FULL PAPER

BJC

British Journal of Cancer (2015) 113, 166–172 | doi: 10.1038/bjc.2015.199

Keywords: prostate cancer; biomarkers; single-nucleotide polymorphisms; biochemical recurrence; castrate metastasis; disease-specific mortality

An analysis of the association between prostate cancer risk loci, PSA levels, disease aggressiveness and disease-specific mortality

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Background: Genome-wide association studies have identified multiple single-nucleotide polymorphsims (SNPs) associated with prostate cancer (PCa). Although these SNPs have been clearly associated with disease risk, their relationship with clinical outcomes is less clear. Our aim was to assess the frequency of known PCa susceptibility alleles within a single institution ascertainment and to correlate risk alleles with disease-specific outcomes.

Methods: We genotyped 1354 individuals treated for localised PCa between June 1988 and December 2007. Blood samples were prospectively collected and de-identified before being genotyped and matched to phenotypic data. We investigated associations between 61 SNPs and disease-specific end points using multivariable analysis and also determined if SNPs were associated with PSA at diagnosis.

Results: Seven SNPs showed associations on multivariable analysis ($P<0.05$), rs13385191 with both biochemical recurrence (BR) and castrate metastasis (CM), rs339331 (BR), rs1894292, rs17178655 and rs11067228 (CM), and rs11902236 and rs4857841 PCa-specific mortality. After applying a Bonferroni correction for number of SNPs ($P<0.0008$), the only persistent significant association was between rs17632542 (*KLK3*) and PSA levels at diagnosis ($P=1.4\times10^{-5}$).

Conclusions: We confirmed that rs17632542 in *KLK3* is associated with PSA at diagnosis. No significant association was seen between loci and disease-specific end points when accounting for multiple testing. This provides further evidence that known PCa risk SNPs do not predict likelihood of disease progression.

Although prostate cancer (PCa) is highly prevalent and prostate-specific antigen (PSA) screening has led to an abundant diagnosis of disease, including many indolent cases, a substantial number of men will still develop symptomatic metastases or die from their cancer. The ability to identify individuals with a more aggressive disease phenotype would result in more appropriate initial treatment strategies. The need for novel biomarkers to add predictive capacity to existing clinical nomograms at time of diagnosis is of upmost relevance.

Inherited germline susceptibility loci, such as single-nucleotide polymorphisms (SNPs) have the potential to be effective biomarkers, not only in screening for disease but also in

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Received 4 February 2015; revised 24 April 2015; accepted 5 May 2015; published online 11 June 2015

contributing to predicting recurrence and response to specific treatments. Since the first PCa genome-wide association study (GWAS) in 2006, there are now over 75 such SNPs associated with disease risk (Eeles *et al*, 2013). Although related to risk, there is far less known about the ability of these SNPs to discriminate aggressive disease. To date, there are only a handful of studies that have looked at the association of PCa risk loci and disease-specific end points (Penney *et al*, 2009; Wiklund *et al*, 2009; Gallagher *et al*, 2010; Pomerantz *et al*, 2011; Szulkin *et al*, 2012; Shui *et al*, 2014). Many of these studies have small cohorts, have variability across institutions, and lack granular data on disease extent, treatment and length of follow-up.

In this study, we aimed to assess the frequency of a large selection of validated PCa susceptibility alleles from previously reported GWAS within a single institution ascertainment of PCa patients with long-term follow-up in order to determine association between risk alleles and disease outcomes, including clinical disease progression and PCa-specific mortality (PCSM).

MATERIALS AND METHODS

Study population. The study population consisted of 1354 men treated for localised PCa at the Memorial Sloan-Kettering Cancer Center between June 1988 and December 2007. Seven hundred and sixty-two individuals identified themselves as being of Ashkenazi Jewish descent, whereas over 90% of the remaining 592 were self-reported non-Jewish Caucasian individuals. Blood samples were drawn and medical records were collected as part of an institutional PCa research database using standardised questionnaires and chart abstraction forms. Pertinent clinical data included disease stage (TNM classification), Gleason score (from needle biopsy), PSA levels and age at diagnosis, as well as dates of biochemical recurrence (BR), development of castration-resistant metastasis (CM), PCSM and overall patient survival.

All patient records were reviewed by physicians to confirm the clinical end points being tested. Age at diagnosis was considered as the date of first positive prostate biopsy. BR was defined as a single measure of PSA $\geqslant 0.2\,\mathrm{ng\,ml^{-1}}$ after radical prostatectomy, and a value of 'nadir $+2$' after other therapy (Stephenson *et al*, 2006; Nielsen *et al*, 2008). CM was defined as time of progression of disease following initiation of antiandrogen therapy. Review of the patient's death certificate and/or medical record identified cause of death. In accordance with an institutional research board-approved protocol, patient identifiers were removed at the time of genetic analysis.

Selection of SNPs and genotyping. A total of 75 susceptibility loci of interest were identified, the majority of which were selected on the basis of being significantly associated with PCa risk from previous published GWAS ($n=67$), the remainder were SNPs associated with PSA levels ($n=6$). Established PCa risk SNPs that we previously evaluated for association with disease end points in a subset of this cohort were excluded (Gallagher *et al*, 2010). SNPs were genotyped using the Mass ARRAY QGE iPLEX system (Sequenom, Inc. San Diego, CA, USA; Gabriel *et al*, 2009). PCR and extension primers for SNPs were designed over three separate multiplex assays using Mass ARRAY Assay Design 3.0 software (Sequenom, Inc). PCR and extension reactions were performed according to the manufacturer's instructions, and extension product sizes were determined by mass spectrometry using the Sequenom iPLEX system. Duplicate test samples and negative controls were also included. In all, 14 of 75 SNPs (19%) failed quality control and were removed from the analysis. The remaining 61 SNPs had an average genotype call rate of 86%, with each SNP being in Hardy–Weinberg equilibrium (Tables 1 and 2). The minor allele frequency in the study cohort ranged from 1 to 49%. The average control rate among duplicate samples was 98%.

Statistical methods. Univariate Cox proportional hazards regression was used to investigate the association between each SNP and BR, CM, and PCSM. Each SNP was analysed under an additive model. The risk allele for each PCa SNP was defined as the allele associated with an increased risk of disease in the literature. Time at risk was calculated from the date of diagnosis to the date of event or date of last contact, and patients without the event were censored at their last follow-up date.

Multivariable analyses were conducted controlling for self-reported Ashkenazi Jewish ancestry; age at diagnosis; biopsy Gleason grade coded as a continuous variable ($1=$ Gleason $<=6$, $2=$ Gleason 7, $3=$ Gleason $>=8$); and clinical stage coded as a continuous variable ($1=$ T1, $2=$ T2, $3=$ T3/4).

Collection of blood samples for genetic testing began in 2000, and therefore, some cases diagnosed before 2000, and who died before 2000 (or who did not participate in blood sampling), were not included in this cohort. This scenario is referred to statistically as 'left truncation.' To account for this, we left-censored the interval from diagnosis to blood draw for each patient.

To address issues of multiple testing, by examining 61 SNPs and applying a Bonferroni correction, statistical significance was defined as $P<0.0008$. All statistical analyses were conducted using Plink (v1.07) and R (v2.9.1) as we have previously described (Willis *et al*, 2012).

RESULTS

One thousand three hundred and fifty-four patients were genotyped. Patient characteristics are presented in Table 3. The median age at diagnosis was 66 years (y) and median pre-operative PSA was $7.3\,\mathrm{ng\,ml^{-1}}$. Treatment at presentation was based on patient and physician preference. The majority of patients (93%) were treated with curative intent: 466 (34%) underwent radical prostatectomy with 804 (59%) receiving radiotherapy (RT) with or without antiandrogen therapy. A majority of patients (61%) had biopsy Gleason score $\geqslant 7$, and 53% of patients with available clinical staging information had $\geqslant$T2 disease. Median (interquartile range) follow-up for survivors was 10.4y (7.2–13.8). At last follow-up, BR was documented in 671 patients (49%), CM in 313 (23%), with 194 (14%) individuals having died from PCa. Median (interquartile range) BR-free survival was 8.1y (2.6–not reached) and median time to CM 21.4y (11.7–23.3). At 5y after PCa diagnosis, 98% of the study population were alive, 91% at 10y, 76% at 15y and 62% at 20y.

Univariate associations between susceptibility loci and PCa outcomes ($P<0.05$) are summarised in Table 4. In all, 2 of 61 SNPs, rs13385191 and rs339331, were associated with an increased risk of BR ($P<0.05$). Three SNPs were associated with CM ($P<0.05$); rs13385191 associated with an increased risk of CM (hazard ratio (HR) $=1.26$), with rs9284813 and rs11067228 both associated with decreased risk of CM (HR $=0.75$ and 0.74, respectively).

Seven SNPs showed associations on multivariable analysis with clinical end points ($P<0.05$). Again rs13385191 (HR $=1.36$; 95% CI $=1.03$–1.81; $P=0.02$) and rs339331 (HR $=1.47$; 95% CI $=1.04$–2.08; $P=0.02$) were associated with an increased risk of BR. Four SNPs, rs13385191, rs1894292, rs17178655 and rs11067228, were associated with CM, with different directions of effect and rs11902236 (HR $=0.78$; 95% CI $=0.62$–0.98; $P=0.03$) and rs4857841 (HR $=0.78$; 95% CI $=0.62$–0.98; $P=0.04$) with PCSM (Table 5). Of note, none of these associations were significant after a Bonferroni correction for multiple testing was applied ($P<0.0008$).

We also asked if any of the SNPs were associated with PSA at diagnosis. One SNP, rs17632542, was significant after multiple test correction ($P=1.7\times10^{-5}$), with carriers of the risk allele [C] more likely to have lower PSA levels at diagnosis (Figure 1).

Table 1. Prostate cancer risk polymorphisms genotyped and analysed in study cohort

SNP	Chr	Pos	Major/minor allele	MAF[a]	Per-allele OR[b]	Candidate gene	Reference
rs1218582	1q21	153100807	AG	0.45	1.06 (1.03–1.09)	*KCNN3*	Eeles *et al*, 2013
rs4245739	1q32	202785465	AC	0.25	0.91 (0.88–0.95)	*MDM4–PIK3C2B*	Eeles *et al*, 2013
rs10187424	2p11	85647807	AG	0.41	0.92 (0.89–0.94)	*GGCX—VAMP8*	Kote-Jarai *et al*, 2011
rs1465618	2p21	43465600	GA	0.23	1.08 (1.03–1.12)	*THADA*	Eeles *et al*, 2013
rs6545977	2p15	63154668	GA	NR	NR	*OTX1—RPL27P5*	Eeles *et al*, 2013
rs13385191	2p24	20751746	GA	0.40	1.15 (1.10–1.21)	*C2orf43*	Takata *et al*, 2010
rs11902236	2p24	10035319	GA	0.27	1.07 (1.03–1.10)	*TAF1B:GRHL1*	Eeles *et al*, 2013
rs3771570	2q37	242031537	GA	0.15	1.12 (1.08–1.17)	*FARP2*	Eeles *et al*, 2013
rs7584330	2q37	238051966	TC	0.22	1.06 (1.02–1.09)	*COL6A3 - MLPH*	Kote-Jarai *et al*, 2011
rs7629490	3p11	87324187	CT	NR	1.06 (1.04–1.09)	*VGLL3 - CHMP2B*	Schumacher *et al*, 2011
rs9284813	3p12	87234859	AG	NR	NR	*VGLL3 - CHMP2B*	Takata *et al*, 2010
rs7611694	3q13	114758314	AC	0.41	0.91 (0.88–0.93)	*SIDT1*	Eeles *et al*, 2013
rs6763931	3q23	142585522	CT	0.45	1.04 (1.01–1.07)	*ZBTB38*	Kote-Jarai *et al*, 2011
rs4857841	3q21	129529333	GA	0.30	1.13 (1.08–1.18)	*EEFSEC*	Lindstrom *et al*, 2012
rs1894292	4q13	74714193	GA	0.48	0.91 (0.89–0.94)	*AFM,RASSF6*	Eeles *et al*, 2013
rs17021918	4q22	95781900	CT	0.34	0.90 (0.87–0.93)	*PDLIM5*	Eeles *et al*, 2013
rs12500426	4q22	95733632	CA	0.46	1.08 (1.05–1.12)	*PDLIM5*	Eeles *et al*, 2013
rs7679673	4q24	106280983	CA	0.45	0.91 (0.88–0.94)	*RPL6P14–TET2*	Eeles *et al*, 2013
rs2121875	5p12	44401301	TG	0.34	1.05 (1.02–1.08)	*FGF10*	Kote-Jarai *et al*, 2011
rs4466137	5q14	83021495	TG	NR	NR	*HAPLN1*	Murabito *et al*, 2007
rs2242652	5p15	1333027	GA	0.19	0.87 (0.84–0.90)	*TERT*	Kote-Jarai *et al*, 2011
rs12653946	5p15	1948829	CT	0.50	1.31 (1.20–1.42)	*IRX4 - IRX2*	Takata *et al*, 2010
rs6869841	5q35	172872032	GA	0.21	1.07 (1.04–1.11)	*FAM44B (BOD1)*	Eeles *et al*, 2013
rs2273669	6p21	109391882	AG	0.15	1.07 (1.03–1.11)	*ARMC2,SESN1*	Eeles *et al*, 2013
rs339331	6q22	117316745	TC	0.31	1.28 (1.17–1.40)	*GPRC6A;RFX6*	Takata *et al*, 2010
rs1933488	6q25	153482772	AG	0.41	0.89 (0.87–0.92)	*RSG17*	Eeles *et al*, 2013
rs651164	6q25	160551785	GA	NR	0.87 (0.83–0.91)	*LOC100289162*	Schumacher *et al*, 2011
rs12155172	7p15	20767731	GA	0.20	1.05 (0.98–1.10)	*RPS26P30–ASS1P11-SP8*	Eeles *et al*, 2013
rs2928679	8p21	23494920	CT	0.42	1.05 (1.01–1.09)	*SLC25A37 NKX3-1*	Eeles *et al*, 2013
rs1512268	8p21	23582408	GA	0.45	1.18 (1.14–1.22)	*SLC25A37 - NKX3-1*	Eeles *et al*, 2013
rs11135910	8p21	25948059	GA	0.16	1.11 (1.07–1.16)	*EBF2*	Eeles *et al*, 2013
rs10086908	8q24	128011937	TC	0.3	0.87 (0.81–0.94)	*POU5F1B, MYC*	Eeles *et al*, 2013
rs12543663	8q24	127993841	AC	0.33	1.08 (1.00–1.16)	*LOC727677, MYC*	Al Olama AA *et al*, 2009
rs13252298	8q24	128164338	AG	NR	0.89 (0.85–0.95)	*FAM84B - SRRM1P1*	Schumacher *et al*, 2011
rs445114	8q24	128392363	TC	0.36	1.14 (1.10–1.19)	*SRRM1P1 - POU5F1B*	Gudmundsson *et al*, 2010
rs16902094	8q24	128320346	AG	0.15	1.21 (1.15–1.26)	*SRRM1P1 - POU5F1B*	Gudmundsson *et al*, 2010
rs817826	9q31	107235855	TC	0.10	1.43 (1.17–1.77)	*RAD23B-KLF4*	Xu *et al*, 2010
rs2252004	10q26	122844709	GT	0.23	1.16 (1.10–1.22)	*NR*	Akamatsu *et al*, 2012
rs11199874	10q26	123022509	GA	0.29	2.9 (2.1–4.1)	*RPL19P16-FGFR2*	Nam *et al*, 2006
rs1938781	11q12	58915110	TC	0.3	1.16 (1.11–1.21)	*FAM111A*	Akamatsu *et al*, 2012
rs11228565	11q13	68735156	GA	0.2	1.23 (1.16–1.31)	*TPCN2 - MYEOV*	Gudmundsson *et al*, 2010
rs7127900	11p15	2233574	GA	0.20	1.22 (1.17–1.27)	*IGF2-INS*	Eeles *et al*, 2013
rs11568818	11q22	101906871	AG	0.44	0.91 (0.88–0.94)	*MMP7*	Eeles *et al*, 2013
rs10875943	12q13	47962277	TC	0.31	1.07 (1.04–1.10)	*TUBA1C-PRPH*	Kote-Jarai *et al*, 2011
rs1270884	12q24	113169954	GA	0.49	1.07 (1.04–1.10)	*TBX5*	Eeles *et al*, 2013
rs1529276	13q33	102726008	TA	NR	NR	*SLC10A2-RPL7P45*	Murabito *et al*, 2007
rs8008270	14q22	52442080	GA	0.18	0.89 (0.86–0.93)	*FERMT2*	Eeles *et al*, 2013
rs7141529	14q24	68196497	AG	0.50	1.09 (1.06–1.12)	*RAD51B*	Eeles *et al*, 2013
rs11650494	17q12	44700185	GA	0.08	1.15 (1.09–1.22)	*GNGT2, ABI3, PHB, SPOP, HOXB13*	Eeles *et al*, 2013
rs7241993	18q23	74874961	GA	0.30	0.92 (0.89–0.95)	*SALL3*	Eeles *et al*, 2013
rs8102476	19q13	38735613	CT	0.46	1.12 (1.08–1.15)	*DPF1 - PPP1R14A*	Gudmundsson *et al*, 2010

Table 1. (Continued)

SNP	Chr	Pos	Major/minor allele	MAF[a]	Per-allele OR[b]	Candidate gene	Reference
rs103294	19q13	54797848	TC	0.30	1.28 (1.21–1.45)	*LILRA3*	Xu *et al*, 2010
rs2427345	20q13	60449006	GA	0.37	0.94 (0.91–0.97)	*GATAS, CABLES2*	Eeles *et al*, 2013
rs6062509	20q13	61833007	AC	0.30	0.89 (0.66–0.92)	*ZGPAT*	Eeles *et al*, 2013
rs742134	22q13	41842773	AG	NR	1.16 (1.01–1.23)	*BIK*	Schumacher *et al*, 2011
rs5759167	22q13	41830156	GT	0.47	0.86 (0.83–0.88)	*RPS25P10-BIK*	Eeles *et al*, 2013

Abbreviations: candidate gene = nearby gene as reported in the cited literature; Chr = chromosome; MAF = minor allele frequency; NR = not reported; OR = previous odds ratio for the SNP as cited by the given paper; Pos = chromosomal location; SNP = single-nucleotide polymorphism.
[a]Data for MAF[a] are taken from the original publication (Ref).
[b]Data for Per-Allele OR are taken from the original publication (Ref). 95% confidence intervals are given in brackets where available.

Table 2. PSA-associated polymorphisms genotyped and analysed in study cohort

SNP	Chr	Pos	Risk allele	RAF	Increase per allele (%)	Candidate gene	Reference
rs401681	5p15	1375087	C	0.55	7	*CLPTM1L*	Gudmundsson *et al*, 2010
rs10788160	10q26	123023539	A	0.31	10.2	*RPL19P16 - FGFR2*	Gudmundsson *et al*, 2010
rs17632542	19q13	51361757	T	0.91	39.1	*KLK3*	Gudmundsson *et al*, 2010
rs11067228	12q24	113556980	A	0.56	8.3	*OSTF1P1-TBX3*	Gudmundsson *et al*, 2010
rs17178655	10q11	51231805	A	0.23	NR	*MSMB*	Xu *et al*, 2010

Abbreviations: candidate gene = nearby gene as reported in the cited literature; Chr = chromosome; Pos = chromosomal location; PSA = prostate-specific antigen. RAF = risk allele frequency; SNP = single-nucleotide polymorphism; Shown are results for alleles that associate with increased (%) levels of PSA. Data are taken from the original publication (Ref).

Table 3. Characteristics of study population

Characteristic	Median (IQR) or frequency (%)
Age at diagnosis (years)	66 (60–71)
Year of diagnosis	
1988–1995	393 (29%)
1996–2000	558 (41%)
2001–2006	403 (30%)
Pre-treatment PSA (ng ml^{-1})	7.3 (4.2–12.9)
Family history PCa	141 (11%)
Biopsy gleason grade	
⩽6	503 (37%)
7	532 (39%)
⩾8	288 (22%)
Unknown	31 (2%)
Clinical stage	
T1	576 (42%)
T2	512 (38%)
T3/4	201 (15%)
Unknown	65 (5%)
Type of treatment	
Radical prostatectomy	466 (34%)
Radiotherapy ± androgen deprivation	804 (59%)
Androgen deprivation alone/WW	84 (7%)

Abbreviations: IQR = interquartile range; PCa = prostate cancer; PSA = prostate-specific antigen; WW = watchful waiting.

Table 4. Univariate associations between SNPs and PCa outcomes under a codominant model ($P<0.05$ by the 2 df test)

SNP	Chr	Gene	Minor allele	MAF	HR (95% CI)	*P* value
Biochemical recurrence						
rs13385191	2	*C2orf43*	A	0.27	1.36 (1.02–1.81)	0.03
rs339331	6	*RFX6/ GPRC6A*	C	0.15	1.45 (1.03–2.02)	0.02
Castrate metastasis						
rs13385191	2	*C2orf43*	A	0.27	1.28 (1.02–1.60)	0.02
rs9284813	3	*VGLL3*	G	0.26	0.75 (0.57–0.98)	0.03
rs11067228	12	*OSTF1P1*	G	0.48	0.74 (0.60–0.93)	0.009

Abbreviations: Chr = chromosome; CI = confidence intervals; HR = hazard ratios; MAF = minor allele frequency; SNP = single-nucleotide polymorphism.

DISCUSSION

Several existing PCa nomograms incorporating clinico-pathological parameters such as Gleason score, TNM stage and PSA aid in predicting likelihood of disease recurrence (Kattan *et al*, 1998; Stephenson *et al*, 2009), however, they are limited in their prognostic capabilities. Novel biomarkers to identify aggressiveness of disease and likelihood of recurrence are required. Although much focus is currently being placed on analysis of somatic mutations in contributing to these predictive models (Erho *et al*, 2013; Karnes *et al*, 2013), germline genetic variants have certain unique advantages. Knowing the inherited genetic predisposition of an individual to develop recurrent disease and metastatic progression at the time of diagnosis would clearly inform decision making regarding best initial treatment strategy and the intensity and approach to follow-up.

Since the first PCa GWAS in 2006 (Amundadottir *et al*, 2006) up through the most recent addition of a further 23 susceptibility loci by the PRACTICAL consortium in 2013 (Eeles *et al*, 2013), over 75 SNPs known to be associated with PCa risk have been identified. The ability of these susceptibility loci, however, to predict disease aggressiveness and clinical outcomes is less clear. Although several studies have reported associations with disease-specific outcomes, results are often conflicting and inconsistent (Penney *et al*, 2009; Wiklund *et al*, 2009; Gallagher *et al*, 2010; Pomerantz *et al*, 2011; Szulkin *et al*, 2012). Most recently, Shui *et al* analysed the association of 47 PCa susceptibility loci with PCSM in a large cohort with over 1000 events and reported association of

Table 5. Multivariate associations between SNPs and PCa outcomes

SNP	Chr	Gene	Minor Allele	MAF	HR (95% CI)	*P* value
Biochemical recurrence						
rs13385191	2	*C2orf43*	A	0.27	1.36 (1.03–1.81)	0.02
rs339331	6	*RFX6/ GPRC6A*	C	0.15	1.47 (1.04–2.08)	0.02
Castrate metastasis						
rs13385191	2	*C2orf43*	A	0.27	1.28 (1.03–1.60)	0.02
rs1894292	4	*AFM,RASSF6*	A	0.42	1.25 (1.01–1.54)	0.03
rs17178655	10	*MSMB*	A	0.21	0.73 (0.55–0.97)	0.03
rs11067228	12	*OSTF1P1*	G	0.48	0.79 (0.63–0.99)	0.04
Prostate cancer-specific mortality						
rs11902236	2	*TAF1B:GRHL1*	A	0.34	0.78 (0.62–0.98)	0.03
rs4857841	3	*EEFSEC*	A	0.31	0.78 (0.62–0.98)	0.04

Abbreviations: Chr = chromosome; CI = confidence interval; HR = hazard ratios; MAF = minor allele frequency; SNP = single-nucleotide polymorphism. **NOTE**: Each SNP was individually assessed in separate multivariable models, controlling for age at prostate cancer diagnosis, PSA at diagnosis, clinical stage and biopsy Gleason grade.

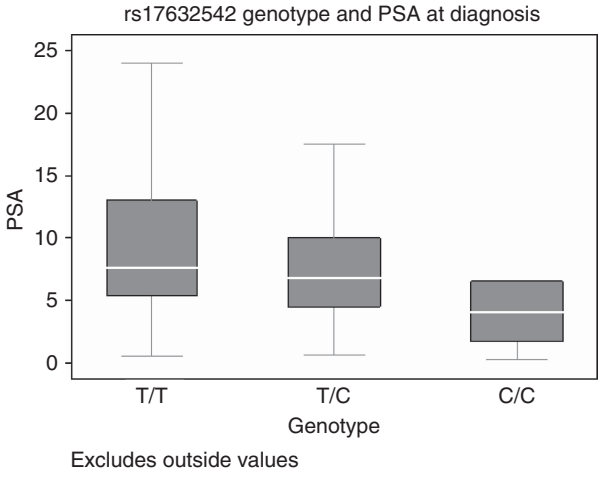

Figure 1. Box plot graph for rs17632542 (*KLK3*) illustrating PSA level at diagnosis with respect to allele (Common T, Het TC, Rare C).

eight SNPs with disease-specific death (Shui *et al*, 2014). In this same study, however, susceptibility loci were not able to distinguish aggressive *vs* non-aggressive disease (Shui *et al*, 2014).

We believe our current study is the first to assess a large number of susceptibility loci with respect to all three clinical end points with extensive follow-up (median 10.4y). We found evidence of associations of several SNPs with all three clinical end points on both univariate and multivariable analyses ($P<0.05$). Importantly, however, when incorporating a Bonferonni correction for multiple testing ($P<0.0008$), the only persistent significant association was with rs17632542, a previously reported *KLK3* variant (Gudmundsson *et al*, 2010; Klein *et al*, 2010; Kote-Jarai *et al*, 2011; Parikh *et al*, 2011), and PSA levels at diagnosis. Thus, the evidence for association at the other SNPs is only suggestive at this point and will need to be replicated in other studies.

rs17632542 lies within exon 4 of the *KLK3* gene and has also previously been associated with PCa risk (Kote-Jarai *et al*, 2011; Parikh *et al*, 2011; Penney *et al*, 2011; Klein *et al*, 2012; Knipe *et al*, 2014). The minor allele (C) causes a non-synonymous amino-acid change from isoleucine to threonine at position 179 (Ile179Thr). In our analysis, carriers of the C allele had a lower PSA at diagnosis; however, there were no associations seen with age at diagnosis, disease stage, Gleason grade, family history of PCa or any of the disease-specific clinical end points. This direction of effect is consistent with other studies such as by Gudmundsson *et al* who reported carriers of the rs17632542-T allele as having higher PSA levels (Gudmundsson *et al*, 2010). Interestingly, we have previously reported that rs17632542-C is associated with decreased PCa risk, (OR = 0.64 (CI = 0.51–0.81) $P=0.00019$). It is plausible that harbouring the rare allele of rs17632542 (C) leads to a direct effect on the function of the PSA protein, possibly through regulatory effects (on transcription of the gene), through altered protein stability or effect on antigenicity and as such detectability in PSA tests. It is also plausible that patients with the rare allele may be less likely to undergo biopsy subsequent to PSA screening because of lower PSA levels, although they may harbour asymptomatic and indolent PCa.

Two other PSA-related SNPs showed associations on multivariable analysis ($P<0.05$). rs11067228 is located in a linkage disequlibrium block that contains the genes *TBX3* (T-Box Transcription Factor 3) and *OSTF1P1* (Osteoclast Stimulating Factor 1 Pseudogene 1), with the common allele (A) being previously associated with higher PSA levels (Gudmundsson *et al*, 2010). The same study reported no association, however, with PCa but an association with a greater probability of having a normal prostate biopsy (Gudmundsson *et al*, 2010). In our study, we observed rs11067228-G to be associated with a lesser chance of development of castrate-resistant disease (HR = 0.79 (CI = 0.63–0.99), $P=0.04$). rs17178655, an intronic variant in the microseminoprotein-β gene (β-*MSP*), was also seen to be associated with development of CM, with carriers of the minor allele (A) less likely to develop CM (HR = 0.73 (CI = 0.55–0.97) $P=0.03$). This SNP had previously been reported by our group to be associated with semen levels of both free and total PSA ($P=0.0027$) but interestingly not levels of β-*MSP* (Xu *et al*, 2010). In contrast with PSA, whereby risk of PCa increases with higher PSA levels, β-*MSP* levels measured in serum, urine and prostate tissue have been shown to be statistically significantly lower in men with PCa and even lower in men with aggressive disease (Nam *et al*, 2006; Whitaker *et al*, 2010).

rs13385191 is located in intron 6 of *C2orf43* (chromosome 2 open reading frame 43) and achieved significance ($P<0.05$) on multivariable analysis for both clinical end points of BR (HR = 1.36 (CI = 1.03–1.81) $P=0.02$) and CM (HR = 1.28 (CI = 1.03–1.60) $P=0.02$). This SNP was initially reported by Takata *et al* in 2010 with the rare allele associated with increased risk of PCa in an Asian population (OR = 1.15 (CI = 1.10–1.21); Takata *et al*, 2010) and subsequently replicated in both European (OR = 1.07 (CI = 1.02–1.12); Lindstrom *et al*, 2012) and Chinese populations (OR = 1.33 (CI = 1.11–1.58); Long *et al*, 2012). Recently, Shui *et al* (2014) reported association of rs13385191 with PCSM, however, with the opposite direction of effect (OR = 0.88 (CI = 0.78–1.00) $P=0.05$). *C2orf43* is a highly conserved gene (Long *et al*, 2012) and as such, may harbour important functional variants in or within close proximity to its location around 2p24.

There were four additional SNPs (rs339331, rs1894292, rs11902236, rs4857841), which showed associations with end points at $P<0.05$. Importantly, however, the allele conferring an increased risk of PCa from previous GWAS studies was, in our analysis, a predictor of less aggressive disease as measured by time to recurrence and disease-specific death (Table 4).

The above results and those from other similar analyses lead us to conclude that susceptibility loci that are associated with initiation and development of PCa are likely to differ from loci that predict disease progression and aggressiveness. The mechanisms and pathways contributing to a more aggressive disease phenotype are still elusive, and additional large-scale discovery studies focusing on disease-specific end points are required. Investigating the cumulative effect of PCa SNPs may well reveal more about the molecular mechanisms of PCa oncogenesis (Jiang *et al*, 2013). As we discover further risk loci, pathway analysis

and computational statistical programmes will hopefully shed further light on these molecular mechanisms. In addition, we must also be aware that SNP function may vary among ethnic populations as has been suggested in other recent work (Jiang *et al*, 2014).

Our study has several limitations: although we report associations of a large selection of PCa risk loci, there are a number of reported GWAS SNPs that due to genotyping failures, were not included in the analysis. We also did not set out to discover any novel susceptibility loci or pathways. However as strengths, we utilised a large sample size with extended follow-up and granular phenotypic data, which includes detailed pathological and treatment variables.

CONCLUSIONS

The ability to discriminate individuals who are more or less likely to harbour an aggressive PCa phenotype and who are predisposed to disease recurrence has long been the focus of attention by the urologic oncology community. Existing nomograms are clinically useful but there is significant potential to increase their accuracy with addition of new biomarkers and individual genetic predictors. In this study, we confirmed that rs17632542 in *KLK3* is associated with PSA at diagnosis confirming reproducibility across multiple cohorts. No significant association was seen between loci and disease-specific end points when accounting for multiple testing. This provides further evidence that known PCa risk SNPs do not predict likelihood of disease progression. Further larger discovery analysis in cohorts with robust clinical end points are required to shed further light on germline predictors of disease recurrence to improve initial management and surveillance strategies.

ACKNOWLEDGEMENTS

This work has been funded by R01 CA175491 from the National Cancer Institute (RJK) and the Robert and Kate Niehaus Clinical Cancer Genetics Initiative at MSKCC (KO). We are grateful to all other ancillary and support staff from the Department of Clinical Genetics MSKCC for such support during the project.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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