Early Onset Prostate Cancer Has A Significant Genetic Component

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BACKGROUND. Prostate cancer (PCa) affects more than 190,000 men each year with ~10% of men diagnosed at ≤55 years, that is, early onset (EO) PCa. Based on historical findings for other cancers, EO PCa likely reflects a stronger underlying genetic etiology.
METHODS. We evaluated the association between EO PCa and previously identified single nucleotide polymorphisms (SNPs) in 754 Caucasian cases from the Michigan Prostate Cancer Genetics Project (mean 49.8 years at diagnosis), 2,713 Caucasian controls from Illumina's iControlDB database and 1,163 PCa cases diagnosed at >55 years from the Cancer Genetic Markers of Susceptibility Study (CGEMS).
RESULTS. Significant associations existed for 13 of 14 SNPs (rs9364554 on 6q25, rs10486567 on 7p15, rs6465657 on 7q21, rs6983267 on 8q24, rs1447295 on 8q24, rs1571801 on 9q33, rs10993994 on 10q11, rs4962416 on 10q26, rs7931342 on 11q13, rs4430796 on 17q12, rs1859962 on 17q24.3, rs2735839 on 19q13, and rs5945619 on Xp11.22, but not rs2660753 on 3p12). EO PCa cases had a significantly greater cumulative number of risk alleles (mean 12.4) than iControlDB controls (mean 11.2; $P = 2.1 \times 10^{-33}$) or CGEMS cases (mean 11.9; $P = 1.7 \times 10^{-5}$). Notably, EO PCa cases had a higher frequency of the risk allele than CGEMS cases at 11 of 13 associated SNPs, with significant differences for five SNPs. EO PCa cases diagnosed at <50 (mean 12.8) also had significantly more risk alleles than those diagnosed at 50–55 years (mean 12.1; $P = 0.0003$).
CONCLUSIONS. These results demonstrate the potential for identifying PCa-associated genetic variants by focusing on the subgroup of men diagnosed with EO disease. *Prostate 72: 147–156, 2012.* © 2011 Wiley Periodicals, Inc.

KEY WORDS: **early onset prostate cancer; polymorphism; association; genetics**

INTRODUCTION

In 2009, prostate cancer (PCa) was the most commonly diagnosed non-cutaneous cancer among men in the United States with an estimated 192,280 new cases and the second leading cause of cancer-related mortality with an estimated 27,360 PCa-related deaths [1]. Although PCa is commonly considered to be a disease of older men, with 63% of men diagnosed over the age of 65, last year over 9% of men diagnosed

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were ≤55 years. The proportion of men diagnosed at younger ages has increased steadily since the introduction of widespread screening with prostate-specific antigen (PSA) and continues to rise despite an apparent stabilization in PCa incidence overall [2,3]. This is despite guidelines for PCa early detection that have previously targeted men starting at age 50 except for those perceived to be at increased risk of disease, that is, those with African American ancestry and/or a family history of PCa, who may begin screening 5–10 years earlier [4,5]. PCa in younger men may have different public health implications, since some data suggest that compared to older men with similar clinical features younger men may be more likely to die of their cancer [6], especially those diagnosed with higher grade or locally advanced disease [7].

Early age at diagnosis is a recognized marker of genetic susceptibility for several hereditary cancers including breast [8], colorectal [9], ovarian [10], and endometrial [11] cancer. Among hereditary PCa families, risk increases with decreasing age of diagnosis of affected relatives [12] and, on average, hereditary PCa is diagnosed 6–7 years earlier than sporadic PCa [6]. Because the lower incidence of PCa at younger ages may indicate the lower overall prevalence of other risk factors for the disease [13], early onset (EO) PCa cases, that is, PCa cases diagnosed ≤55 years of age, may provide an especially rich sub-group of men among whom to search for genes associated with PCa risk.

Several recent genome-wide association studies (GWAS) have provided statistically significant evidence for multiple independent loci associated with PCa [14–21]. Efforts to distinguish those loci that may be associated with aggressive PCa, a clinically important form of PCa that is most likely to impact survival, have also been pursued [22–25]. In this study, we evaluate the evidence for association between risk of EO PCa and 14 single nucleotide polymorphisms (SNPs) distributed across 10 chromosomes in a sample of 754 unrelated Caucasian American EO PCa cases from the University of Michigan Prostate Cancer Genetics Project (UM-PCGP) who were diagnosed at ≤55 years and 2,713 Caucasian controls. These SNPs were selected based on having the strongest evidence in the published literature supporting an association with PCa and, for rs1571801, with aggressive PCa [14,16,17,19,25,26]. We found significant evidence for an association ($P < 0.05$) between EO PCa and 13 of the 14 SNPs, with the direction of the association consistent with prior reports. Further, we show that the EO PCa cases had a significantly greater average number of risk alleles across these SNPs than the 1,163 PCa cases from the Cancer Genetic Markers of Susceptibility Study (CGEMS) study who were diagnosed with disease after age 55.

SUBJECTS AND METHODS

Study Subjects

The study population consists of 754 unrelated Caucasian American participants in the UM PCGP diagnosed with histologically confirmed PCa (*International Classification of Diseases for Oncology* code C61.9) at ≤55 years of age. Dates of diagnosis were obtained from the date of diagnostic biopsy for 96.6% of cases, with the date of diagnosis for the remaining cases determined from the date of trans-urethral resection of the prostate, date of radical prostatectomy or physician's note. The majority of cases (95%) were diagnosed between November, 1993 and February, 2006. Men were aged 27–55 years at diagnosis, with average and median ages of 49.8 and 50 years, respectively. Cases completed self-administered questionnaires that collected information on family history of prostate and other cancers, medical history, and demographic factors. In addition, detailed clinical information relating to the diagnosis and treatment of PCa, including Gleason score from biopsy, tumor stage, and PSA level at diagnosis, was available from medical records. Peripheral blood samples for preparation of DNA were drawn from all subjects for genotyping. All study procedures have been approved by the University of Michigan Institutional Review Board and were conducted in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations. Written informed consent was obtained from all study participants prior to participation.

Genotyping EO PCa Cases

Fourteen SNPs were selected for genotyping based on published reports identifying them as significantly associated with PCa: rs2660753 on 3p12, rs9364554 on 6q25, rs10486567 on 7p15, rs6465657 on 7q21, rs6983267 and rs1447295 on 8q24, rs1571801 on 9q33, rs10993994 on 10q11, rs4962416 on 10q26, rs7931342 on 11q13, rs4430796 on 17q12, rs1859962 on 17q24.3, rs2735839 on 19q13, and rs5945619 on Xp11.22. Applied Biosystems TaqMan™ SNP assay system was used to genotype individual DNA samples with allelic discrimination performed on an ABI PRISM 7900HT Sequence Detection System. Any SNPs remaining undetermined by the assay were directly sequenced on an Applied Biosystems 3100 Genetic Analyzer using Big Dye version 1.1 chemistries for a final overall average 99.1% call completion, with no individual SNP below 95.6% completion. Quality control included duplicate genotyping of 5% of the

samples, distributed evenly among TaqMan™ genotyping batches. Out of 897 duplicate paired genotype calls, 16 genotype calls were discrepant, corresponding to 98.2% agreement overall. In total 658 cases had complete genotype data on all 14 SNPs.

iControlDB and CGEMS Study Subjects

An independent set of 2,713 unrelated controls of Caucasian ancestry with available genotype data were obtained from Illumina's iControlDB database (www.illumina.com/science/icontroldb.ilmn), that is, iControls, as a comparison group for the UM EO PCa cases. Subjects were anonymous but had information available on age, sex, and ancestry. iControls were genotyped with Illumina's HumanHap550v1 (referred to as V1 subjects; n = 1,197) or HumanHap550v3 (referred to as V3 subjects; n = 1,516) genome-wide genotyping platforms. The use of iControls in genetic association studies has been documented previously [27,28]. Genotype data for all 14 SNPs considered in this study were available in both V1 and V3 iControl samples. For SNP rs5945619, located on the X chromosome, the allele from each male and a single randomly chosen allele from each female in the iControls constituted the iControl sample for this SNP.

Caucasian PCa cases (n = 1,163) and screened controls (n = 1,113) in the CGEMS GWAS were included as additional comparison groups to our EO PCa cases. PCa cases and controls in the initial CGEMS GWAS were participants in the control arm of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial [29]. All CGEMS cases and controls were PSA screened; cases were 55–74 years at diagnosis, with an average age at diagnosis >60 years. CGEMS samples were genotyped using the Illumina HumanHap300 and HumanHap240 chips. Data were available for all 14 SNPs investigated in this report.

Statistical Analyses

The observed genotype distributions for each SNP were tested for consistency with Hardy–Weinberg equilibrium (HWE) expected proportions using 1-degree-of-freedom Pearson chi-square tests in the EO PCa cases and iControls, respectively, using the PLINK software [30] version 1.06 (available from www.pngu.mgh.harvard.edu/purcell/plink). Unconditional logistic regression models were used to evaluate the association between EO PCa and SNP genotypes assuming a multiplicative, that is, log-additive, genetic inheritance model. Statistical significance was assessed using 1-degree-of-freedom likelihood-ratio tests. Both two-sided and one-sided hypothesis tests were performed, with the direction of the one-sided test determined from prior published studies (see Table II for relevant references). Statistical analyses were performed using SAS version 9.1.3 software package (SAS Institute, Cary, NC). Similar analyses were performed using screened controls from the CGEMS GWAS as the comparison group. Finally, allelic-based likelihood ratio tests were used to systematically test for differences in the ''risk'' allele frequencies between EO PCa cases and PCa cases diagnosed after age 55 years, that is, CGEMS PCa cases, for each SNP.

To assess whether the cumulative number of risk alleles across the 13 PCa-associated SNPs was associated with EO PCa and whether EO PCa cases carry more risk alleles on average than older-onset PCa cases, we calculated the total number of risk alleles in each case and each control sample (i.e., EO PCa and CGEMS PCa cases and iControls and CGEMS controls). SNP rs2660753 was not included in these calculations as it was not significantly associated with PCa in the current study, the CGEMS study, nor in a large study of 7,370 PCa cases and 5,742 controls by the PRACTICAL consortium [15]. Individual subjects missing genotype data for any of the 13 SNPs used in calculating the sum of risk alleles were excluded from this calculation. Unconditional logistic regression models were used to test whether there were significant differences in the total number of risk alleles between EO PCa cases and iControls and between EO PCa and CGEMS PCa cases. Unconditional logistic regression was also used to evaluate the performance of iControls as a reference group by comparing the distribution of allele frequencies between iControls and CGEMS controls.

Finally, case-only analyses were performed to assess whether SNP genotypes were associated with clinical features observed in our EO PCa cases, specifically, age at diagnosis, biopsy, or pathological Gleason score, and serum PSA at diagnosis. Single-SNP case-only analyses were performed using logistic regression (for dichotomous outcomes: family history and aggressive disease) and Spearman's rank correlation (for continuous outcomes: age, Gleason score, and pre-treatment serum PSA at diagnosis). Disease aggressiveness has been defined previously [31] and is similar to the definition used by the International Consortium for Prostate Cancer Genetics [32]. Analyses were repeated using the cumulative number of risk alleles as a continuous predictor of clinical features of EO PCa.

RESULTS

The majority (63.8%) of EO PCa cases reported a positive family history of PCa, with over 40% having

TABLE I. Sample Characteristics for 754 Early-Onset Prostate Cancer Cases

	EO cases		
Clinical features	n = 754	%	Range
Age at diagnosis (years)[a]	49.8 (3.9)	50.5	34–55
34–45	105	13.9	
46–50	272	36.1	
51–55	377	50.0	
Number of affected family members[a,b]	1.4 (1.8)	1	0–17
0	273	36.2	
≥1	481	63.8	
Family history of prostate cancer			
1st degree relative (confirmed)	310	41.1	
2nd degree relative (confirmed)	83	11.0	
1st or 2nd degree relative (unconfirmed)	73	9.7	
3rd degree relative (unconfirmed)	15	2.0	
Serum PSA (ng/ml)[a,c]	22.9 (218.7)	5.4	0.3–5428
<4.0	172	24.0	
4.0–9.9	403	56.1	
≥10	143	19.9	
Gleason Score[a]	6.4 (0.9)	6.0	3–10
3–6	446	61.0	
7	227	31.1	
8–10	58	7.9	
Aggressive disease[d]			
No	529	70.2	
Yes	225	29.8	

[a]For age at diagnosis, number of affected family members, and serum PSA statistics given are: mean (SD), median, and range.
[b]Total numbers of affected family members, not including the proband.
[c]Serum prostate-specific antigen (PSA) measured at diagnosis prior to treatment.
[d]Aggressive disease defined as in Lange et al. [1].

a confirmed, first-degree affected relative (Table I). Clinically, 76% of cases presented with serum PSA level at diagnosis ≥4.0 ng/ml and 8.1% had clinical Gleason scores 8–10. Overall, 29.8% of men with EO disease were diagnosed with aggressive PCa, as defined in Lange et al. [31] All SNPs in EO PCa cases and 13 of 14 SNPs in iControls had genotype frequencies consistent ($P > 0.001$) with HWE. Among iControls, SNP rs4430796 had an observed genotype distribution inconsistent with HWE ($P = 4.4 \times 10^{-4}$, deficit of heterozygotes compared to expectation). Upon further inspection, 112/1,197 V1 samples had a missing genotype call (compared to 4/1,516 V3 samples) for this SNP. Sample-specific testing for HWE revealed that the genotype distribution for rs4430796 was consistent with HWE among V3 samples, but not among V1 samples ($P = 6.1 \times 10^{-4}$).

Thirteen of the 14 studied SNPs, excluding rs2660753, demonstrated evidence ($P < 0.05$) of association with EO PCa. All 13 associated SNPs had a direction of effect consistent with previous reports (Table II). Ten of the 13 SNPs remained statistically significant after strict application of the Bonferroni correction for multiple testing to the one-sided test results (i.e., $P_{(\text{one-sided})} < 0.0036$), while all 13 SNPs remained statistically significant after applying Holm's less conservative sequential rejection method (i.e., the Holm–Bonferroni method) for multiple testing [33] (data not shown). Similar results were obtained when CGEMS controls were utilized as the reference group (Table III). SNP rs4430796 was significantly associated with EO PCa when using either V1 ($P = 1.8 \times 10^{-4}$, OR = 1.28) or V3 ($P = 3.2 \times 10^{-6}$, OR = 1.34) iControl samples. No significant evidence for an association was observed between rs2660753 and EO PCa using the combined iControl samples or when restricting the iControl samples to V1 ($P = 0.88$, OR = 0.99) or V3 ($P = 0.06$, OR = 0.84) samples. In addition, the odds ratio observed between EO PCa and rs2660753 based on V3 control samples was in the opposite direction to the previous report by Eeles et al.[14] Finally, the total number of risk alleles observed in each subject (measured as the sum of risk alleles across 13 SNPs excluding rs2660753) was strongly associated with EO PCa ($P = 2.1 \times 10^{-33}$, Table IV and Fig. 1).

TABLE II. Early Onset Prostate Cancer Risk Associated With SNPs When Compared with iControlDB Public Controls

SNP	Chr.	Alleles[a]	Frequency of risk allele: Early-onset cases (n = 754)	Frequency of risk allele: iControls (n = 2,713)	OR (95% CI)[b]	*P* (two-sided)	*P* (one-sided)[c]
rs2660753 [14]	3p	C/T	0.127	0.140	0.90 (0.76, 1.07)	0.22	0.89
rs9364554 [14]	6q	C/T	0.317	0.259	1.32 (1.17, 1.50)	1.3×10^{-05}	6.3×10^{-06}
rs10486567 [16]	7p	A/G	0.806	0.761	1.30 (1.12, 1.49)	4.0×10^{-04}	2.0×10^{-04}
rs6465657 [14]	7q	T/C	0.495	0.451	1.19 (1.06, 1.34)	0.0029	0.0015
rs1447295 [26]	8q	C/A	0.134	0.099	1.41 (1.18, 1.67)	1.7×10^{-4}	8.7×10^{-5}
rs6983267 [19]	8q	T/G	0.606	0.499	1.55 (1.38, 1.75)	1.9×10^{-13}	9.6×10^{-14}
rs1571801 [25]	9q	G/T	0.274	0.245	1.16 (1.02, 1.32)	0.024	0.012
rs4962416 [16]	10q	A/G	0.326	0.295	1.15 (1.02, 1.30)	0.023	0.012
rs10993994 [16]	10q	C/T	0.470	0.422	1.21 (1.08, 1.36)	0.0011	5.5×10^{-04}
rs7931342 [14]	11q	T/G	0.578	0.544	1.15 (1.03, 1.30)	0.017	0.0083
rs1859962 [17]	17q	T/G	0.534	0.474	1.26 (1.12, 1.41)	6.4×10^{-05}	3.2×10^{-05}
rs4430796 [17]	17q	C/T	0.571	0.499	1.31 (1.17, 1.47)	3.2×10^{-06}	1.6×10^{-06}
rs2735839 [14]	19q	A/G	0.888	0.849	1.39 (1.18, 1.67)	1.9×10^{-04}	9.3×10^{-05}
rs5945619 [14]	Xp	T/C	0.432	0.364	1.33 (1.13, 1.57)	7.9×10^{-04}	3.9×10^{-04}

[a]Reference allele/allele associated with increased risk of prostate cancer in prior studies.
[b]Odds ratio for each additional copy of risk allele (as identified in previous studies) assuming a multiplicative model.
[c]One-sided test for direction of alternative hypothesis determined by prior study.

The frequencies of previously defined risk alleles were higher among the younger EO PCa cases than the older CGEMS cases. Specifically, the risk allele in 11 of the 13 SNPs was more common among EO PCa cases (Table III). This difference was statistically significant ($P < 0.05$) for five of the 11 SNPs. EO PCa cases had significantly more total risk alleles across the 13 SNPs than the PCa cases from the CGEMS

TABLE III. Distribution of SNP Allele Frequencies in Early Onset Prostate Cancer Cases Compared to CGEMs Cases and Controls

SNP	Chr.	Alleles [a]	Early onset cases allele freq.[b]	CGEMS controls (n = 1,101): Allele freq.[b]	CGEMS controls (n = 1,101): OR (95% CI)[c]	CGEMS controls (n = 1,101): *P*[d]	CGEMS cases (n = 1,176): Allele freq.[b]	CGEMS cases (n = 1,176): % Change[e]	CGEMS cases (n = 1,176): *P*[d]
rs2660753	3p	C/T	0.127	0.120	1.06 (0.87,1.30)	0.53	0.127	0.0	0.99
rs9364554	6q	C/T	0.317	0.291	1.13 (0.98,1.30)	0.097	0.293	+8.2	0.13
rs10486567	7p	A/G	0.806	0.770	1.23 (1.05,1.47)	0.010	0.792	+1.8	0.30
rs6465657	7q	T/C	0.495	0.460	1.14 (1.00,1.30)	0.046	0.472	+4.9	0.17
rs1447295	8q	C/A	0.134	0.101	1.39 (1.13,1.70)	0.0020	0.144	−6.9	0.36
rs6983267	8q	T/G	0.606	0.489	1.60 (1.40,1.84)	3.7×10^{-12}	0.555	+9.2	0.0021
rs1571801	9q	G/T	0.274	0.242	1.18 (1.02,1.37)	0.029	0.285	−3.9	0.47
rs4962416	10q	A/G	0.326	0.264	1.34 (1.16,1.55)	6.4×10^{-05}	0.321	+1.6	0.76
rs10993994	10q	C/T	0.470	0.368	1.49 (1.31,1.70)	2.0×10^{-09}	0.413	+13.8	5.6×10^{-04}
rs7931342	11q	T/G	0.578	0.498	1.37 (1.20,1.56)	2.5×10^{-06}	0.543	+6.4	0.033
rs1859962	17q	T/G	0.534	0.486	1.21 (1.06,1.38)	0.0045	0.532	+0.4	0.92
rs4430796	17q	C/T	0.571	0.501	1.32 (1.15,1.51)	4.9×10^{-05}	0.541	+5.5	0.082
rs2735839	19q	A/G	0.888	0.844	1.45 (1.20,1.79)	1.4×10^{-04}	0.864	+2.8	0.027
rs5945619	Xp	T/C	0.432	0.336	1.50 (1.24,1.82)	3.2×10^{-05}	0.413	+4.6	1.7×10^{-04}

[a]Reference allele/allele associated with increased risk of prostate cancer as identified in prior studies, that is, the ''risk'' allele.
[b]Frequency of risk allele.
[c]Odds ratio and 95% confidence interval estimating the risk of prostate cancer associated with each additional copy of a risk allele, assuming a log-additive genetic model.
[d]Two-sided *P*-value.
[e]Percent change in risk allele frequency (+/− increase or decrease, respectively) in EO compared to CGEMS Cases.

TABLE IV. Cumulative Number of Risk Alleles for 13 SNPs in EO Cases, iControlDB Controls, CGEMS Cases, and CGEMS Controls

	EO cases[a,b] (n = 754)	EO cases 50–55 years[c] (n = 459)	EO cases < 50 yrs[c] (n = 295)	iControls[a,d] (n = 2,713)	CGEMS cases[b] (n = 1,1760	CGEMS controls[d] (n = 1,101)
Mean	12.39	12.13	12.81	11.16	11.92	10.97
SD/SE	2.36/0.092	2.42/0.12	2.22/0.14	2.34/0.047	2.30/0.070	2.39/0.075

[a]EO cases compared to iControls ($P = 1.6 \times 10^{-32}$).
[b]EO cases compared to CGEMS cases ($P = 4.4 \times 10^{-05}$).
[c]EO cases diagnosed at <50 years versus EO cases diagnosed between 50 and 55 years ($P = 0.00030$).
[d]iControls versus CGEMS controls ($P = 0.033$).

study, with 12.42 risk alleles on average compared to 11.92 in CGEMS cases ($P = 1.7 \times 10^{-5}$, Table IV). In EO PCa case-only analyses, the frequency of the risk allele at three SNPs, rs1048656 ($P = 0.012$), rs1099399 ($P = 0.0087$), and rs1859962 ($P = 0.037$), was significantly greater in men diagnosed with PCa prior to age 50 (n = 295) than in men diagnosed with PCa between the ages of 50 and 55 years (n = 459). Across all 13 associated SNPs, there was significant evidence for more total risk alleles in EO PCa cases diagnosed prior to age 50 (12.81 risk alleles on average) than in men diagnosed with PCa between the ages of 50 and 55 (12.13 risk alleles on average; $P = 0.0003$, Table IV).

There was no significant evidence for any association between individual SNPs or total number of risk alleles measured across the 13 associated SNPs and pre-diagnostic serum PSA. The number of risk alleles at rs2735839 was significantly negatively correlated, after Bonferroni correction, with biopsy Gleason score (Spearman's correlation = −0.12, $P = 0.0016$). One SNP was nominally significantly correlated ($P < 0.05$) with Gleason score (rs1859962, Spearman's correlation = −0.080, $P = 0.033$). We found significant evidence for a negative correlation between the cumulative number of risk alleles across the 13 SNPs associated with EO PCa and biopsy Gleason score (Spearman's correlation = −0.085, $P = 0.032$).

DISCUSSION

We performed a replication-based genetic association study for 14 SNPs previously reported to be associated with PCa in a sample of 754 Caucasian American EO PCa cases from the UM-PCGP and 2,713 Caucasian American public controls from Illumina's iControlDB database. We found significant evidence ($P < 0.05$) for an association between EO

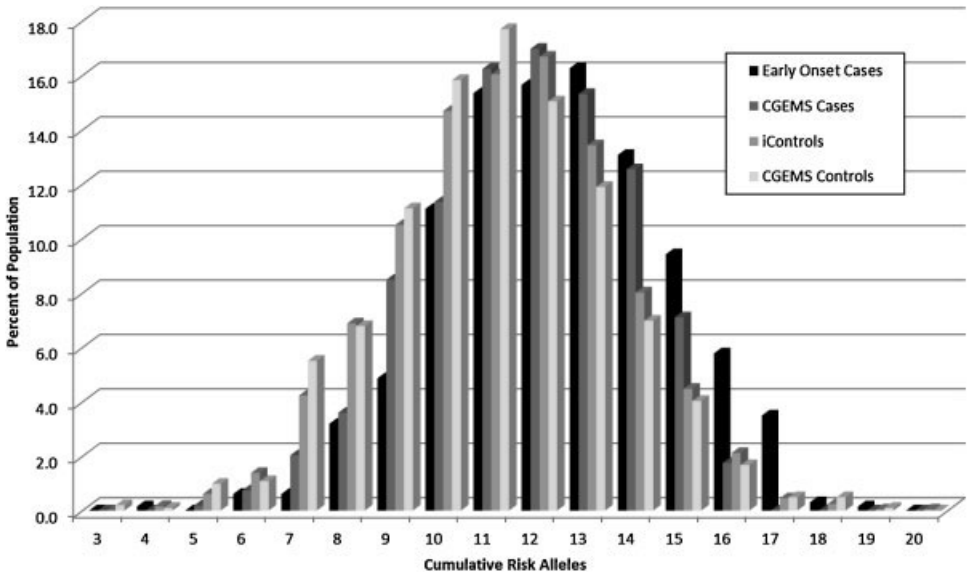

Fig. 1. The distribution of total number of risk alleles across 13 SNPs in early onset prostate cancer cases compared to iControlDB controls, CGEMS cases, and CGEMS controls.

PCa and 13 of the 14 SNPs, but not rs2660753, with similar direction of effect as in previous reports [14,16,17,19,25,26]. For 11 of the 13 SNPs, the association observed in the younger EO PCa cases was stronger than those in the existing literature, which reflect older case populations.

To our knowledge our study is the first to report replication for an association between rs1571801 and PCa, although we did not find any significant evidence to support an increased frequency of the risk allele in PCa cases with aggressive disease [data not shown]. The association of rs1571801 with PCa was first identified in a GWAS for aggressive PCa using combined participants from the Cancer of the Prostate in Sweden (CAPS) and CGEMS studies [25]. Interestingly, as reported in Duggan et al. [25], the frequency of the risk allele for rs1571801 was greater in non-aggressive than in aggressive cases among CGEMS samples. SNP rs1571801 was one of only two SNPs (the other being rs1447295) with a higher risk allele frequency in CGEMS compared to EO PCa cases. Given that the association between rs1571801 and PCa was previously identified in a study that included CGEMS cases, the higher frequency of the risk allele in CGEMS cases compared to our EO PCa cases may be explained by the *winner's curse* phenomenon [34].

SNPs rs4430796 and rs1859962 were first identified by deCODE Genetics from a targeted follow-up study to their original GWAS [26] that was initiated in response to reports of linkage evidence to chromosome 17 in UM-PCGP and John's Hopkins University PCa pedigrees [17,31]. A subset of the UM-PCGP subjects who were included in the reported linkage analysis on chromosome 17 was also included in this current association study. The association of PCa risk with two SNPs at 8q24, rs1447295 and rs6983267, was first reported by deCODE Genetics [26] and the CGEMS GWAS study that expanded on the initial findings at 8q24 [19], respectively. Two SNPs, rs10486567 and rs4962416, were first reported in a follow-up study to the initial GWAS by the CGEMS study [16]. We note that our current study includes only those CGEMS PCa cases included in the initial GWAS [19]. The remaining seven SNPs (rs2660753, rs9364554, rs6465657, rs10993994, rs7931342, rs2735839, and rs5945619) were first reported in the early-onset and familial PCa GWAS by Eeles et al. [14] and subsequently followed-up in a confirmatory study in 7,370 PCa cases and 5,742 controls by the PRACTICAL consortium [15]. In this follow-up study, strong supporting evidence for six of the seven SNPs (excluding rs2660753) was reported.

We used a public control population of Caucasian Americans that have been genotyped in several studies using different genotyping platforms than the platform used to genotype our study cases. The iControls were genotyped on Illumina's HumanHap550v1 and HumanHap550v3 Beadchip genome-wide SNP platforms compared to the UM-PCGP cases, who were genotyped using Applied Biosystems TaqMan assays. It is possible that the use of different genotyping platforms in cases and controls could have led to a systematic bias or batch effect in genotyping calls. Public controls were selected for use in this study based on the limited availability of unrelated controls for genotyping to UM-PCGP investigators and the availability of a large number of reference samples' control genotypes though Illumina's iControlDB database. We note that the iControlDB samples have been genotyped using the same Illumina Beadchip technology used in several recent PCa genome-wide studies. While we cannot definitively rule out the possibility of bias resulting from a batch genotyping effect, we note that the direction of the association between EO PCa and 13 SNPs was consistent with previous reports. Further, the allele frequencies for the SNPs were similar between the two independent V1 and V3 iControlDB samples. Prior GWA studies that have included the iControlDB samples have not noted the presence of any major genotype call bias in their reports [27,28]. Most importantly, we note that our results did not differ when we used the CGEMS controls' genotype distribution as the reference group (Table III) and that our case-only results are not subject to any such possible bias.

EO PCa has been shown to be significantly associated with increased family history of the disease, providing evidence of a stronger underlying genetic etiology of EO disease than for late-onset disease. To date, several multistage GWA studies for PCa have been conducted using a variety of rules for PCa case inclusion. The GWAS based on younger (i.e., $\leq$60 years) PCa cases and cases with a positive family history of PCa by Eeles et al. [14] demonstrated the increased power for detecting SNPs associated with PCa that can be achieved by including cases with enriched genetic susceptibility to the disease. Eeles et al.[14] found significant evidence of association for seven novel SNPs ($P < 1.0 \times 10^{-7}$) in their stage 1 results in addition to the widely reported PCa susceptibility loci on chromosomes 8q24 and 17q. Although an association with age of diagnosis has been tested and rejected in many prior studies, including the original GWAS reports that identified the SNPs we have tested, the men included in all of these studies were much older than those included in the present study. On average, our EO PCa cases were diagnosed at 49.8 years, $\sim$7 years younger than the PCa cases in the study by Eeles et al. Furthermore,

>60% of our cases had a family history of PCa in 1st or 2nd degree relatives (52.1% confirmed, 9.7% unconfirmed). We found significant evidence ($P < 0.05$) supporting the association between EO PCa and 13 of 14 SNPs studied, with the same direction of effects as in previous reports. We showed that the frequency of risk alleles, for both individual SNPs and in aggregate across SNPs, is significantly greater in EO PCa cases than in CGEMs cases who were diagnosed at >55 years. Interestingly, we also found significant evidence that the trend of more risk alleles in younger cases compared to older cases existed among just our EO PCa cases suggesting that the cumulative impact of common genetic risk factors are particularly important in men diagnosed with PCa prior to their 50th birthday.

In summary, our results provide strong evidence that SNPs associated with overall PCa are also likely to be associated with EO PCa and that studies focused on EO PCa could be a particularly powerful resource for future association studies focusing on PCa. From a clinical perspective, these findings suggest that common genetic variants play an increased role in EO PCa, relative to later onset PCa, and that greater emphasis should be placed on measuring the cumulative impact of these variants on EO PCa. It is likely that novel common and rare high-penetrant genetic variants exist and have yet to be identified that will be particularly important in EO and familial PCa. We are in the process of performing a GWAS and high-throughput sequencing efforts that will focus on this important set of patients with EO PCa.

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