

Genome-wide association of familial prostate cancer cases identifies evidence for a rare segregating haplotype at 8q24.21

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Acknowledgements

This research was supported by National Cancer Institute Grant U01 CA 89600 (Support for the ICPCG), and a grant from the Center for Inherited Disease Research supported the genotyping aspect of the study (contract no. HHSN268201200008I; PI Cannon-Albright). The Geneva Coordinating Center at the University of Washington conducted quality control aspects of the study and submitted public facing data to the database of Genotypes and Phenotypes (dbGaP) at the National Center for Biotechnology Information.

The authors acknowledge the Keith and Susan Warshaw Fund, the Maurice Warshaw Fund, the C. Scott Watkins Fund, and the Tenuity Family Fund. The FHCRC portion of the study was supported by NIH grants R01 CA080122, R01 CA056678, and R01 CA092579, Fred Hutchinson Cancer Research Center and the Prostate Cancer Foundation. Funding for the iCOGS infrastructure (PRACTICAL data) came from: the European Community's Seventh Framework Programme under grant agreement n° 223175 (HEALTH-F2-2009-223175) (COGS), Cancer Research UK (C1287/A10118, C1287/A 10710, C12292/A11174,

C1281/A12014, C5047/A8384, C5047/A15007, C5047/A10692, C8197/A16565), the National Institutes of Health (CA128978) and Post-Cancer GWAS initiative (1U19 CA148537, 1U19 CA148065 and 1U19 CA148112 - the GAME-ON initiative), the Department of Defence (W81XWH-10-1-0341), the Canadian Institutes of Health Research (CIHR) for the CIHR Team in Familial Risks of Breast Cancer, Komen Foundation for the Cure, the Breast Cancer Research Foundation, and the Ovarian Cancer Research Fund. The Biomedical Research Centre at The Institute of Cancer Research (UK) acknowledges the support of the National Institute of Health Research, the Royal Marsden NHS Foundation Trust, and Prostate Cancer UK.

Abstract

Previous genome-wide association studies (GWAS) of prostate cancer risk focused on cases unselected for family history and have reported over 100 significant associations. The International Consortium for Prostate Cancer Genetics (ICPCG) has now performed a GWAS of 2,511 (unrelated) familial prostate cancer cases and 1,382 unaffected controls from 12 member sites. All samples were genotyped on the Illumina 5M+exome single nucleotide polymorphism (SNP) platform. The GWAS identified significant evidence for association for SNPs in 6 regions previously associated with prostate cancer in population-based cohorts including 3q26.2, 6q25.3, 8q24.21, 10q11.23, 11q13.3, and 17q12. Of note, SNP rs138042437 ($p = 1.7e-8$) at 8q24.21 achieved a large estimated effect size in this cohort (odds ratio=13.3). 116 previously sampled affected relatives of 62 risk allele carriers from the GWAS cohort were genotyped for this SNP, identifying 78 additional affected carriers in 62 pedigrees. A test for an excess number of affected carriers among relatives exhibited strong evidence for co-segregation of the variant with disease ($p=8.5e^{-11}$). The majority (92%) of risk allele carriers at rs138042437 had a consistent estimated haplotype spanning approximately 100 kilo-bases of 8q24.21 that contained the minor alleles of 3 rare SNPs (dosage minor allele frequencies <1.7%), rs183373024 (PRNCR1), previously associated SNP rs188140481, and rs138042437 (CASC19). Strong evidence for co-segregation of a SNP on the haplotype further characterizes the haplotype as a prostate cancer predisposition locus.

Keywords: prostate cancer, aggressive prostate cancer, familial, GWAS, ICPCG

Introduction

More than 100 SNPs have been identified through genome-wide association studies (GWAS) to date as being significantly associated with prostate cancer risk in case-control datasets. These studies mainly included prostate cancer cases that were unselected based on family history (Amundadottir 2006; Duggan 2007; Gudmundsson 2007a; Gudmundsson 2007b; Haiman 2007; Yeager 2007; Eeles 2008; Gudmundsson 2008; Salinas 2008; Sun 2008; Thomas 2008; Kote-Jarai 2008; Gudmundsson 2009; Haiman 2011; Kote-Jarai 2011; Schumacher 2011; Gudmundsson 2012; Eeles 2013; Al Olama 2014; Al Olama 2015). Three additional studies have identified another five SNPs associated with aggressive prostate cancer (Helfand 2015; Berndt 2015; Al Olama 2013).

The ICCPG has now conducted a GWAS of familial prostate cancer in a large international case-control dataset. Twelve member sites contributed one case from each studied high-risk prostate cancer pedigree along with matched unrelated population controls. A total of 2,511 cases and 1,382 geographically and frequency age-matched controls without a history of prostate cancer were genotyped on the Illumina 5M+exome SNP platform. A subset of 1,394 of the prostate cancer cases were identified as having aggressive disease by clinically documented cancer features. Separate analyses for all prostate cancer cases and the aggressive prostate cancer cases were conducted.

A SNP in the 8q24.21 region, rs138042437, exhibiting strong association (odds ratio, OR=13.3; $p = 1.7e-8$) and in high LD ($r^2 = 1.0$ (Gudmundsson 2012)) with a previously reported SNP, rs188140481, was an ideal candidate for further investigation. The rare allele of SNP rs138042437 was tested for co-segregation with disease in 116 affected relatives of 62 index cases from the GWAS study that were available from ICCPG member sites; 78 additional case carriers and 38 non-carriers were observed in 48 of 62 typed families. The marker achieved showed a strong association with disease ($p=8.5e^{-11}$) in a test

for an excess number of affected carrier relatives (43.9 expected by chance), further characterizing the haplotype containing the SNP as a prostate cancer predisposition locus.

Subjects and Methods

Sample cohort

The ICPCG consists of multiple international groups focused on the study of high-risk prostate cancer pedigrees and represents the largest available collect of familial prostate cancer cases and pedigrees. Twelve member sites of the ICPCG consortium provided samples for analysis. These sites included Cancer Council Victoria (Australia), Centre de Recherche sur les Pathologies Prostatiques (CeRePP), Fred Hutchinson Cancer Research Center -Seattle (FHCRC), University of Tampere (Finland), Johns Hopkins University (JHU), University of Ulm (Germany), Institute of Cancer Research, U.K. (ICR), Louisiana State University Health Sciences Center - New Orleans (LSU), The Mayo Clinic (Mayo), University of Michigan (Michigan), Northwestern University (Northwestern), and University of Utah (Utah). Selection of cases and controls was limited to Caucasians (race self-reported). Each site recruited study participants according to their own protocols; however, in order to define a set of familial cases, sites were asked to supply only 1 case from previously sampled pedigrees having at least 3 related cases with an average age at diagnosis ≤ 75 years. Sites selected the sampled case from each eligible pedigree that was clinically most aggressive or had the earliest age at diagnosis. Controls were selected to be male, unrelated to cases and each other, and with no prior diagnosis of cancer. Controls were not matched 1:1 to cases, but were selected such that the overall distribution of race and birth-year was similar between selected cases and controls. Only 9 of 12 sites contributed controls. However, principal components analysis (PCA) of genotype data identified that cases from the 3 sites without controls were found to be

of similar genetic background to cases at other sites and were merged accordingly. The PCA outcomes that were used to merge sites is shown in Supplemental Figure 1. The specific merging of controls by site is indicated in Table 1, which also shows the composition of cases and controls contributed by each site. All sites collected samples under approval from institutional review boards at their respective institutions, and informed consent was obtained for all study participants.

Phenotypes

Standardized criteria for determining prostate cancer status were imposed to address the potential for clinical heterogeneity across the 12 sites. All prostate cancer cases were confirmed by death certificate or medical record. Cases were designated as aggressive if they had: i) regional or distant stage at diagnosis (stage T3, T4, N1, or M1, based on pathology if radical prostatectomy was done; otherwise, based on clinical stage), ii) tumor Gleason score 8-10 at diagnosis, or poorly differentiated or non-differentiated grade if Gleason score was unavailable, iii) diagnostic PSA > 20 ng/ml, or iv) died from metastatic prostate cancer before age 65. These criteria were previously used to define aggressive disease by the ICPCG (Schaid 2006; Christensen 2007). Cases not meeting these criteria were considered less-aggressive prostate cancer. According to this definition there were 1394 aggressive cases and 1096 less-aggressive cases and 21 of 'unknown' aggressive status (not analyzed) among the set of cases passing quality control. Two phenotypes were analyzed for GWAS, all prostate cancer and aggressive cases versus less-aggressive cases, applying the ICPCG definition.

Genotype data

All participants were genotyped on the Illumina HumanOmni5Exome-4v1-1+exome SNP array at the Center for Inherited Disease Research (CIDR). The exome portion of the dataset was analyzed and reported separately. Quality control was performed at the time of genotyping by CIDR in conjunction

with the GENEVA Coordinating Center at the University of Washington (Laurie 2010). The quality control procedure removed 43 participants because of previously undocumented relationships closer than 3rd degree. PCA of approximately 150,000 SNPs identified a relatively homogeneous subset of the cases and 53 outliers were removed. All individuals had call rates greater than 99%. Quality control removed 57 cases and 39 controls, leaving 2511 cases and 1382 controls for analysis. SNP markers were filtered out according to HWE p-value < 0.0001, minor allele frequency (MAF < 0.01), > 1 Mendelian error detected in a set of duplicated samples, and a missing call rate $\geq 2\%$, which led to the removal of approximately 45% of markers (of which a large proportion were monomorphic), leaving 2,567,713 SNPs to serve as the imputation basis. The genomic inflation parameter (λ) was 0.98 for the observed genotype data indicating that population stratification and cryptic relatedness between study subjects is well controlled in this data set (Price 2010). The GENEVA Coordinating Center also imputed genotypes for SNPs that were observed in the 1000 Genomes Project that did not occur on the SNP array. Imputation used IMPUTE2 software (Howie 2010; Delaneau 2011) and estimated haplotypes using a reference panel of 1092 samples from International HapMap Consortium Phases 2 and 3 haplotypes. The target imputation set included approximately 27.7M SNPs, which were analyzed in GWAS. Approximately 66% of the resulting target SNPs had MAF < 1% (dosage) or IMPUTE INFO score ≤ 0.3 , leaving approximately 9.4M SNPs for analysis.

GWAS was performed with PLINK software v1.9 (Purcell 2007) using logistic regression analysis at each imputed marker and including study site assignment. The first four PCA eigenvectors estimated during quality control were included as covariates because a test of association indicated a significant relationship between affection status and eigenvectors 1, 2, and 4. Following the widely accepted standard for interpreting GWAS (Pe'er 2008), significance was established for SNPs with $p < 5e^{-8}$. Regional association plots were generated with LocusExplorer software (Dadaev 2015). Details of the publicly

available datasets that were used in preparation of the figure are described elsewhere (Al Olama 2015). Linkage disequilibrium (LD) estimates between markers originated from 1000 Genomes Project phase 3 data (May 2013 release; 1000 Genomes Consortium 2014). Human genome version 19 coordinates are given throughout.

Identifying independent signals in regions of association

Because of the potential for linkage disequilibrium between markers, we analyzed each region containing multiple significant SNPs with forward step-wise regression and elastic net in order to identify SNPs with an independent contribution to the signal at each region. Markers were selected from each region with GWAS $p < 0.01$ (to reduce the number of simultaneously analyzed markers); all were within 500-kb of the significant flanking markers in the region. For the forward selection analysis, the 'step' function in R (base package) was employed for model development which included study site assignment and the first four eigenvectors estimated during quality control as covariates. Although step-wise selection is commonly used in GWAS, there are several problems that compromise its value for high-dimensional data: the resulting regression coefficients are biased away from zero, it does not adequately handle correlated markers, and there is an inflated risk of capitalizing on chance features of the data. For these reasons, we also used the elastic net method, which is designed to select variables of interest by addressing the high-dimensionality of the data set and correlated markers. The 'glmnet' package in R (Friedman 2010; Tibshirani 2012) was used for the elastic net analysis; study site and the PCA eigenvectors were retained in all models. We selected SNPs from the elastic net analysis that had non-zero coefficients after tuning α and λ penalties by 10-fold cross-validation (x100) with the 'caret' R package.

Comparison of familial prostate cancer outcomes with previously associated SNPs

We compared the ORs estimated for SNPs previously reported to be associated with prostate cancer with the ORs estimated for this study. We used the following statistic to test for significant departure between the ORs of the two studies being compared,

$$Z = \frac{\log(OR_1) - \log(OR_2)}{(SE_1^2 + SE_2^2)^{1/2}}$$

where OR_1 is the previously reported OR for a SNP, OR_2 is the estimated OR from the current study for that SNP, and SE_1 and SE_2 are the standard errors of the ORs. The statistic Z follows a normal distribution. A low p-value indicates a substantial departure between the two estimated ORs. Positive Z scores indicate a stronger effect size in the previous study and a negative Z score indicates a stronger effect size in the familial cohort.

Investigation of a high OR SNP

The SNP rs138042437 (referred to as kgp28696802 in the imputed dataset), which had a high OR in the GWAS, was followed up to assess segregation of the rare allele with prostate cancer. The frequency of the SNP was estimated from genotype data for all available cases and controls from the PRACTICAL Consortium (members of the consortium can be found in the Supplemental Table 1) to gauge effect size in cases not ascertained on the basis of their family history of prostate cancer. Sanger sequencing of additional relatives for rs138042437 used Qiagen Multiplex Master Mix following the Qiagen protocol with a 64 degree annealing temperature using forward primer [gatcagagtggccagaatgg] and reverse primer [ggagaagacagagactgaagaagg], which produced a product size of 492 bases.

A test of over-transmission of the rare allele at rs138042437 to affected relatives assumes a null hypothesis of random segregation and that the number of pedigrees where specified individuals share a rare variant is the sum of independent Bernoulli trials with differing probabilities of success depending

on the configuration of observed carriers in each pedigree. The exact distribution of the statistic was computed with the probability of success for each Bernoulli trial (pedigree) defined as the probability of each configuration of carriers in the pedigree as determined by RVsharing program (RVsharing R software package, (Bureau 2014)), conditional on the proband carrying the variant. The RVsharing program computes the probability that specified carriers of a rare variant (assumed to be <1%) in the pedigree share the variant, assuming the variant has entered the pedigree only once (Bureau 2014). The probability mass function (pmf) for the number of sharing cases was calculated for each pedigree. The pmf for the total number of sharing cases summed over all pedigrees was then computed simply by taking the convolution of the pedigree pmfs.

Haplotypes at the 8q24.21 locus were estimated from imputed genotypes at 8q24.21 using SHAPEIT2 software (Delaneau 2011). Haplotypes were also estimated from genotypes derived from targeted sequencing data of the 8q24.21 locus in an independent set of 56 prostate cancer cases, also ascertained from ICPCG member sites but not included in the GWAS dataset. Haplotypes were estimated from the targeted sequencing data in order to identify the presence of other rare or novel variants that might not be present in the imputed markers. Targeted sequencing data capture used a targeted Agilent SureSelect Custom capture library designed to collect all coding and non-repetitive non-coding bases in the 8q24.21 locus. Sequence data were generated following the protocol for Illumina HiSeq 101 Cycle Paired End Sequencing (v4) until an average read depth of 100 was achieved for intended capture bases. Reads were aligned with BWA v0.7.12 (Li 2010), variants were called with GATK v3.5 (McKenna 2010), and variant annotation was produced by Annovar (2015-06-17) (Wang 2010).

Results

All prostate cancer

Figure 1 shows the Manhattan plot of the GWAS results. Figure 2 shows a QQ-plot for all the GWAS statistics. The QQ-plot shows slight departure of the distribution of test statistics from the expected distribution ($\lambda=1.08$) that is likely an artifact of imputation and not indicative of population stratification between cases and controls (since no inflation was apparent in the observed genotype data, $\lambda=0.98$). The GWAS identified multiple significant SNPs at 6 regions including 3q26.31, 6q24.3, 8q24.21, 10q11.23, 11q13.3, and 17q12. Table 2 reports the most significant SNP from each of these regions and Supplemental Table 2 shows the 135 significant SNPs ($p < 5e^{-8}$) that occurred in the GWAS.

For the regions with multiple significant SNPs, a forward selection and elastic net analysis in each region identified which SNPs within the region contributed an independent signal. The analyzed regions were 6q25.3, 8q24.21, 10q11.23, 11q13.3, and 17q12. The 3q26.31 region had only one significant SNP and was not investigated further. SNPs contributing independent signals in these regions were all previously published and validated, or proximal and in high LD with associated SNPs. Table 3 shows the markers included by these two selection approaches and the nearest previously reported SNP, if different. The overlap of the forward selection and elastic net analysis at each region retained only one SNP in the final model for each region, with the exception of the 8q24.21 region, which retained nine SNPs, four of which were also significant in the GWAS. A benefit of elastic net is that it allows highly correlated SNPs to be selected in a model, whereas forward selection might select only one. This makes sense when it is statistically impossible to distinguish among highly correlated SNPs, any of which could be a functional SNP, or any of which could be highly correlated with a functional SNP. Elastic-net achieve this by imposing a penalty on the absolute values of the SNP regression coefficients,

which shrinks the unimportant coefficients to zero, while also shrinking the larger coefficients. This avoids the overly optimistic results from forward selection, while causing the coefficients from elastic net to be closer to zero than those from forward selection, as illustrated in Table 3. Figure 3 shows the locations of significant markers from the GWAS that were retained by the variance reduction methods applied to genotype data in the region for the 8q24.21 locus as well as the approximate LD structure of the region.

The SNP rs138042437 ($p=1.7e^{-8}$, OR=13.3, 95% confidence interval [5.4, 32.7], minor allele=G, MAF=0.015) exhibited the highest estimated effect size of any marker in the GWAS and is in high LD ($r^2=1$) with previously reported SNP rs188140481 (Gudmundsson 2012) ($p=5e^{-9}$, OR=9.4, 95% confidence interval [4.4, 19.9], minor allele=A, MAF=0.016), and SNP rs183373024 ($p=2.9e^{-9}$, OR=7.6, 95% confidence interval [3.9, 14.9], minor allele=G, MAF=0.017). For rs138042437, familial cases in this study had MAF=2.2%, compared to 0.2% for controls. Population estimates of global minor allele frequency are 0.4% in 1000 Genomes Project SNP annotation (1000 Genomes Project Consortium 2012) and 0.2% in dbSNP annotation (Sherry 2001). The MAF of the rare allele for rs138042437 in the prostate cancer case-control dataset from the PRACTICAL Consortium was 2.2% (out of 22,898 alleles) for cases and 1.1% (out of 23,054 total alleles) for controls (OR = 1.90, 95% confidence interval (1.71, 2.12)). The comparatively high frequency of the rare allele at rs138042437 in the much larger set of controls from the PRACTICAL Consortium dataset than controls in this study indicates that the estimated allele frequency in the controls is most likely too low, hence the magnitude of the estimated effect size for the marker is likely over-stated in this dataset.

Known predisposition genes

We also scanned for association signals at SNPs within known predisposition genes for prostate cancer, *BRCA1* (Agalliu 2009), *BRCA2* (Edwards 2003), and *HOXB13* (Ewing 2012). Association at *BRCA1/2* genes was modest (most extreme $p=1e^{-3}$). For *HOXB13*, the SNP rs138213197 ($p = 2.8e^{-7}$; OR = 4.7), representing the G84E mutation, had a similar estimated effect size to a recent meta-analysis of prostate cancer in 120,617 men (OR = 4.5, 95% confidence interval [3.28, 6.20]; Huang 2014).

Comparison of effect size for significant SNPs observed in non-familial datasets

Ninety-one of the 103 SNPs previously reported and validated as significantly associated with prostate cancer risk in GWAS of cases and controls not ascertained on the basis of family history (summarized in Al Olama, et al., 2014), were available for analysis. The Z statistic compared the estimated ORs between the current study and the original report of a significant association for each SNP. Supplemental Figure 2 shows all of the outcomes of the comparisons of ORs for 91 of the SNPs previously associated with prostate cancer. For the large majority of SNPs, the effect size (OR) did not differ significantly between the originally reported estimate and the one observed for the familial prostate cancer cases vs. controls comparisons. Only three SNPs had a significantly higher estimated risk among the familial cases compared to controls: rs7584330 at 2q37 in *MLPH* (OR=1.26, previously reported OR=1.06 (Al Olama 2014); $Z = -2.9$), rs9364554 at 6q25 in *SLC22A3* (OR=1.35, previously reported OR=1.28 (Eeles 2008), $Z=-3.5$), and rs7931342 at 11q13 near *MYEOV* (OR=1.38, previously reported OR=1.27 (Eeles 2007), $Z=-2.7$). However, the outcomes of these comparisons appear to fall within an expected distribution that would be achieved by chance, suggesting that increased ORs may likely represent false positives, and also suggesting that most of the risk SNPs detected in population-based GWAS apply to our study with cases enriched for prostate cancer family history.

Aggressive prostate cancer

A Manhattan plot showing the outcomes from the GWAS of aggressive cases (n=1394) and less-aggressive cases (n=1096) appears in Figure 4, and Figure 5 shows the corresponding QQ-plot ($\lambda=1.08$). No markers were significant ($p < 5e^{-8}$) in this analysis. Similarly, none of the SNPs previously associated with aggressive disease were significant in this GWAS; Specifically, SNPs rs2735839 (chr19:51.36 Mb; $p=0.110$; Helfand 2015), rs11672691 (chr19:41.99 Mb; $p=0.020$; Al Olama 2013), rs11704416 (chr22:40.44 Mb; $p = 0.017$; Al Olama 2013), rs35148638 (chr5:86.61 Mb; $p=0.33$; Berndt 2015), and rs78943174 (chr3:175.25 Mb; $p=0.34$; Berndt 2015).

Testing for segregation of rs138042437 in relatives

The GWAS identified 112 carriers of the rare allele for rs138042437 (107 cases and 5 controls). Given the evidence of association of SNP rs188140481 (in high LD with rs138042437; $r^2=1.0$) with prostate cancer presented in a previous GWAS study (Gudmundsson 2012), the very high estimated effect size of the variants in this data, and the availability of already sampled affected relatives, 116 affected relatives of 62 rare-allele carriers in the GWAS cases were tested for the presence of the rare allele; 78 additional affected carriers and 38 non-carriers were observed. The 116 affected relatives included 79 siblings (56 carriers), 3 half-siblings (3 carriers), 9 avunculars (5 carriers), 20 cousins (13 carriers), and 5 more distant relatives (1 carrier). The exact p-value for the observed sharing calculated from the convolution pmf was $8.5e^{-11}$. For reference, the mean and standard deviation of the distribution were 43.9 and 5.0 respectively, which, using the Normal approximation would give $p=6e^{-12}$. The extreme p-value indicates strong evidence for segregation of the rare allele in affected relatives of index carriers.

Haplotypes were estimated for 1,083 imputed markers falling within 10-Kb of 3 significant markers in high-LD at the 8q24.21 locus (rs183373024, rs188140481, and rs138042437 ($r^2=1.0$, $MAFs < 1.7\%$)), defined as 128.094-128.218 Kb. All 112 risk-allele carriers at rs138042437 (107 cases and 5

controls) also carried the risk allele at rs183373024 and rs188140481. An identical haplotype was estimated for 98 cases and 5 controls (92% of risk allele carriers) that contained the rare allele of all 3 SNPs and no other rare variants (MAFs<2%). The complete haplotype is given in Supplemental Table 3. Haplotypes were also estimated from genotypes of 56 prostate cancer cases (in an independent study of cases ascertained from ICPCG member sites and not included in the GWAS dataset) with targeted sequencing data available at the 8q24 region in order to test for the presence of other rare variants not captured in the imputed genotype data that may be detectable with targeted sequence data. 7 carriers of the risk alleles at rs183373024, rs188140481, and rs138042437, were identified among the 56 cases who shared an identical estimated haplotype spanning 128,103,679-128,223,746 bp on chromosome 8 that did not contain any other rare or novel variants. Two of 112 carriers observed in the GWAS dataset (both were cases), 3 of 116 additionally sampled affected relative carriers, and 1 of 7 sequenced case carriers, were homozygous for the rare allele. No determination of the presence of a deletion in the homozygous carriers could be made from available data.

In terms of aggressiveness, the 3 SNPs on the haplotype rs183373024, rs188140481, and rs138042437 had high effect sizes in the GWAS of all prostate cancer (ORs \geq 7.6), and low effect sizes in the aggressive GWAS (ORs=0.9). Investigation of clinical variables that accompanied the GWAS samples did not distinguish affected risk-allele carriers at rs138042437 from non-carrier cases in the ICPCG data set. Affected carriers were not different with respect to age at diagnosis (average age for carriers = 60.45 years, non-carriers = 60.35; t-test p = 0.9), ICPCG aggressiveness status (aggressive carriers = 37%, aggressive non-carriers = 35%; χ^2 p=0.7), or Gleason score (high=7+, moderate or low <7; high Gleason carriers = 63%, high Gleason non-carriers = 60%; χ^2 p=0.6).

Discussion

This GWAS of familial prostate cancer cases and matched controls independently detected significantly associated SNPs in 6 regions, all of which had been previously associated with prostate cancer risk in earlier GWAS and confirmation studies. Of particular interest, however, is that SNPs rs183373024, rs188140481, and rs138042437 at 8q24.21 exhibited very high risk estimates in this study (ORs ≥ 7.6). SNP rs188140481 was previously associated with prostate cancer in a GWAS conducted on 1,795 Icelanders where SNPs were imputed from whole genome data (Gudmundsson 2012). Two of the SNPs occur in long noncoding RNAs, CASC19 (rs138042437) and PRNCR1 (rs183373024), which are a predominant feature of the 8q24.21 region (Xiang 2015; Han 2016). The rare alleles of all 3 SNPs were observed in 107 familial cases in this GWAS population, 62 cases of which had 116 additionally sampled affected relatives available who were subsequently genotyped; the variant was observed in 78 additional relatives ($p=8.5e^{-11}$), indicating strong evidence for co-segregation of the variants with prostate cancer in these families.

A consistent haplotype of 3 rare SNPs was observed among the majority of risk allele carriers in the GWAS cohort at the 8q24.21 locus, rs183373024, rs188140481, and rs130842437, in LD block 2 of the 8q24.21 region (Al Olama 2009). A consistent haplotype was also identified for 7 risk allele carriers for the 3 SNPs in a set of 56 additional familial prostate cancer cases with targeted sequencing data available for the 8q24.21 region. The fact that no additional novel variants were detected among the set of variants on the haplotype estimated from the targeted sequence data indicates either high fidelity of the imputation strategy, or low fidelity of targeted sequencing, in terms of capturing the diversity of the region. It may be possible that further rare variation exists on the risk haplotype that is not observable through either high density SNP genotyping or targeted sequencing, but may be observable with the application of whole genome sequencing of carriers.

It is not clear which of the 3 variants on the risk haplotype conveys an alteration in risk for prostate cancer, either singly or in combination. However, a recent investigation of ENCODE ChipSeq data using the FunciSNP R package identified that rs183373024 occurs at a FoxA1 binding site (Hazelett 2013), which could plausibly indicate regulation of genomic features in the region. Furthermore, SNP rs183373024 is in the long non-coding RNA PRNCR1, upregulation of which has recently been shown to enhance cellular proliferation in colon cancer (Yang 2016). The SNP rs138042437 resides within a POLR2A binding site indicating that this SNP may impact transcription of nearby genomic features (Chen 2015). Despite the fact that biological activity is not currently well understood for most long non-coding RNAs, some progress is being made. For instance, another long noncoding RNA in the 8q24.21 region, CARLo-5 (*CCAT1* locus), has recently been shown through functional investigation to affect cell-cycle regulation and tumor development (Kim 2014) indicating that long noncoding RNAs can have substantial impact on currently cryptic biological processes.

No significant differences were observed when considering clinical variables (ie, age at diagnosis, ICPCG aggressiveness status, and Gleason score) for carriers and non-carriers of the risk alleles at rs138042437, and the 3 SNPs on the risk haplotype did not show even nominal evidence of association in the case-case aggressiveness GWAS comparisons. Furthermore, no significant differences were observed in a previous investigation that reported results by age at diagnosis and aggressiveness status for carriers and non-carriers of rs188140481 in the Icelandic population (Gudmundsson 2012). Taken together, these observations suggest that the haplotype is not characterized as a marker for more aggressive disease.

Of particular note, the 3 SNPs on the haplotype exhibited large estimated effect sizes in the GWAS (ORs ≥ 7.6), larger in magnitude than the recently discovered predisposition gene for prostate cancer, *HOXB13* (rs138213197; OR=4.7; $p=2.8e-7$), which was characterized as a prostate cancer

predisposition gene on the basis of demonstrated segregation of a deleterious variant in prostate cancer pedigrees (Huang 2012).

Comparisons of the estimated ORs between previously reported prostate cancer risk SNPs and the estimates from this familial cases vs. unaffected control dataset did not have detectable differences for the majority of SNPs tested, indicating similar risks for the majority of known prostate cancer associated SNPs in this study population. The GWAS of familial aggressive cases did not reveal any novel significant findings and did not statistically confirm SNPs previously associated with aggressive disease, possibly indicating a lack of statistical power for the aggressive phenotype in this dataset.

The major strengths of a GWAS including familial cases are the ability to detect SNPs with larger effect sizes using a modest sample size and the availability of previously sampled affected relatives who can be subsequently analyzed to test for segregation. Here, the strengths of this design were sufficient to compensate for the lower statistical power of the design compared to larger case-control datasets. One way to interpret the findings of the study is to consider that if functional validation of the 3 candidate SNPs is achieved, which appears at least probable in the context of other functional investigations of the 8q24.21 region, the study will have at least partially explained prostate cancer susceptibility in 48 families with multiple affected men. Fine-mapping of this region in a large multiethnic cohort will add further insight on these variants/haplotypes and help to determine the most likely causal variants. In summary, the strong evidence for co-segregation of the risk haplotype with prostate cancer further characterizes the haplotype as a predisposition locus for prostate cancer.

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Tables

Table 1. Description of the study population by site. Sites that did not contribute controls were analyzed with other sites of similar genetic background; groups analyzed together are denoted by capital letters in ‘Analysis grouping’. Numbers in the table indicate the number of cases or controls from each site and the numbers in parentheses indicate those that passed quality control procedures.

GROUPNAME	Analysis grouping	Cases (QC)	Aggressive (QC)	Controls (QC)	Total (QC)
FINLAND	A	112 (112)	59 (59)	94 (93)	206 (205)
AUSTRALIA	B	166 (163)	92 (90)	169 (165)	335 (328)
LSU	C	17 (17)	17 (17)	0 (0)	17 (17)
MAYO	C	174 (171)	124 (122)	135 (135)	309 (306)
JHU	D	169 (166)	86 (85)	0 (0)	169 (166)
NORTHWESTERN	D	217 (212)	71 (70)	179 (177)	396 (389)
ICR	E	565 (544)	304 (296)	339 (333)	904 (877)
FHCRC	F	127 (123)	96 (93)	122 (113)	249 (236)
MICHIGAN	F	394 (389)	308 (303)	0 (0)	394 (389)
UTAH	G	364 (352)	63 (61)	160 (142)	524 (494)
CEREPP	H	83 (83)	69 (69)	72 (72)	155 (155)
GERMANY	I	180 (179)	129 (129)	152 (152)	332 (331)
Total	-	2568 (2511)	1418 (1394)	1422 (1382)	3990 (3893)

Table 2. Most significant SNP in 6 regions from the genome-wide association of 2,511 prostate cancer cases and 1,382 controls.

Chromosome	Base pair position (hg19)	Accession ID	Minor allele	Minor allele frequency	Odds Ratio	Lower 95% CI	Upper 95% CI	p-value
3	170,074,517	kgp1923988	C	0.192	1.41	1.25	1.59	3.4e-08
6	160,842,537	rs3123636	C	0.356	1.37	1.23	1.51	2.8e-09
8	128,077,146	kgp9645322	A	0.044	2.39	1.81	3.16	8.2e-10
10	51,549,496	rs10763567	A	0.489	1.31	1.19	1.44	5.6e-10
11	6,9012,244	rs12270641	T	0.450	1.40	1.27	1.55	1.4e-11
17	36,102,381	rs11651052	A	0.435	1.37	1.24	1.51	2.5e-10

Table 3. Results for forward selection and elastic-net analyses for SNPs selected by both methods.

References: ^a(Eeles 2008), ^a b(AI Olama 2009), ^b (Gudmundsson 2007a), ^c (Gudmundsson 2012), ^d (AI Olama 2009), ^e (Haiman 2007), ^f (Amundadottir 2006), ^g (Eeles 2007), ^h (Gudmundsson 2007b).ⁱ

Cytogenetic band	Nearest genomic feature [†]	Accession number (bp position, hg19)	Accession number for equivalent previously published SNP (bp position, hg19)	Forward selection coefficient (standard error)	Elastic net coefficient
6q25.3	SLC22A3	*rs3123636	^a rs9364554 (160.83)	0.28 (0.05)	0.005

		(160.84 Mb)	Mb)		
8q24.21	Intergenic (10 kb upstream of PCAT2)	*rs77541621 (128.08 Mb)	^b rs6983561 (128.10 Mb)	0.68 (0.15)	0.103
	PRNCR1	*rs56005245 (128.11 Mb)	^c rs16901979 (128.12 Mb)	0.39 (0.07)	0.036
	Intergenic, 50Kb downstream of PRNCR1, 50-Kb upstream of CASC19	rs2392725 (128.16 Mb)	Unreported	-0.29 (0.05)	-0.018
	CASC19	*rs138042437 (128.208 Mb)	^d rs188140481 (128.19 Mb)	2.21 (0.46)	0.143
	400 bases downstream of CASC19	rs79797202 (128.210 Mb)	Unreported	-0.47 (0.14)	-0.016
	Intergenic, 10-Kb	rs144940621 (128.215 Mb)	Unreported	0.88 (0.27)	0.096

	downstream of CASC19				
	CASC8/ CASC21	*rs4506170 (128.32 Mb)	^e rs620861 (128.34 Mb)	0.31 (0.07)	0.027
	CASC8	rs12682374 (128.41 Mb)	^f rs6983267 (128.41 Mb)	-0.19 (0.05)	-0.004
	Intergenic, 20- Kb downstream of CASC8	rs7841251 (128.52 Mb)	^g rs1447295 (128.48 Mb)	-0.26 (0.07)	-0.012
10q11.23	TIMM23	*rs10763567 (51.54 Mb)	^e rs10993994 (51.49 Mb)	-0.27 (0.05)	-
		rs72795883 (51.57 Mb)		-	0.26
11q13.3	Intergenic (75- Kb upstream of MYEOV)	*rs12270641 (69.01 Mb)	^h rs7931342 (68.99 Mb)	0.33 (0.05)	0.024
17q12	HNF1B	*rs11651052 (36.10 Mb)	ⁱ rs4430796 (36.10 Mb)	0.31 (0.05)	0.044

*SNP was significant in the genome-wide association of all prostate cancer cases versus controls.

†Refseq annotation names.

Figure captions

Fig 1 Manhattan plot of genome-wide association of 2,511 familial prostate cancer cases and 1,382 controls

Fig 2 QQ-plot of genome-wide association results from 2,511 familial prostate cancer cases and 1,382 controls using observed genotypes from the Illumina 5M SNP platform

Fig 3 Genomic features within the 8q24.21 region. The top plot shows the location of the plotted region on chromosome 8 with respect to cytogenetic bands. The x-axis of all subsequent plots is base-pair position, denoted along the bottom of the figure. For the main plot, the y-axis is $-\log_{10}(\text{p-value})$ from the GWAS. Triangles represent SNPs tested in the GWAS. Significant SNPs with an independent signal in the variance reduction analyses are labeled, have outlined triangles, and are assigned different colored shading; the colored shading is also applied to other SNPs in the region in high linkage disequilibrium (LD), where darker color indicates higher LD. The curved lines are normalized LD curves and indicate the general strength and position of LD between the labeled SNPs and other SNPs in the region. The next plot shows the density of genotyped SNPs in the region, followed by a heat map of r^2 estimates between labeled SNPs and other SNPs in the region, where darker shading indicates higher LD. The next plot shows the position of established LD blocks in the 8q24.21 region (Al Olama 2009). The next plot shows ENCODE histone modification binding sites with colors representing different tracks using the same color scheme as the UCSC genome browser (<https://genome.ucsc.edu>), and the height represents strength of the signal. The next plot shows a heat map of ENCODE DNase binding sites, with darker color representing higher sensitivity. The next plot shows a heat map of expression in a prostate cancer cell

line, with darker color representing higher expression. Finally, the genomic features in the region are given (RefSeq ID)

Fig 4 Genome-wide association of 1,394 aggressive versus 1,096 non-aggressive familial prostate cancer cases

Fig 5 QQ-plot of genome-wide association results from 1,394 aggressive versus 1,096 non-aggressive familial prostate cancer cases