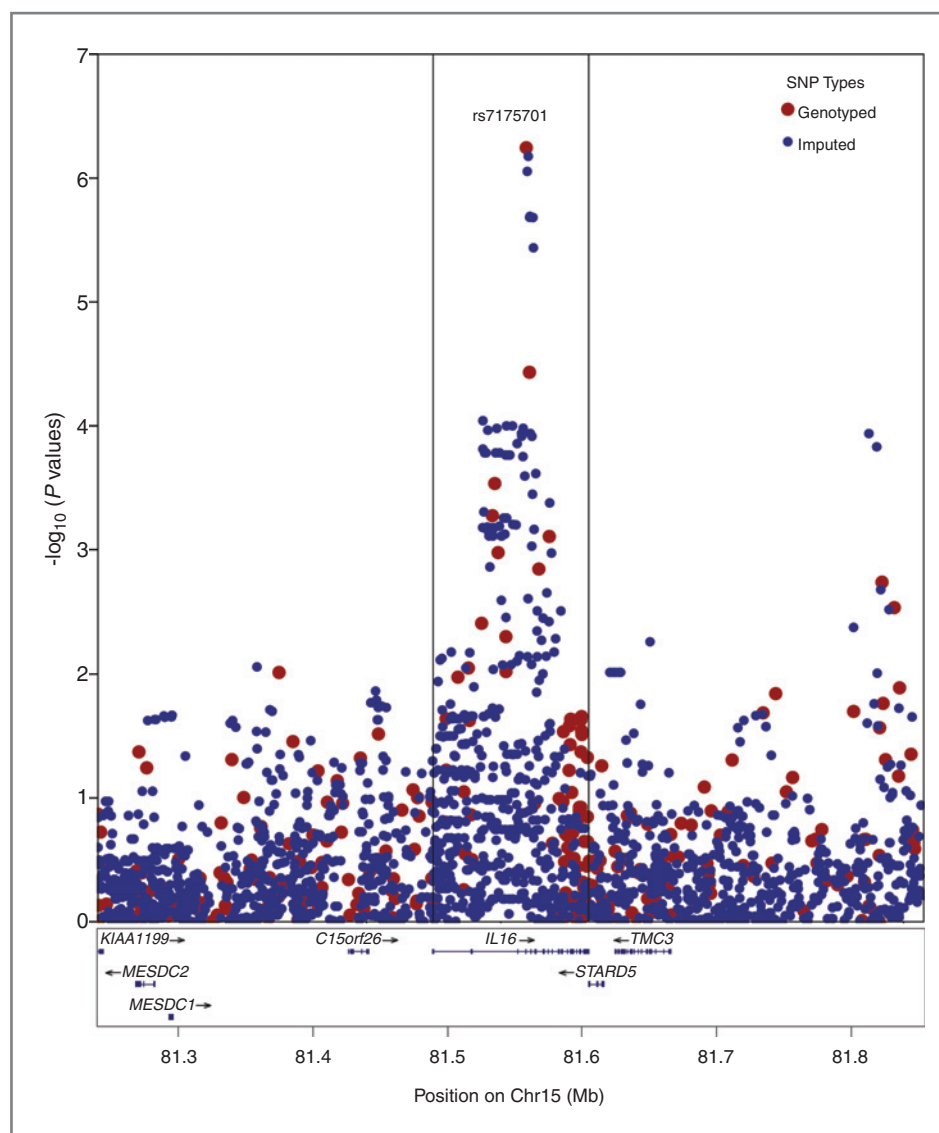
<sup>c</sup>Asymptotic  $P$  values were estimated using logistic regression, adjusting for age and global WAA.

<sup>d</sup>Empirical  $P$  values were obtained by adaptive permutation, adjusting for age and global WAA.

<sup>e</sup>Asymptotic  $P$  values were estimated using logistic regression adjusting for age, local 15q26.3 WAA, and global WAA.

<sup>f</sup> $P$  values of logistic regression adjusting for the SNPs, age, and global WAA.

Figure 1.  $-\log_{10} P$  value plot of genotyped and imputed SNPs in *IL16* ( $\pm 250$  kb) region in Washington, D.C. AA discovery samples. The position of the *IL16* is indicated by 2 vertical lines.



risk in the replication sample set remained significant (Table 4). rs7175701 again was the most significant with an OR of 1.48 (95% CI 1.22–1.79). To test if these SNPs are independently associated with prostate cancer, we tested for association conditioning on rs7175701, and we found that 2 SNPs, rs4616256 and rs11556218, remained significant. When the association was tested conditioning on rs11556218, 7 other SNPs were significant. When we conditioned on both rs7175701 and rs11556218, only rs4616256 remained significant. These 3 SNPs were weakly correlated with each other ( $r^2 < 0.12$ ) in our discovery and replication samples.

Because the association tests in discovery and replication sample sets showed striking differences, we examined MAF differences between cases and controls in both sample sets and tested for heterogeneity between the 2 sample sets (Table 5). In the discovery samples, the MAF of rs7175701 and the SNPs around it are very different

between the cases and controls, but the MAF of these SNPs are similar between cases and control in the replication sample set. The MAF difference between cases and controls for rs11556218 is larger in the replication sample set, but consistent in the 2 sample sets. Table 5 reveals the results of the test of heterogeneity. Heterogeneity in SNP association was observed for 5 of the SNPs with  $P_H < 0.10$ .

We conducted additional association tests stratifying the subjects by age and PSA levels in the pooled sample set to control the confounding effects of age and PSA levels. Although the association was not as strong because of smaller sample size, the results tended to show a similar pattern of association, and rs7175701 showed the strongest association in 3 of these stratified analyses ( $P = 6.7 \times 10^{-5}$ , for all cases and controls with age  $\geq 60$ ;  $P = 1.4 \times 10^{-5}$ , for cases with age  $\geq 60$  and all controls;  $P = 2.8 \times 10^{-5}$  for cases with PSA  $\leq 10$  ng/mL and all controls). An

**Table 3.** Results of association tests for Chicago replication samples

SNP	Position <sup>a</sup>	MA <sup>b</sup>	OR (95% CI)	Asymptotic <i>P</i> value <sup>c</sup>	Empirical <i>P</i> value <sup>d</sup>
rs12907134	81525319	A	0.13 (0.68–1.23)	0.55	0.75
rs7179134	81533378	A	1.01 (0.74–1.39)	0.93	0.86
rs7180245	81535149	A	0.96 (0.70–1.33)	0.82	0.75
rs7175701	81558623	C	1.03 (0.78–1.38)	0.82	0.86
rs8026245	81561001	G	0.91 (0.67–1.24)	0.55	0.86
rs11637363	81567973	C	1.09 (0.82–1.46)	0.55	0.86
rs4616256	81575754	A	1.18 (0.79–1.75)	0.42	0.75
rs4072111	81578139	A	0.90 (0.49–1.66)	0.74	0.86
rs11556218	81598269	G	1.63 (1.12–2.38)	0.01	0.008
rs11325	81601340	T	1.20 (0.89–1.62)	0.22	0.25
rs1131445	81601782	C	0.84 (0.61–1.15)	0.28	0.28

<sup>a</sup>Base pair position on the chromosomal is based on NCBI build 37/hg19.

<sup>b</sup>Minor alleles.

<sup>c</sup>Asymptotic *P* values were estimated using logistic regression, adjusting for age and global WAA.

<sup>d</sup>Empiric *P* values were obtained by adaptive permutation, adjusting for age and global WAA.

interesting pattern was observed for SNP association analyses of cases with PSA > 10 ng/mL and all controls, 2 SNPs were significantly associated with prostate cancer risk ( $P = 0.02$  for rs4616256 and  $P = 0.03$  for rs1131445). Moreover, when cases (PSA ≤ 10 ng/mL) and cases (PSA > 10 ng/mL) were analyzed, only rs12907134 was significant ( $P = 0.01$ ). No SNP was significantly associated with prostate cancer risk when the analysis was restricted to younger prostate cancer cases (age < 60) and all controls.

## Discussion

Several GWAS have found candidate loci for prostate cancer, but the majority of these studies were conducted in men of European descent. Only one GWAS has been conducted to find prostate cancer risk loci in men of African descent (24). With the exceptions of the strong association of 8q24 and 17q12 variants, association studies in men of African ancestry have not replicated the SNPs identified in men of European ancestry (7, 8, 43–45). The possible explanations for this failure include differences

**Table 4.** Results of association tests in the pooled analysis

SNP	Position <sup>a</sup>	MA <sup>b</sup>	OR (95% CI)	Asymptotic <i>P</i> value <sup>c</sup>	Empirical <i>P</i> value <sup>d</sup>	Multivariate analysis <sup>e</sup>		
						rs7175701	rs11556218	rs7175701 and rs11556218
rs12907134	81525319	A	0.76 (0.62–0.94)	0.01	0.01	0.99	0.02	0.95
rs7179134	81533378	A	1.33 (1.09–1.63)	0.006	0.005	0.62	0.007	0.61
rs7180245	81535149	A	1.33 (1.08–1.63)	0.007	0.008	0.56	0.006	0.52
rs7175701	81558623	C	1.48 (1.22–1.79)	$7.52 \times 10^{-5}$	$5.47 \times 10^{-5}$	NA	$1.23 \times 10^{-4}$	NA
rs8026245	81561001	G	1.33 (1.09–1.61)	0.004	0.005	0.73	0.002	0.95
rs11637363	81567973	C	1.32 (1.08–1.60)	0.005	0.004	0.39	0.03	0.51
rs4616256	81575754	A	1.50 (1.16–1.96)	0.002	0.002	0.04	0.001	0.01
rs4072111	81578139	A	0.79 (0.52–1.20)	0.26	0.21	0.31	0.21	0.20
rs11556218	81598269	G	1.42 (1.11–1.80)	0.005	0.005	0.02	NA	NA
rs11325	81601340	T	1.14 (0.94–1.39)	0.20	0.25	0.78	0.85	0.25
rs1131445	81601782	C	0.84 (0.69–1.03)	0.10	0.17	0.18	0.49	0.65

<sup>a</sup>Base pair position on the chromosomal is based on NCBI build 37/hg19.

<sup>b</sup>Minor alleles.

<sup>c</sup>Asymptotic *P* values were estimated using logistic regression, adjusting for age and global WAA.

<sup>d</sup>Empirical *P* values were obtained by adaptive permutation, adjusting for age and global WAA.

<sup>e</sup>*P* values of logistic regression adjusting for the SNPs, age, and global WAA.

**Table 5.** MAF in cases and controls and results of the test for heterogeneity

SNP	Position <sup>a</sup>	MA	Discovery		Replication		Pooled		Heterogeneity P value <sup>b</sup>
			Cases	Controls	Cases	Controls	Cases	Controls	
rs12907134	81525319	A	0.29	0.38	0.34	0.38	0.31	0.38	0.19
rs7179134	81533378	A	0.43	0.34	0.36	0.36	0.41	0.35	0.03
rs7180245	81535149	A	0.43	0.33	0.35	0.36	0.40	0.34	0.01
rs7175701	81558623	C	0.59	0.43	0.51	0.49	0.56	0.46	0.002
rs8026245	81561001	G	0.46	0.35	0.37	0.37	0.42	0.36	0.005
rs11637363	81567973	C	0.48	0.39	0.44	0.42	0.46	0.40	0.11
rs4616256	81575754	A	0.21	0.13	0.17	0.16	0.19	0.14	0.04
rs4072111	81578139	A	0.05	0.06	0.06	0.06	0.05	0.06	0.50
rs11556218	81598269	G	0.23	0.19	0.22	0.16	0.22	0.18	0.35
rs11325	81601340	T	0.43	0.41	0.43	0.38	0.43	0.40	0.64
rs1131445	81601782	C	0.28	0.31	0.29	0.32	0.28	0.31	0.96

<sup>a</sup>Base pair position on the chromosomal is based on NCBI build 37/hg19.

<sup>b</sup>Test of heterogeneity between the discovery and replication sample set based on  $\chi^2$  statistics. Very similar results were obtained from the Breslow-Day tests.

in LD patterns, small sample size, heterogeneous clinical characteristics, and environmental differences. Fine mapping of previously identified regions has found other SNPs that show stronger association with prostate cancer than the GWAS SNPs (46). Thus, this study attempted to replicate the GWAS finding by fine mapping and using larger AA samples than many other replication studies.

SNP rs4072111 identified first in the GWAS in EAs (1) was not significantly associated with prostate cancer risk in either of our AA sample sets. Only 2 replication studies, one conducted in AAs and the other in EAs, examined the association of this SNP with prostate cancer, but neither study found significant association (7, 47). Differences in the LD pattern between African- and European-descent populations probably explain these different results (Supplementary Fig. S2A–S2D). In the 1000 Genomes data for European populations, rs4072111 is not in high LD with other *IL16* SNPs that we examined, but in general these SNPs are in higher LD in European populations than in African-descent populations.

Although the "exact" replication of rs4072111 was not observed, we were successful in "local" replication, revealing that several other *IL16* variants are independently associated with prostate cancer risk in 2 independent samples of AAs. In the discovery sample set, the association tests of genotyped and imputed SNPs revealed that rs7175701 (intron 4) had the strongest signal of association. The genotyped SNP, rs4616256 (intron 9), was also significantly associated, even after conditioning on rs7175701. The association of these 2 intronic SNPs with cancer have not been reported previously, but they are associated with *IL16* gene expression in an expression quantitative trait loci (eQTL) GWAS (28). Although association tests of imputed and genotyped SNPs revealed that no imputed SNPs were more strongly asso-

ciated with prostate cancer than rs7175701, we did observe several imputed SNPs which remained significant even after conditioning on rs7175701, including 2 SNPs (rs16972963 and rs12899457) in a gene desert region more than 200 Kb downstream of *IL16*. When we attempted to replicate the findings with carefully chosen SNPs, we found evidence of another independent risk-causing SNP for prostate cancer, rs11556218. The rs11556218 T/G polymorphism is located in exon 6 and is a missense substitution between asparagine (*Asn*) and lysine (*Lys*). In Chinese population, the G allele of rs11556218 is associated with increased risk of colorectal and gastric cancer (29), and nasopharyngeal carcinoma (30). In the pooled sample set, 3 weakly linked SNPs (rs7175701, rs4616256, and rs1155218) were independently associated with prostate cancer.

The contrasting results of the association tests between our 2 clinical sample sets probably resulted from a combination of factors and illustrate limitations of this study. First, sample size for both clinical sample sets was small and there may not have been sufficient statistical power to show consistent association. A follow-up study that includes more subjects would increase statistical power.

Second, the differences in association could be attributed to differences in WAA proportion in 2 sample sets. The C allele of rs7175701 is more common in West African (0.57 in HapMap YRI) than non-African populations (0.46 in artificial seawater and 0.24 in CEU), and more Washington, D.C. prostate cancer cases had high global WAA estimates (>90%) than Chicago prostate cancer cases (36.5% in Washington, D.C. and 23.6% in Chicago cases; Supplementary Fig. S5). The frequency of rs7175701 allele C, however, was not different between the discovery and replication sample sets (50% in discovery cases and controls combined and 49% in the replication set), and the mean global WAA between discovery and replication

cases was not statistically different ( $P = 0.19$ ). The heterogeneity in global WAA proportion in the 2 data sets could be explained by the enrichment of local 15q26.3 WAA in Washington, D.C. samples, so we conducted association tests further adjusting for local WAA in addition to global WAA. When local WAA was adjusted, the association of rs7175701 became stronger ( $P = 9.8 \times 10^{-8}$ , compared with  $P = 5.7 \times 10^{-7}$ ). Although we controlled for global WAA in our analyses, we also explored the association using only subjects with global WAA greater than 80% in the pooled data set. The SNP rs7175701 was still significantly associated with prostate cancer risk ( $P = 0.002$ ), again suggesting that the association between rs7175701 and prostate cancer risk was not confounded by population stratification.

Third, there are multiple pathways to prostate cancer initiation and progression, so the differences in the clinical characteristics between 2 sample sets could explain the differing results. The Chicago replication samples were recruited more recently than the Washington, D. C. discovery samples, and prostate cancer was detected through more aggressive PSA screening with a lower cutoff for biopsy in some of replication samples. The characteristics of cases in the discovery and replication sample sets are, however, similar and the mean age and PSA levels between 2 sample sets are not statistically different ( $P = 0.336$  for age and  $P = 0.342$  for PSA). We also noted that the replication controls had slightly higher mean PSA, so we tested association excluding control subjects with PSA > 2.5 ng/mL. The results of association tests were the same as the results of tests including all the replication controls and rs11556218 showed the strongest association. The stratified analysis in pooled data set based on PSA levels showed similar results with the strongest association of rs7175701, confirming the validity of our results despite the heterogeneity between the sample sets.

To further understand the role of rs7175701 in gene expression and interaction, we browsed SCAN, a database of genetic and genomic data on eQTL (48), because the true causal loci are likely to be eQTLs (49). SCAN predicts that this SNP affects the expression of *IL16* and 8 other genes in HapMap CEU and a total of 18 genes in YRI lymphoblastoid cell lines. Five of the 18 genes (*DNMT3A*, *ITGA1*, *SLC30A1*, *SSTR2*, and *TRIM35*) are expressed in prostate cancer cells and another gene *ZNF281* could be a target of *SOX4*, a transcription factor that is overexpressed in tumor cells (50). Seven other genes (*FKRP*, *HCP5*, *PELO*, *PPARGC1B*, *PRCC*, *RHBDD2*, and *ZMAT3*) are expressed or involved in other types of cancer. It is noteworthy that zinc is involved in the function of 3 of these genes, *SLC30A1* (or *ZNT1*, Zinc transporter), *ZMAT3* (Zinc finger protein), and *ZNF281* (Zinc finger protein). Zinc may play roles in prostate cancer development (51, 52) and zinc transporters are downregulated in prostate cancer cells of AAs (53). Interestingly, only one gene (*ITGA1*) has a possible role in inflammatory response. When we searched the SCAN database for rs4616256 and

rs11556218, SCAN did not predict that these 2 SNPs affect expression of any gene that is expressed in prostate cancer cells or other types of cancer cells.

Effects of *IL16* variants on expression of *IL16* in prostate cancer cells and other tumor cells and the association of *IL16* variants with prostate cancer aggressiveness and clinical characteristics deserve further investigation. The expression of *IL16* in prostatectomy tumor specimens from prostate cancer patients is positively correlated with Gleason score and pathologic stage (54), and high expression of *IL16* has been observed in other types of tumor cells (55, 56). The serum levels of IL-16 protein are also elevated in patients with other types of cancers, especially patients in advanced stages of cancer (57, 58).

Although the exact mechanism is unclear and additional functional studies are warranted, our study provides evidence that *IL16* polymorphisms play a role in prostate cancer susceptibility in AAs. Many of the significantly associated SNPs are linked to rs7175701, which is an eQTL that is associated with expression of genes related to prostate cancer or other types of cancers. Another associated variant, rs11556218, consists of an amino acid change that may affect function of the gene. Our findings are significant given that there has been limited focus on the role of *IL16* genetic polymorphisms on prostate cancer risk. To our knowledge, this is the first study that showed the association of these variants with cancer in African-descent populations. Identifying genetic variants affecting the expression and interaction of *IL16* and other gene expressed in tumor cells is important for understanding the role of *IL16* in tumor growth.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** C. Ahaghotu, R.A. Kittles

**Development of methodology:** R.A. Kittles

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** A.B. Murphy, J. Newsome, M. Ruden, C. Ahaghotu, R.A. Kittles

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** K. Batai, A.B. Murphy, R.A. Kittles

**Writing, review, and/or revision of the manuscript:** K. Batai, A.B. Murphy, C. Ahaghotu, R.A. Kittles

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** R.A. Kittles

**Study supervision:** A.B. Murphy, R.A. Kittles

**Other (designing primers and other related tasks for preparation of DNA samples, performing SNP genotyping and analysis, and providing background information on *IL16* gene):** E. Shah

#### Web Resources

SPSmart SNP Database (<http://spsmart.cesga.es/>)

SCAN SNP and CNV Annotation Database (<http://www.scandb.org/newinterface/about.html>)

#### Acknowledgments

The authors would like to thank all the participants from Washington, D.C. and Chicago, IL. The authors also thank Drs. Nathan Ellis and Wendy Hernandez for helpful discussions and manuscript edits.



## Grant Support

This work was supported by the Department of Defense (DAMD W81XWH-07-1-0203) and NCI/NIH (RC2-CA148085-01 and U01-CA136792-02S1).

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Received June 14, 2012; revised July 27, 2012; accepted August 13, 2012; published OnlineFirst August 24, 2012.

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