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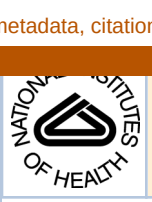

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Genetic polymorphism and prostate cancer aggressiveness: A case-only study of 1536 GWAS and candidate SNPs in African Americans and European Americans

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Abstract

BACKGROUND—Genome-wide association studies have established a number of replicated single nucleotide polymorphisms (SNPs) for susceptibility to prostate cancer (CaP), but it is unclear whether these susceptibility SNPs are also associated with disease aggressiveness. This study evaluates whether such replication SNPs or other candidate SNPs are associated with CaP aggressiveness in African-American (AA) and European-American (EA) men.

METHODS—A 1,536 SNP panel which included 34 genome-wide association study (GWAS) replication SNPs, 38 flanking SNPs, a set of ancestry informative markers, and SNPs in candidate genes and other areas was genotyped in 1,060 AA and 1,087 EA men with incident CaP from the North Carolina-Louisiana Prostate Cancer Project (PCaP). Tests for association were conducted using ordinal logistic regression with a log-additive genotype model and a 3-category CaP aggressiveness variable.

RESULTS—4 GWAS replication SNPs (rs2660753, rs13254738, rs10090154, rs2735839) and 7 flanking SNPs were associated with CaP aggressiveness ($P<0.05$) in 3 genomic regions: one at

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No potential conflicts of interest were disclosed.

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3p12 (EA), 7 at 8q24 (5 AA, 2 EA), and 3 at 19q13 at the kallilkrein-related peptidase 3 (*KLK3*) locus (2 AA, 1 AA and EA). The *KLK3* SNPs also were associated with serum prostate-specific antigen (PSA) levels in AA ($p < 0.001$) but not in EA. A number of the other SNPs showed some evidence of association but none met study-wide significance levels after adjusting for multiple comparisons.

CONCLUSIONS—Some replicated GWAS susceptibility SNPs may play a role in CaP aggressiveness. However, like susceptibility, these associations are not consistent between racial groups.

Keywords

SNP; Prostate cancer aggressiveness; African American; Genome-wide association

INTRODUCTION

Prostate cancer (CaP) is the most common cancer diagnosed in men living in developed countries and the second leading cause of cancer death among men in the US(1,2). African Americans (AA) have substantially higher CaP incidence and mortality rates than European Americans (EA)(2–5) and age and family history are well established CaP risk factors for both AA and EA (6,7).

Extensive evidence supports a genetic component for CaP susceptibility. Quantitative estimates from twin studies indicate that 42% of the variation in CaP risk may be attributed to genetic components—higher than for any other type of common cancer (8). In particular, a number of genome-wide association studies (GWAS) have identified and replicated single nucleotide polymorphisms (SNPs) associated with CaP risk (9–18), which have been summarized in a recent meta-analysis(19). Several studies have provided evidence that some SNPs associated with susceptibility in EA may also show association in AA (18,20). A large study of AA by Haiman et al.(21) found that about half of SNPs identified from GWAS studies of other ethnic groups also showed association for risk of CaP in AA men. In addition, nearby SNPs may show stronger associations with risk in AA (18,20), perhaps reflecting ethnic-specific LD structure at risk loci.

It remains unclear whether the growing number of well-replicated susceptibility SNPs emerging from case-control GWAS are also associated with disease aggressiveness among men with CaP. A few studies have examined this question for GWAS susceptibility SNPs among EA but the relationships have not been consistent from study to study(22,23). If susceptibility and aggressiveness are biologically correlated (use similar gene pathways), then GWAS results on susceptibility may be useful in identifying candidate SNPs for aggressiveness, particularly in candidate genes. Chen and Witte suggest that beyond conventional p values, additional information such as SNP functionality and conservation should be included when ranking SNPs for further study (24). Existing studies of aggressiveness have yet to examine large numbers of AA where disease aggressiveness is higher. Furthermore, AA have different patterns of linkage disequilibrium that may provide additional insight into causative allele identification.

Candidate gene and GWAS of copy number variations (CNVs) have provided some evidence of loci contributing to CaP aggressiveness, although most have come from studies of men of European ancestry(23,25–31). The inability to accurately identify men with aggressive vs. indolent CaP has generated increased interest in identifying genetic markers associated with aggressive disease(32,33).

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Here we report on CaP aggressiveness from a large, population-based case-only study of AA and EA men. We examine both a set of replicated susceptibility SNPs from GWAS and SNPs flanking those regions along with a wider set of candidate SNPs chosen based on GWAS results, linkage, and candidate gene studies and incorporating both functional predictions and ethnic-specific LD relationships.

SUBJECTS AND METHODS

Study population

PCaP is a multi-disciplinary, population-based, case-only study designed to investigate racial differences in CaP that has been previously described(18,34). In North Carolina (NC), prostate cancer cases were diagnosed between July 2004 and October 2007. In total 1,655 NC men were eligible and 1,031 NC men participated (62.3%) in the PCaP study including 505 AA (62.1% participation rate) and 526 EA (64.9% participation rate). In Louisiana (LA), prostate cancer cases were diagnosed between May 2004 and June 2005 (Pre-Hurricane Katrina PCaP cohort) and between January 2006 – July 2009 (Post-Hurricane Katrina PCaP cohort). In total, 293 LA men were eligible for the Pre-Katrina cohort and 213 participated (72.7%) in PCaP including 119 AA (70.8% participation rate) and 94 EA (79.0% participation rate). In total, 1,614 LA men were eligible for the Post-Katrina cohort and 1,014 participated (70.7%) in PCaP including 506 AA (62.6% participation rate) and 508 EA (70.7% participation rate). In summary, 1,130 men self-reporting as black/African American (AA) and 1,128 men self-reporting as white/Caucasian/Caucasian American/ European American (EA) between 40–79 years of age with newly diagnosed, histologically-confirmed, adenocarcinoma of the prostate were enrolled in North Carolina (NC) or Louisiana (LA). Study questionnaire information was collected via a structured in-home interview conducted by trained study nurses that included information on date of birth, self-described race and ethnicity, family history of CaP, and detailed information on demographic characteristics, diet, and health history. Medical records were abstracted for information related to the CaP screening and diagnosis including total serum prostate-specific antigen (PSA) (defined as the PSA value closest and within 1 year prior to diagnosis date), tumor stage at diagnosis (stage number was derived from stage as reported in the medical record –see Statistical Analysis section) and clinical Gleason score (sum of primary and secondary Gleason grade). Tumor aggressiveness was classified using clinical Gleason score, stage, and PSA as: (1) high aggressive (Gleason score ≥ 8, or PSA > 20 ng/ml, or Gleason score = 7 and stage cT3–cT4) (N=370 subjects (17%): 212 AA and 158 EA); (2) low aggressive (Gleason score < 7 and stage cT1–cT2, and PSA < 10 ng/ml) (N=1054 subjects (49%): 469 AA and 585 EA); or (3) intermediate aggressive (all other cases) (N=643 subjects (30%): with 331 AA and 312 EA) (Table 1). Sufficient medical records data was not available to classify aggressiveness category (designated as unknown) for approximately 4% of the study population (N= 80 subjects: 48 AA and 32 EA). Abstraction of medical record data for details of prostate cancer treatment and long term follow-up of outcome are currently underway. DNA was available on 1,060 (94%) AA and 1,087 (96%) EA men who are the subjects of this study (Table 1). Age at diagnosis was derived from self-reported date of birth and date of diagnostic biopsy as indicated in the medical record and rounded to the nearest full year. Informed consent was obtained from all subjects prior to blood and questionnaire collection.

SNP selection

A set of 1,536 SNPs were selected using the SNPinfo web server, which incorporates ethnic-specific linkage disequilibrium (LD) information, GWAS P-value results, and SNP functional predictions(35). An important feature of SNPinfo is the ability to specify the source population for the GWAS data used (in this case European) so that the appropriate

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LD structure is used for SNP selection. The Tagster algorithm(36) was used to select multiple population tag SNPs, in this case for both AA and EA. SNPinfo also allows for the incorporation of disease-associated P-values to aid in SNP prioritization. This study used data from Cancer Genetic Markers of Susceptibility (CGEMS) project, which used a 550K GWAS array on 1,171 CaP cases and 1,157 controls of European descent(11). The smaller of the two P values was used for each SNP in the GWAS: either the "susceptibility" P value reported by CGEMS based on case-control analysis, or the "aggressiveness" P value that was computed by our group using the aggressiveness phenotype reported by CGEMS for the 1,171 cases in a case-only analysis.

The SNP selection procedure had 6 different selection processes. The first process made use of primary and supplementary data from published GWAS and GWAS replication studies to identify 286 SNPs associated with CaP. These SNPs include a special subset of 34 "replication SNPs," i.e. SNPs that were the primary findings of either CaP GWAS or replication studies available at the time the panel was constructed.

The second selection process was based on candidate genes. Literature was reviewed to construct a list of 848 genes of interest in CaP etiology or aggressiveness. Five hundred and forty two candidate genes were eliminated using SNPinfo and only those candidate genes that met one of the following criteria were retained: They contained CGEMS GWAS SNPs that had P-values < 0.05 (based on susceptibility or aggressiveness), or they failed the first criterion but had insufficient SNP coverage in the 550K GWAS panel to adequately assess the gene. Insufficient gene coverage was defined as < 50% of common SNPs (MAF ≥ 0.05 in Europeans) reported in dbSNP for that gene in high LD (r^2 ≥0.80) with at least one SNP in the 550K GWAS panel. Three hundred and six genes were retained and SNPinfo's GenePipe was used to preferentially select 583 SNPs that were predicted to be functionally important, had small P-values, or were multi-population LD tag SNPs. GenePipe parameter values for this selection were as follows: gene upstream region = 5 kb, gene downstream region = 1 kb, MAF = 0.05 for all SNPs, weight = 3 for any predicted functional SNP and small *P*-value SNPs, weight = 1 for all other SNPs, r^2 threshold = 0.8, minimum number of SNPs tagged by each selected tag SNP = 3, minimum number of tag SNPs/gene = 1, and maximum number of tag SNPs/gene = 5.

The third genome-wide selection process prioritized SNPs in the CGEMS 550K panel with small P-values (based on either susceptibility or aggressiveness) that were either themselves predicted to have functional effects or were in high LD with a SNP that was predicted to have functional effects. 6,034 CGEMS SNPs had $P \leq 0.01$. SNPinfo's GenomePipe identified 41,755 SNPs that were in high LD ($r^2 \geq 0.8$) with at least one of these SNPs. From the 41,755 SNPs, SNPinfo selected 379 common SNPs (MAF ≥ 0.05) that were predicted to be functional by at least one of the biological function prediction methods. Functional prediction methods incorporated into SNPinfo include Polyphen, SNPs3D, SIFT, TRANSFAC®, evolutionary conservation and miRNA target site activity (34).

The fourth selection process was based on reported linkage regions. Forty three non-overlapping linkage regions were identified that have been reported to be associated with CaP. SNPinfo's LinkagePipe was used in conjunction with the CGEMS P values (susceptibility or aggressiveness) to select a maximum of 7 of the most significant SNPs from each of the 43 linkage regions.

The fifth process capitalized on the overlap between small P-value SNPs reported in multiple CaP GWAS studies. At the time of this study, the only publicly available CaP GWAS data other than CGEMS was for the Framingham Study(37). Fifty eight SNPs were included that had association P-values <0.01 in both the CGEMS and Framingham GWAS.

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Finally, to control for population stratification, 50 ancestry informative SNP markers (AIMs) were selected using allele frequency data in HapMap phase I+II populations (http://hapmap.ncbi.nlm.nih.gov). Twenty five of these SNPs were monoallelic (variant allele frequency (VAF) = 0) in CEU (US residents with ancestry from Northern and Western Europe, collected by the Centre d'Etude du Polymorphisme Humain (CEPH)), rare in Asians (HapMap CHB plus JPT) (VAF < 0.01) but common in populations of African ancestry (YRI, Yoruban from Nigeria, VAF > 0.65 and AA VAF > 0.25). Additionally 25 SNPs were monoallelic (VAF = 0) in YRI, rare in Asians (VAF < 0.05), but common in CEU (VAF > 0.5).

Genotyping

DNA was extracted from blood samples (n=1,785) or buccal cells (n=140) by the UNC-Chapel Hill Biospecimen Processing Facility, or from lymphocytes immortalized by the UNC-Chapel Hill Tissue Culture Facility (n=222). Genotyping was performed by the NIH Center for Inherited Disease Research (CIDR) using a custom designed 1,536 SNP Illumina GoldenGate array. Genotyping took place in 2 phases (phase 1: 933 samples and phase 2: 1,315 samples) as samples became available. Genotyping in phase 1 included a set of 22 blinded PCaP sample duplicates, 29 HapMap sample duplicates and 19 HapMap trios. Phase 2 included a set of 29 blinded PCaP sample duplicates, 19 HapMap sample duplicates and 11 HapMap sample trios. A total of 100 PCaP samples overlapped between phase 1 and phase 2 to monitor genotyping quality. For both phases the HapMap samples included representation of CEU and YRI ancestral groups. Allele calling was conducted using Illumina's Genotyping Module version 1.0.10 in GenomeStudio 1.0.2.20706. The genotype intensity cluster plots were visually inspected for each SNP. Genotypes with an Illumina GenCall (GC) score below 0.25 were assigned as missing.

Statistical Analysis

Association of individual SNP with a 3-category CaP aggressiveness variable intended to capture disease severity (where low was coded as 0, intermediate as 1 and high as 2; see 'Study Population' description for detailed definition of aggressiveness category) and 4-category tumor stage variable: category 1 - T1a, T1b, T1c, T1(Not Otherwise Specified, NOS), T1C (N=1163 subjects (54%): 570 AA and 593 EA); category 2 - T2(NOS), T2a, T2b, T2 a or b, T2c (N=895 subjects (42%): 441 AA and 454 EA); category 3 -T3/4(NOS), T3b, T3a, 3A and category 4 - T4 (categories 3 and 4 (T3/T4 and T4) were combined due to small sample size, N=37 subjects (2%): 19 AA and 18 EA) were tested using ordinal logistic regression adjusting for recruitment site (NC or LA). Stage category was derived from stage as report in the medical record. Of the 2147 subjects genotyped, 2096 (97.6%) had stage available, while 51 (2.4%) did not. Of the 2096 subjects with stage, 2015 subjects (96.1%) had pathological stage data. For the 81 subjects (3.9%) missing pathological stage, clinical stage was used. Additionally for a small portion of subjects (N=51 subjects, 2.4%) neither pathological nor clinical stage was available; these subjects were designated 'unknown' (Table 1). A log-additive genotypic model was assumed where genotypes were coded as 0, 1, or 2 according to the number of minor alleles carried for each SNP. Association between SNPs and either log transformed PSA value or Gleason score were tested with a linear mixed model adjusting for site (NC or LA) as a random variable. SNP effect on age at diagnosis was tested using a Cox proportional hazards model. The proportion of European or West African ancestry was used to correct for population stratification in all tests. Ancestry proportion was estimated using STRUCTURE(38) and AIMs. HapMap genotype data for 209 independent individuals from CEU, YRI, CHB and JPT populations were used to assist individual ancestry estimation. Statistical tests for GWAS and flanking SNPs were not corrected for multiple comparisons because these SNPs were selected based on *a priori*

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evidence of CaP association, but other SNPs were evaluated against study-wide significance levels.

RESULTS

Forty one PCaP research subjects were excluded because of failed genotyping due to poor sample quality and 78 research subjects were excluded due to missing aggressiveness data. In total, 2,028 research subjects with high quality genotype data were available for analysis, consisting of 449 AA and 480 EA from NC, and 548 AA and 551 EA from LA. Two hundred and twenty two SNPs (14%) in phase 1 and 248 SNPs (16%) in phase 2 were excluded due to poor clustering pattern or parent-parent-child (P-P-C) heritability errors identified using HapMap trios which resulted in 1,247 SNPs with high quality genotype data that were available for men in both genotyping phases. The overall research subject genotyping call rate was 99.96% and 99.91%, respectively for phase 1 and phase 2. In both phases, the reproducibility rate was 99.99% based on blind duplicates and the overall P-P-C heritability based on HapMap trios was 99.98%.

Thirty six ancestry informative markers (AIMs) had high quality genotyping data and were used for ancestry estimates (Supplementary Figures 1A and B). Self-identified EA men (n=1,031) had an average proportion of European ancestry of 0.97±0.08 and self-identified AA men (n=997) had an average proportion of African ancestry of 0.90±0.16 and European ancestry of 0.08±0.15.

The 1,536 SNP panel included 34 replication SNPs identified from CaP GWAS. Eight additional SNPs (Supplementary Table 2) that were part of the 1,536 SNP panel were subsequently replicated in GWAS studies and thus increased to 42 the total number of GWAS replication SNPs included on the panel. The panel included an additional 45 “flanking” SNPs that were within the same gene or intergenic region as replication SNPs and could be useful in dissecting LD structure, particularly in AA. Genotype data were available for 34 replication SNPs and 38 flanking SNPs, after exclusion of SNPs based on genotype quality criteria. Of the 34 replication SNPs, 4 were significantly associated (P <0.05) with CaP aggressiveness in either AA or EA: one on 3p12, two on 8q24, and one on 19q13 (Table 2). Seven of the 38 flanking SNPs were significant at an unadjusted P-value threshold of 0.05, in AA or EA: 5 on 8q24 and two on 19q13.

The chromosome 19q13 SNPs were located in and around the kallikrein gene family. In AA, the 3 SNPs had an association with PSA levels but not with age (Table 3). Among AA, SNPs rs266870 and rs2735839 demonstrated evidence suggestive of an association (p=0.05) for stage and Gleason score, respectively. In EA, there was no association of the 3 SNPs with either PSA or age, however all 3 were associated with Gleason score. Additionally, SNP rs266870 showed association with stage.

Of the remaining 1,139 SNPs with high quality genotype data that were not AIMs, replicated SNPs, or flanking SNPs, 54 showed evidence of association ($P < 0.05$) in AA and 52 showed evidence of association in EA (Supplementary Table 1). However, only two of these SNPs showed evidence of association in both groups and none of the SNPs met study-wide significance (corresponding to unadjusted P value of 4.13×10^{-5}).

DISCUSSION

Approximately 1 in 6 American men will be diagnosed with CaP in their lifetime, however less that 3% will die from their disease(2). More than 90 percent of CaP diagnosed in the US were identified at an early stage from widespread PSA screening(39), but it remains unclear whether early detection actually reduces mortality from CaP(40,41). Although death rates

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for CaP have declined since 1991–1994, disparities in both incidence and mortality rates between African- and European-American men remain, with AA more likely to develop and die from CaP(2). Standard CaP treatment is costly and has been associated with considerable comorbidity, which includes urinary incontinence and sexual impotence, and subsequent decreases in quality of life(33,39). Therefore the identification of factors that put men at high risk for aggressive CaP is important for public health. Although genetic factors have been associated with CaP susceptibility, their utility as potential markers for CaP severity and their applicability across different populations is not known. In a large study of 3055 AA men with CaP, Haiman et al.(21) found that there was no evidence of an association between the total number of GWAS susceptibility alleles that an individual carried and the severity of CaP, but this does not exclude the possibility that individual SNPs may have association with disease severity.

In this report, we describe a case-only analysis of CaP aggressiveness in 1,060 AA and 1,087 EA collected by PCaP: One of the largest single cohorts of AA with CaP analyzed to date for genetic determinants of aggressiveness. Significant strengths of this study include the population-based composition of the cohort which makes research findings generalizable, the inclusion of a large number of African American men, and the extensive clinical and epidemiological characterization of research subjects. A central goal of this analysis was to determine if genetic variants associated with disease susceptibility identified in GWAS of men of European descent were also associated with disease aggressiveness in either AA or EA. In addition, CaP aggressiveness alleles were examined in other candidate genes and regions implicated in CaP, with a focus on possibly functional SNPs. While several studies have examined GWAS replication SNPs for their association with CaP disease severity in European men(14,22,23), studies in AA and have typically been focused on a few specific gene pathways or genomic regions (e.g., 8q24) and have used case-control or linkage analyses to evaluate *susceptibility* to advanced disease(21,25,42–45). This study is one of the first to examine the association of an extensive set of GWAS replication SNPs with aggressiveness in a case-only analysis of a large number of AA with incident CaP.

We found evidence that some susceptibility SNPs may be associated with disease aggressiveness. A GWAS replication SNP (rs2660753) on chromosome 3p11 between hypothetical gene LOC285232 and CHMP2B was associated with aggressiveness in EA, but not AA. This SNP was not predicted to have functional effects and lies in an intergenic region of 3p approximately 8.7 kb downstream from LOC285232 and 166 kb upstream from CHMP28, its nearest gene neighbors. The 8q24 region, one of a few genomic regions evaluated in both AA and EA for association with CaP susceptibility (9–11,21,29,46,47) and disease severity (21,29,48–50), held a number (N=7) of the significant SNPs in this cohort. Of these, two (rs13254738 and rs10090154) were GWAS replication SNPs and both SNPs were associated with PCaP aggressiveness in AA but not EA. Of the remaining 5 significant flanking SNPs in 8q24, 3 were associated in AA and two in EA. The direction of effect was not always consistent between AA and EA, however such ethnic flip flops of the risk allele may reflect ethnic differences in LD structure (18).

In addition, 3 SNPs in and around kallikrein gene family member kallikrein-related peptidase 3 (*KLK3*) on chromosome 19q13 also were associated with CaP aggressiveness which includes one replication SNP rs2735839 (Table 2). *KLK3* is responsible for the production of prostate-specific antigen (PSA). Serum PSA level, in combination with other clinical parameters, is used often to assess CaP severity as it was in the PCaP study. Of the 3 *KLK3* related SNPs, rs1058205 and rs2735839 were in high LD in populations of both African descent (Africans from Southwestern US, ASW) and European descent (Utah residents of Northern and Western European descent, CEU). This high LD was evident in

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the consistency of the results for both SNPs when evaluated for association with prostate cancer aggressiveness in PCaP.

A sub-analysis of the 3 *KLK3* SNPs associated with CaP aggressiveness for their association with serum PSA, stage, Gleason score and age at diagnosis was conducted to further understand the relationship between *KLK3* SNPs and CaP (Table 3). All three 19q13 SNPs were significantly associated with serum PSA levels in AA (Table 3). The direction of the effect was unexpected although consistent for all three SNPs, the alleles that were associated with decreased aggressiveness (Table 2) were also associated with increased serum PSA. In contrast, among EA, only one SNP had an association with aggressiveness, none had an association with PSA level, and all 3 SNPs were significantly associated with Gleason score – a relationship that was not found in AA. Only the *KLK3* replication SNP rs2735839 has been previously evaluated in EA for its association with other clinicopathological features of CaP(22,23), however results have been inconsistent. The association between the *KLK3* locus, PSA levels, and disease aggressiveness has been the subject of a recent review (23) and data from non-PSA screened cohorts(17) and a functional study of *KLK3* alleles(51) support a possible dual role for this locus.

In addition to *KLK3*, only one other CaP GWAS replication SNP and two of the remaining SNPs surveyed in our panel have been previously associated with disease severity. With the exception of the *KLK3* SNPs, we did not find evidence of association. We also evaluated a wide array of candidate gene and other SNPs for association with CaP aggressiveness and while some yielded P-values < 0.05, in either or both AA and EA groups, none reached study-wide significance.

The use of CaP aggressiveness at diagnosis may be a weak surrogate for CaP survival –the true outcome of interest. CaP aggressiveness at diagnosis may be influenced by screening and access to health care, and both may vary by racial group; thus there is the possibility of misclassification of CaP aggressiveness within the PCaP study. For example, frequently screened individuals are more likely to be diagnosed with CaP at an earlier stage, low Gleason grade, and low PSA level and therefore are more likely to be classified as having low aggressive disease at diagnosis. However some of these individuals may have rapidly progressing disease that is only evident after diagnosis. The ongoing collection of outcome data for most PCaP research subjects will allow for more accurate classification in the future.

Efforts are underway to conduct a meta-analysis of more than 30 GWAS SNPs for their association with CaP severity among a set of prostate cancer studies(32). A key goal of such an effort is long-term follow-up to ensure cancer outcomes are available for analysis. Such joint research in diverse populations will be important for establishing genetic markers of CaP disease severity that may help guide treatment decisions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical characteristics of PCaP research subjects with available DNA

	African American		European American	
	NC	LA	NC	LA
No. of subjects	458	602	493	594
Age at diagnosis				
Median	60	63	64	65
Range	41–79	43–79	41–79	42–79
Median PSA, ng/ml	6	6	5	5
PSA levels, N(%)				
<4	40 (8.7)	67 (11.1)	81 (16.4)	92 (15.5)
4–10	286 (62.4)	327 (54.3)	329 (66.7)	373 (62.8)
10–20	76 (16.6)	94 (15.6)	62 (12.6)	61 (10.3)
>20	52 (11.3)	62 (10.3)	20 (4.1)	35 (5.9)
Unknown	4 (0.9)	52 (8.6)	1 (0.2)	33 (5.6)
Gleason Score, N (%)				
≤6	242 (52.8)	344 (57.1)	314 (63.7)	360 (60.6)
7	159 (34.7)	174 (28.9)	128 (26.0)	162 (27.3)
8–10	56 (12.2)	82 (13.6)	50 (10.1)	68 (11.4)
Unknown	1 (0.2)	2 (0.3)	1 (0.2)	4 (0.7)
Pathologic stage*, N (%)				
T1	276 (60.3)	294 (48.8)	284 (57.6)	309 (52.0)
T2	169 (36.9)	272 (45.2)	203 (41.2)	251 (42.3)
T3–T4	11 (2.4)	8 (1.3)	4 (0.8)	14 (2.4)
Unknown	2 (0.4)	28 (4.6)	2 (0.4)	20 (3.4)
Aggressiveness, n (%)				
Low	209 (45.6)	260 (43.2)	284 (57.6)	301 (50.7)
Intermediate	157 (34.3)	174 (28.9)	142 (28.8)	170 (28.6)
High	92 (20.1)	120 (19.9)	66 (13.4)	92 (15.5)
Unknown	0 (0)	48 (8.0)	1 (0.2)	31 (5.2)

*For the 81 subjects (3.9%) missing pathological stage, clinical stage was used.

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Table 2

CaP aggressiveness associated SNPs at P-value threshold of 0.05 in either of AA and EA for SNPs in GWAS reported regions

SNP	Chr	Position	Type*	Nearest Gene#	Reference genotype	AA		EA	
						P	OR	P	OR
rs2660753	3p12.1	87193364	replication	*LOC285232‖CHMP2B*	CC	0.173	1.1(0.9–1.3)	0.007	1.5(1.1–1.9)
rs2293850	8q24.21	127450879	flanking	*LOC100130158‖FAM84B*	CC	0.017	0.8(0.7–0.9)	0.078	0.8(0.7–1.0)
rs6993569	8q24.21	128153279	flanking	*FAM84B‖POU5F1P1*	GG	0.404	0.9(0.8–1.1)	0.031	1.5(1.0–2.1)
rs13254738	8q24.21	128173525	replication	*FAM84B‖POU5F1P1*	AA	0.032	1.2(1.0–1.4)	0.306	1.1(0.9–1.3)
rs1456305	8q24.21	128196434	flanking	*FAM84B‖POU5F1P1*	AA	0.048	1.3(1.0–1.7)	0.370	1.1(0.9–1.5)
rs10505476	8q24.21	128477298	flanking	*FAM84B‖POU5F1P1*	CC	0.766	0.9(0.8–1.2)	0.045	1.2(1.0–1.5)
rs6999921	8q24.21	128510110	flanking	*POU5F1P1‖MYC*	AA	0.005	0.7(0.5–0.9)	0.051	1.3(0.9–1.8)
rs10090154	8q24.21	128601319	replication	*POU5F1P1‖MYC*	CC	0.043	1.2(1.0–1.5)	0.601	0.9(0.7–1.2)
rs266870	19q13.33	56043746	flanking	*KLK15‖KLK3*	TT	0.049	0.8(0.7–1.0)	0.015	1.2(1.0–1.5)
rs1058205	19q13.33	56055210	flanking	*KLK3*	TT	0.004	0.8(0.6–0.9)	0.485	0.9(0.7–1.2)
rs2735839	19q13.33	56056435	replication	*KLK3‖KLK2*	GG	0.005	0.8(0.6–0.9)	0.248	0.9(0.7–1.1)

*: "flanking" indicates SNPs located at nearby of GWAS reported replication SNP.

#: symbol "‖" indicates a intergenic SNP located between 2 specified genes.

"replicated SNPs", i.e. SNPs that were the primary findings of GWAS and replication studies, represented 21 different chromosomal regions (see Supplementary Table 1). Not all of these SNPs had been established as replicated SNPs at the time of selection.

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Table 3

Association test results by race between phenotype (PSA, stage, Gleason score and age) and 3 SNPs in *KLK3* gene.

Phenotype	Statistic	AA			EA		
		rs266870TT*	rs1058205TT	rs2735839GG	rs266870TT	rs1058205TT	rs2735839TT
PSA	coefficient**	0.17	0.16	0.14	0.01	−0.02	0.02
	P	0.001	0.0004	0.002	0.7	0.7	0.8
Stage	OR(95% CI)	0.8(0.6–1.0)	0.9(0.7–1.1)	0.9(0.8–1.1)	1.2(1.0–1.5)	0.9(0.7–1.2)	0.9 (0.7–1.1)
	P	0.05	0.3	0.6	0.01	0.5	0.2
Gleason	Coefficient**	0.03	0.08	0.09	−0.10	0.13	0.14
	P	0.6	0.07	0.05	0.009	0.02	0.02
Age	OR(95% CI)	1.0(0.9–1.1)	1.0(0.9–1.1)	0.9(0.9–1.1)	1.0(0.9–1.1)	1.0(0.9–1.2)	0.9(0.8–1.1)
	P	0.7	0.6	0.7	1	0.6	0.6

*SNP rs number and reference genotype

**Regression coefficient from linear mixed model