

Genome-wide Association Study Identifies a Genetic Variant Associated with Risk for More Aggressive Prostate Cancer

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Abstract

Background: Of the 200,000 U.S. men annually diagnosed with prostate cancer, approximately 20% to 30% will have clinically aggressive disease. Although factors such as Gleason score and tumor stage are used to assess prognosis, there are no biomarkers to identify men at greater risk for developing aggressive prostate cancer. We therefore undertook a search for genetic variants associated with risk of more aggressive disease.

Methods: A genome-wide scan was conducted in 202 prostate cancer cases with a more aggressive phenotype and 100 randomly sampled, age-matched prostate-specific antigen screened negative controls. Analysis of 387,384 autosomal single nucleotide polymorphisms (SNPs) was followed by validation testing in an independent set of 527 cases with more aggressive and 595 cases with less aggressive prostate cancer, and 1,167 age-matched controls.

Results: A variant on 15q13, rs6497287, was confirmed to be most strongly associated with more aggressive ($P_{\text{discovery}} = 5.20 \times 10^{-5}$, $P_{\text{validation}} = 0.004$) than less aggressive disease ($P = 0.14$). Another SNP on 3q26, rs3774315, was found to be associated with prostate cancer risk; however, the association was not stronger for more aggressive disease.

Conclusions: This study provides suggestive evidence for a genetic predisposition to more aggressive prostate cancer and highlights the fact that larger studies are warranted to confirm this supposition and identify further risk variants.

Impact: These findings raise the possibility that assessment of genetic variation may one day be useful to discern men at higher risk for developing clinically significant prostate cancer. *Cancer Epidemiol Biomarkers Prev*; 20(6); 1196–203. ©2011 AACR.

Introduction

More than 217,000 American men were predicted to be diagnosed with prostate cancer last year (1). The majority of patients present with localized tumors that will remain indolent; however, 20% to 30% of patients will have a clinically significant, more aggressive form of the disease

and current screening practices cannot predict who these high-risk men will be.

Genome-wide association studies (GWAS) of prostate cancer have identified numerous genetic variants associated with overall risk of disease (2–10) and more than 30 variants have subsequently been validated (11–22). Few GWAS have analyzed data according to indices of more aggressive prostate cancer (e.g., advanced stage and/or high tumor grade; refs. 9, 23) and in most instances, risk variants found to be associated with aggressive disease have also been associated with less aggressive prostate cancer (23, 24). However, a recent study by Xu and colleagues (25) found that the minor allele of single nucleotide polymorphism (SNP), rs4054823, located on 17p12, was present more frequently in cases with aggressive compared with indolent disease ($P = 2.1 \times 10^{-6}$) and that the homozygous minor allele genotype was more frequent in cases with poorly differentiated tumors (Gleason score ≥ 8) or higher-stage disease ($\geq pT3b$). Results from linkage analyses have also suggested that risk loci exist for aggressive hereditary prostate cancer (26–31); however, the study by Xu and colleagues is the first to provide evidence that genetic variants may also increase risk

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for an aggressive phenotype of the more common sporadic form of the disease.

To search for genetic variants associated with risk of more aggressive prostate cancer, we completed a GWAS on this distinct phenotype and validated our findings in an independent dataset that included both more aggressive and less aggressive prostate cancer cases.

Materials and Methods

Study subjects

The GWAS and validation study populations consist of participants from previous population-based, case-control studies of prostate cancer (study I and study II) in residents of King County, Washington, for which methods have been previously described (32, 33). Briefly, study I cases were diagnosed between January 1, 1993, and December 31, 1996, and were 40 to 64 years of age at diagnosis. Study II cases were diagnosed between January 1, 2002, and December 31, 2005, and were 35 to 74 years of age at diagnosis. Overall, 2,244 eligible prostate cancer patients were identified, 1,754 (78.2%) were interviewed, and blood samples yielding sufficient DNA for genotyping were drawn from 1,457 (83.1%) cases who completed a study interview. A comparison group of controls without a self-reported physician's diagnosis of prostate cancer was identified by random digit dialing. Controls were frequency matched to cases by 5-year age groups. A total of 2,448 eligible men were identified; 1,645 (67.2%) were interviewed, and of these men blood samples were drawn and DNA prepared from 1,352 (82.2%) by using standard protocols. For these analyses, only Caucasian participants with DNA were included (1,324 cases and 1,267 controls).

Subjects completed in-person questionnaires that collected data on family structure and cancer history, medical history, and social and demographic factors. Clinical information on cases, including Gleason score, tumor stage and prostate-specific antigen (PSA) level at diagnosis, was obtained from the Seattle-Puget Sound SEER cancer registry. Vital status and underlying cause of death for cases were obtained through the SEER registry. Death certificates were also reviewed to confirm underlying cause of death. For study I cases, disease recurrence or progression was queried through self-administered surveys and medical record reviews completed in 2005. All cases in the GWAS were from study I and met one or more of the following criteria: Gleason score ≥ 7 (4 + 3); regional or distant stage; diagnostic PSA value of 50 ng/mL or more; evidence of progression/recurrence; or prostate cancer-specific death (Table 1). Controls were randomly selected from a subset of study I controls ($n = 400$) who had a serum PSA value of 2.0 ng/mL or less and reported a negative PSA and/or DRE (digital rectal examination) screening history within the 5-year period prior to study enrollment, and were frequency matched to cases on age (5-year age groups). Cases in the validation phase were defined as having more aggressive disease on the basis of one or more of the following: Gleason

score ≥ 7 (3 + 4); regional or distant stage; diagnostic PSA value of 20 ng/mL or more; evidence of progression/recurrence; and/or prostate cancer-specific death.

All study procedures were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board and genotyping was approved by the National Human Genome Research Institute. Written informed consent was obtained from all study participants prior to participation.

Genotyping

A genome-wide scan was carried out using the Affymetrix Human Mapping 500K array set according to the manufacturer's protocol (Affymetrix, Inc.). From this GWAS, 9 SNPs with $P \leq 1 \times 10^{-4}$ were chosen for validation and genotyped using the Applied Biosystems SNPlex Genotyping System (Applied Biosystems). Identification of the specific SNP alleles was carried out using the ABI 3730xl DNA Analyzer with GeneMapper software. Each batch of DNA aliquots genotyped incorporated similar numbers of case and control samples, and laboratory personnel were blinded to the case-control status of samples.

Statistical analysis

Standard GWAS quality control procedures were executed on the discovery dataset (34). Samples with a missing call rate of more than 2% were filtered from the analysis. Principle components analysis (35) was used to test for population structure and 2 samples identified as outliers were removed from the study. Age-adjusted ORs and 95% CIs were calculated assuming a log-additive genetic model. Likelihood ratio P values were calculated for each SNP by comparing the full model to the reduced model (without the SNP). SNPs with a likelihood ratio $P \leq 1 \times 10^{-4}$ were considered as potential hits and investigated for quality by checking clusters plots, missing call rates, minor allele frequencies, and departure from Hardy-Weinberg equilibrium (34). Analyses were carried out using R version 2.11.1.

Quality control for the validation dataset included genotyping of 142 blind duplicate samples distributed across all genotyping batches. There was 99.3% or more agreement between blind duplicate samples for all 9 SNPs. Polytomous regression models adjusted for age were used to test a log-additive model of cases stratified by disease aggressiveness (less vs. more) compared with controls. Replication was declared if the direction of the effect was the same as in the initial GWAS and a 1-sided $P \leq 0.0056$ (P value of 0.05/9) was observed. To test for significant differences in risk estimates for more versus less aggressive disease, a Wald χ^2 test was used ($P_{\text{heterogeneity}}$). Analyses were carried out using SAS version 9.1.3.

Results

The clinical characteristics of cases included in the GWAS and the validation study are presented in

Table 1. Clinical characteristics of the discovery and validation study participants

Characteristics	Discovery study		Validation study		
	More aggressive cases ^a (N = 202), n (%)	Controls (N = 100), n (%)	More aggressive cases ^b (N = 527), n (%)	Less aggressive cases (N = 595), n (%)	Controls (N = 1,167), n (%)
Age at reference date, y					
35–49	17 (8.4)	8 (8.0)	46 (8.7)	40 (6.7)	100 (8.6)
50–54	39 (19.3)	20 (20.0)	68 (12.9)	86 (14.4)	158 (13.5)
55–59	70 (34.7)	34 (34.0)	105 (19.9)	159 (26.7)	310 (26.6)
60–64	76 (37.6)	38 (38.0)	148 (28.1)	172 (28.9)	295 (25.3)
65–69			85 (16.1)	68 (11.4)	160 (13.7)
70–74			75 (14.2)	70 (11.8)	144 (12.3)
Gleason score					
2–4	3 (1.5)		10 (1.9)	57 (9.6)	
5–6	74 (36.6)		76 (14.4)	536 (90.1)	
7 = 3 + 4	79 (39.1)		300 (56.9)		
7 = 4 + 3	20 (9.9)		56 (10.6)		
8–10	45 (22.3)		84 (15.9)		
Missing	1 (0.5)		1 (0.2)	2 (0.3)	
Stage					
Local	65 (32.2)		370 (70.2)	595 (100)	
Regional	121 (59.9)		140 (26.6)		
Distant	16 (7.9)		17 (3.2)		
Diagnostic PSA level, ng/mL					
<4.0	18 (8.9)		47 (8.9)	115 (19.3)	
4.0–9.9	95 (47.0)		279 (52.9)	354 (59.5)	
10.0–19.9	30 (14.8)		92 (17.5)	69 (11.6)	
20.0–49.9	21 (10.4)		51 (9.7)		
≥50.0	22 (10.9)		27 (5.1)		
Missing	16 (7.9)		31 (5.9)	57 (9.6)	
Progression/recurrence ^c					
No	69 (34.2)		71 (46.1)	192 (74.7)	
Yes	97 (48.0)		48 (31.2)		
Missing	36 (17.8)		35 (22.7)	65 (25.3)	
Prostate cancer-specific death					
No ^d	164 (81.2)		496 (94.1)	595 (100.0)	
Yes	38 (18.8)		31 (5.9)		

^aAge less than 65 years at diagnosis, diagnostic PSA level ≥ 50 ng/mL, regional/distant stage, Gleason score ≥ 7 (4 + 3), progression/recurrence event, and/or prostate cancer-specific death.

^bDiagnostic PSA level ≥ 20 ng/mL, regional/distant stage, Gleason score ≥ 7 , progression/recurrence event, and/or prostate cancer-specific death.

^cData available for study I participants only.

^dIncludes patients who are alive, died of other causes, or their vital status is unknown.

Table 1. Of the 202 cases in the GWAS, selection in order of inclusion criteria yielded 65 (32.2%) who had a Gleason score ≥ 7 (4 + 3), 101 (50%) who had regional or distant stage disease, 1 (0.5%) who had a diagnostic PSA value of 50 ng/mL or more, and 35 (17.3%) who had a recurrence/progression event. Of the 527 more aggressive cases in the validation study, 440 (83.5%) had a Gleason score ≥ 7 (3 + 4), 39 (7.4%) had regional or distant stage disease, 20 (3.8%) had a diagnostic PSA value of 20 ng/mL or more, 25 (4.7%) had a recur-

rence/progression event, and 3 (0.6%) died of prostate cancer.

In the GWAS, no variant reached genome-wide significance ($P < 1 \times 10^{-7}$); however, 9 variants showed some evidence of association with more aggressive disease ($P < 1 \times 10^{-4}$; Table 2). These were then evaluated in an independent population-based dataset. In this validation study of 527 more aggressive cases, 595 less aggressive cases, and 1,167 age-matched controls, the risk estimate for rs6497287 on 15q13 was validated and the magnitude

Table 2. Results of 9 SNPs selected for validation of an association with more aggressive prostate cancer

SNP (Major/Minor allele)	Chromosome	Gene	500 K GWAS			Validation study								
			Controls (N = 100)		More aggressive ^a (N = 202)	Controls (N = 1,167)			More aggressive ^b (N = 527)			Less aggressive (N = 595)		
			MAF	OR		95% CI	MAF	OR	95% CI	P ^c	MAF	OR	95% CI	P ^d
rs3774315 (A/G)	3q26	TNFSF10	0.15	2.50	1.55-4.03	7.34 × 10 ⁻⁵	0.25	1.17	0.99-1.38	0.03	1.25	1.08-1.46	0.002	0.45
rs8015211 (T/C)	14q22	FBXO34	0.25	2.25	1.53-3.32	1.64 × 10 ⁻⁵	0.33	1.12	0.96-1.30	0.08	0.99	0.85-1.15	0.56	0.17
rs12433615 (A/G)	14q22		0.23	2.16	1.47-3.18	4.06 × 10 ⁻⁵	0.31	1.10	0.94-1.28	0.12	1.10	0.95-1.28	0.10	0.97
rs1188069 (T/G)	14q22		0.13	2.52	1.55-4.11	6.38 × 10 ⁻⁵	0.20	1.12	0.94-1.34	0.11	1.02	0.86-1.22	0.40	0.39
rs2341883 (A/G)	14q22		0.24	2.06	1.41-3.02	1.01 × 10 ⁻⁴	0.32	1.20	1.03-1.40	0.01	1.19	1.03-1.38	0.01	0.96
rs6497287 (T/C)	15q13	HERC2	0.01	8.92	2.10-37.9	5.20 × 10 ⁻⁵	0.06	1.46	1.10-1.94	0.004	1.17	0.88-1.56	0.14	0.17
rs1150069 (C/A)	16q23	WWOX	0.41	0.42	0.28-0.62	9.43 × 10 ⁻⁶	0.30	1.01	0.85-1.19	0.54	1.11	0.95-1.29	0.90	0.34
rs4628973 (A/G)	16q24		0.38	0.35	0.24-0.54	4.62 × 10 ⁻⁷	0.30	1.02	0.86-1.20	0.58	0.89	0.76-1.04	0.07	0.16
rs225061 (G/T)	Xp21	IL1RAPL1	0.28	0.59	0.44-0.81	9.71 × 10 ⁻⁴	0.22	0.95	0.73-1.23	0.34	0.81	0.63-1.05	0.06	0.32

Abbreviation: MAF, minor allele frequency. Values in bold are significant after adjustment for multiple comparisons.

^aDiagnostic PSA level ≥ 50 ng/mL, regional/distant stage, Gleason Score ≥ 7 (4 + 3), recurrence/progression event and/or prostate cancer-specific death.

^bDiagnostic PSA level ≥ 20 ng/mL, regional/distant stage, Gleason score ≥ 7 (3+4), recurrence/progression event and/or prostate cancer-specific death.

^cTwo-sided P value.

^dOne-sided P value.

^eHeterogeneity P value for the difference in ORs for more versus less aggressive prostate cancer.

of the risk estimate was stronger in cases with aggressive prostate cancer. Under a log-additive model, carriers of the rs6497287 minor allele had an OR of 1.46 (95% CI, 1.10–1.94; $P = 0.004$) for aggressive disease and an OR of 1.17 (95% CI, 0.88–1.56; $P = 0.14$) for less aggressive disease; however, a test for heterogeneity was not significant ($P_{\text{heterogeneity}} = 0.17$). The SNP, rs2341883 on 14q22, was associated with elevated risk estimates of both more and less aggressive prostate cancer (OR = 1.20; 95% CI, 1.03–1.40; $P = 0.01$ and OR = 1.19; 95% CI, 1.03–1.38; $P = 0.01$, respectively); however, these results did not remain significant after adjustment for multiple testing. Finally, rs3774315 on 3q26 was more strongly associated with less aggressive disease (OR = 1.25; 95% CI, 1.08–1.46; $P = 0.002$) than more aggressive disease (OR = 1.17; 95% CI, 0.99–1.38; $P = 0.03$; Table 2).

To search for further evidence that these SNPs were associated with prostate cancer risk, we did a combined analysis of all the more aggressive cases and controls (Supplementary Table S1) and also examined the Cancer Genetic Markers of Susceptibility (CGEMS) dataset (10, 36). No SNPs achieved genome-wide significance ($P < 1 \times 10^{-7}$) in the combined analysis; however, the strongest risk estimate observed was for rs6497287, which is consistent with results from the independent validation component described earlier in the text. In the CGEMS dataset, SNP rs2341883 (14q22) was directly genotyped and no association was observed for either overall or more aggressive prostate cancer. SNP rs6497287 (15q13) was not genotyped in CGEMS; however, rs6497287 is located in *HERC2* and data for 15 SNPs across this gene were available. On further investigation it was found that one of these SNPs, rs7183877, is in perfect linkage disequilibrium (LD) with rs6497287; however, neither this nor the remaining SNPs showed significant evidence of an association with prostate cancer risk. Variant rs3774315 also was not genotyped in CGEMS, but a SNP in strong LD with this variant, rs3136594 ($r^2 = 0.85$), was evaluated. In addition, data for 6 other SNPs located in *TNFSF10*, which rs3774315 tags, were also available. Although no variant was more strongly associated with aggressive disease, three were associated with overall prostate cancer risk (rs3815496: dominant OR = 1.23; 95% CI, 1.05–1.46; $P = 0.01$; rs3136597: trend OR = 0.79; 95% CI, 0.65–0.96; $P = 0.02$; rs4894559: dominant OR = 1.25; 95% CI, 1.06–1.48; $P = 0.009$).

Discussion

Over the past few decades PSA screening has not only increased the number of prostate cancer diagnoses, but has also increased the number of patients who are overtreated for indolent disease. Two large, randomized trials recently published interim results on PSA screening in relation to prostate cancer-specific mortality (37, 38). Although their main findings were divergent, both studies concluded that screening was associated with substantially increased risks of overdiagnosis and

overtreatment. Therefore, the ability to target screening to men at higher risk for more aggressive disease may allow for early detection of those at higher risk for developing clinically significant prostate cancer. It may also reduce the number of men at low risk for clinically significant disease whose quality of life and function (e.g., urinary, sexual) may be impacted by intensive treatment that may be unnecessary. Recently, numerous GWAS have discovered genetic variants that are associated with overall disease risk; however, these variants are not uniquely associated with risk of more clinically significant prostate cancer nor have they been shown to be clinically useful for predicting patient outcomes (12, 13, 39–41).

To date, few studies of sporadic prostate cancer have focused on identifying variants associated with more aggressive disease (9, 23–25), and although initial results looked promising, most associations were subsequently shown not to be unique to this phenotype (23, 24). One reason for this could be the inclusion of all Gleason score 7 disease in the criteria for defining aggressiveness. Gleason 7 tumors are a heterogeneous group that are less aggressive than Gleason score 8–10 tumors (42, 43), which may have attenuated associations with aggressiveness. Therefore, defining aggressive disease using stricter phenotype criteria (e.g., Gleason score 8–10, regional/distant stage) may reduce heterogeneity and strengthen results. Lack of statistical power due to limited sample size is also an issue in these studies, especially to detect less common variants or those with weak to modest effects. As the proportion of cases meeting a stricter aggressive disease definition will be small in most studies (e.g., 15%–30% of all cases), collaborative efforts will be needed to obtain enough samples to achieve sufficient power.

In the study presented here, we found evidence that SNP rs6497287 is more strongly associated with more aggressive than with less aggressive disease. It must be noted though that a significant difference in risk estimates between the two phenotypes was not observed. Whether this is because of the limited sample size or the fact that this SNP is actually associated with overall disease risk will have to be determined in larger studies. Nonetheless, this variant highlights an interesting prostate cancer candidate gene. SNP rs6497287 is located in *HERC2*, a large 93-exon gene on 15q11–13. This gene functions as a determinant of human iris color (44, 45) and has recently been shown to have a role in the DNA damage response. *HERC2* protein both mediates the specificity of E3 ubiquitin ligase binding (46) and acts as an E3 ligase itself, regulating XPA (47) and BRCA1 protein levels (48). It is interesting to note that *HERC2* translocates from the cytoplasm to the nucleus to participate in the functions described earlier and the rs6497287 variant is located close to the nuclear localization signal of *HERC2*. Given the role *HERC2* plays in the DNA damage response, mutations within this gene may compromise genomic stability and thereby increase cancer risk.

Similar to previous studies (23, 24), we also identified a SNP, rs3774315, that was associated with more aggressive disease in the GWAS, but was subsequently found to be associated with overall prostate cancer risk in the validation dataset. This variant is located on 3q26 in the *TNFSF10* gene, whose protein product TRAIL plays a key role in apoptosis (49). Although the association we observed was not observed in CGEMS, 3 other *TNFSF10* variants were found to be associated with overall disease risk in CGEMS, providing evidence that this gene may play a role in prostate cancer development and should be investigated further.

There are a number of strengths and limitations of our study that should be noted when interpreting these results. Significant strengths include the focus on aggressive prostate cancer and the population-based resource for confirmation. The genetic heterogeneity of prostate cancer is well known; therefore, to reduce heterogeneity and increase the likelihood of identifying aggressive prostate cancer risk variants, cases with clinical features associated with adverse outcomes were chosen for the GWAS. Results from this phase were then validated in a dataset comprising a more widely used definition of aggressive disease, including all cases with a Gleason score ≥ 7 tumor. This definition was used by CGEMS (9) and although Gleason score 7 tumors are heterogeneous and there are differences between the CGEMS study and the one presented here, CGEMS is the only publicly available GWAS dataset that allows assessment of risk for more aggressive prostate cancer to which our findings can be compared. Another advantage of this study is that more than 380,000 SNPs were examined compared with the previous study by Xu and colleagues in which approximately 27,000 SNPs were examined for associations with aggressive disease (25). A major limitation of this GWAS is the small sample size due to funding constraints. It should be noted that the small proportion of samples in the GWAS stage and the small proportion of SNPs carried through to the independent replication stage of this study provided comparable power as would be obtained from jointly analyzing the data from both stages (50). Although it is encouraging that both the independent replication and joint analyses highlighted rs6497287, the power of this study is limited and the results here need to be replicated in larger independent datasets.

In summary, we have found evidence that a genetic variant, rs6497287, is more strongly associated with

aggressive prostate cancer. This finding lends strength to the hypothesis that there is a genetic susceptibility to clinically significant prostate cancer. As is the prediction for indolent prostate cancer, many low- to moderate-penetrant common risk alleles may contribute to the inherited predisposition for more aggressive disease. Therefore, because of the relatively small proportion of patients with aggressive versus indolent disease in most prostate cancer study populations, future collaborative efforts will be needed to attain genome-wide statistical power for finding genetic variants that uniquely distinguish men at higher risk for the more aggressive disease phenotype. Once this goal is realized, genetic variants may be useful for stratification of men who may benefit most from early detection and screening programs. At diagnosis, this information may also be helpful for identifying those patients who, based on their genetic risk profile, may warrant more aggressive initial therapy and also those who can be spared unnecessary therapy, thereby avoiding treatment-related morbidity. In addition to potential public health and clinical benefits, identification of variants associated with more aggressive disease may broaden our knowledge of the underlying biology of prostate cancer, providing insight into possible preventative and therapeutic targets.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010;60:277–300.
2. Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, et al. A common variant associated with prostate cancer in European and African populations. *Nat Genet* 2006;38:652–8.
3. Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, Jugurnauth SK, et al. Multiple newly identified loci associated with prostate cancer susceptibility. *Nat Genet* 2008;40:316–21.
4. Eeles RA, Kote-Jarai Z, Olama AA, Giles GG. Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. *Nat Genet* 2009;41:1116–21.

5. Gudmundsson J, Sulem P, Gudbjartsson DF, Blondal T, Gylfason A, Agnarsson BA, et al. Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. *Nat Genet* 2009;41:1122–6.
6. Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, et al. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet* 2007;39:631–7.
7. Gudmundsson J, Sulem P, Rafnar T, Bergthorsson JT, Manolescu A, Gudbjartsson D, et al. Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. *Nat Genet* 2008;40:281–3.
8. Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, Manolescu A, et al. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet* 2007;39:977–83.
9. Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N, et al. Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet* 2008;40:310–5.
10. Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet* 2007;39:645–9.
11. Camp NJ, Farnham JM, Wong J, Christensen GB, Thomas A, Cannon-Albright LA. Replication of the 10q11 and Xp11 prostate cancer risk variants: results from a Utah pedigree-based study. *Cancer Epidemiol Biomarkers Prev* 2009;18:1290–4.
12. FitzGerald LM, Kwon EM, Koopmeiners JS, Salinas CA, Stanford JL. Analysis of recently identified prostate cancer susceptibility loci in a population-based study: associations with family history and clinical features. *Clin Cancer Res* 2009;15:3231–37.
13. Kote-Jarai Z, Easton DF, Stanford JL, Ostrander EA, Schleutker J, Ingles SA, et al. Multiple novel prostate cancer predisposition loci confirmed by an international study: the PRACTICAL Consortium. *Cancer Epidemiol Biomarkers Prev* 2008;17:2052–61.
14. Salinas CA, Kwon E, Carlson CS, Koopmeiners JS, Feng Z, Karyadi DM, et al. Multiple independent genetic variants in the 8q24 region are associated with prostate cancer risk. *Cancer Epidemiol Biomarkers Prev* 2008;17:1203–13.
15. Schumacher FR, Feigelson HS, Cox DG, Haiman CA, Albanes D, Buring J, et al. A common 8q24 variant in prostate and breast cancer from a large nested case-control study. *Cancer Res* 2007;67:2951–6.
16. Severi G, Hayes VM, Padilla EJ, English DR, Southey MC, Sutherland RL, et al. The common variant rs1447295 on chromosome 8q24 and prostate cancer risk: results from an Australian population-based case-control study. *Cancer Epidemiol Biomarkers Prev* 2007;16:610–2.
17. Sun J, Purcell L, Gao Z, Isaacs SD, Wiley KE. Association between sequence variants at 17q12 and 17q24.3 and prostate cancer risk in European and African Americans. *Prostate* 2008;68:691–7.
18. Suuriniemi M, Agalliu I, Schaid DJ, Johanneson B, McDonnell SK, Iwasaki L, et al. Confirmation of a positive association between prostate cancer risk and a locus at chromosome 8q24. *Cancer Epidemiol Biomarkers Prev* 2007;16:809–14.
19. Wang L, McDonnell SK, Slusser JP, Hebbing SJ, Cunningham JM, Jacobsen SJ, et al. Two common chromosome 8q24 variants are associated with increased risk for prostate cancer. *Cancer Res* 2007;67:2944–50.
20. Waters KM, Le Marchand L, Kolonel LN, Monroe KR, Stram DO, Henderson BE, et al. Generalizability of associations from prostate cancer genome-wide association studies in multiple populations. *Cancer Epidemiol Biomarkers Prev* 2009;18:1285–9.
21. Xu J, Kibel AS, Hu JJ, Turner AR, Pruett K, Zheng SL, et al. Prostate cancer risk associated loci in African Americans. *Cancer Epidemiol Biomarkers Prev* 2009;18:2145–9.
22. Zheng SL, Sun J, Wiklund F, Smith S, Stattin P, Li G, et al. Cumulative association of five genetic variants with prostate cancer. *N Engl J Med* 2008;910–19.
23. Duggan D, Zheng SL, Knowlton M, Benitez D, Dimitrov L, Wiklund F, et al. Two genome-wide association studies of aggressive prostate cancer implicate putative prostate tumor suppressor gene DAB2IP. *J Natl Cancer Inst* 2007;99:1836–44.
24. Sun J, Zheng SL, Wiklund F, Isaacs SD, Li G, Wiley KE, et al. Sequence variants at 22q13 are associated with prostate cancer risk. *Cancer Res* 2009;69:10–5.
25. Xu J, Zheng SL, Isaacs SD, Wiley KE, Wiklund F, Sun J, et al. Inherited genetic variant predisposes to aggressive but not indolent prostate cancer. *Proc Natl Acad Sci U S A* 2010;107:2136–40.
26. Chang BL, Isaacs SD, Wiley KE, Gillanders EM, Zheng SL. Genome-wide screen for prostate cancer susceptibility genes in men with clinically significant disease. *Prostate* 2005;64:365–661.
27. Schaid DJ, McDonnell SK, Zarfes KE, Cunningham JM, Hebbing S, Thibodeau SN, et al. Pooled genome linkage scan of aggressive prostate cancer: results from the International Consortium for Prostate Cancer Genetics. *Hum Genet* 2006;120:471–85.
28. Schaid DJ, Stanford JL, McDonnell SK, Suuriniemi M, McIntosh L, Karyadi DM, et al. Genome-wide linkage scan of prostate cancer Gleason score and confirmation of chromosome 19q. *Hum Genet* 2007;121:729–35.
29. Slager SL, Zarfes KE, Brown WM, Lange EM, McDonnell SK. Genome-wide linkage scan for prostate cancer aggressiveness loci using families from the University of Michigan prostate cancer genetics project. *Prostate* 2006;66:173–9.
30. Stanford JL, McDonnell SK, Friedrichsen DM, Carlson EE, Kolb S, Deutsch K, et al. Prostate cancer and genetic susceptibility: a genome scan incorporating disease aggressiveness. *Prostate* 2006;66:317–25.
31. Witte JS, Goddard KA, Conti DV, Elston RC, Lin J, Suarez BK, et al. Genome-wide scan for prostate cancer-aggressiveness loci. *Am J Hum Genet* 2000;67:92–9.
32. Agalliu I, Salinas CA, Hansten PD, Ostrander EA, Stanford JL. Statin use and risk of prostate cancer: results from a population-based epidemiologic study. *Am J Epidemiol* 2008;168:250–60.
33. Stanford JL, Wicklund KG, McKnight B, Daling JR, Brawer MK. Vasectomy and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 1999;8:881–6.
34. Laurie CC, Doherty KF, Mirel DB, Pugh EW, Bierut LJ, Bhangale T, et al. Quality control and quality assurance in genotypic data for genome-wide association studies. *Genet Epidemiol* 2010;34:591–602.
35. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. *PLoS Genet* 2006;2:e190.
36. The National Cancer Institute Cancer Genetic Markers of Susceptibility (CGEMS) project. 2005; Available from: <http://cgems.cancer.gov/>
37. Andriole GL, Crawford ED, Grubb RL III, Buys SS, Chia D, Church TR, et al. Mortality results from a randomized prostate-cancer screening trial. *N Engl J Med* 2009;360:1310–9.
38. Schroder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, et al. Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med* 2009;360:1320–8.
39. Kader AK, Sun J, Isaacs SD, Wiley KE, Yan G, Kim ST, et al. Individual and cumulative effect of prostate cancer risk-associated variants on clinicopathologic variables in 5,895 prostate cancer patients. *Prostate* 2009;69:1195–205.
40. Wiklund FE, Adami HO, Zheng SL, Stattin P, Isaacs WB, Gronberg H, et al. Established prostate cancer susceptibility variants are not associated with disease outcome. *Cancer Epidemiol Biomarkers Prev* 2009;18:1659–62.
41. Kraft P, Hunter DJ. Genetic risk prediction—are we there yet? *N Engl J Med* 2009;360:1701–3.
42. Roehl KA, Han M, Ramos CG, Antenor JV, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. *J Urol* 2004;172:910–4.
43. Wright JL, Salinas CA, Lin DW, Kolb S, Koopmeiners J, Feng Z, et al. Prostate cancer specific mortality and Gleason 7 disease differences in prostate cancer outcomes between cases with Gleason 4 + 3 and Gleason 3 + 4 tumors in a population based cohort. *J Urol* 2009;182:2702–7.

44. Kayser M, Liu F, Janssens AC, Rivadeneira F, Lao O, van Duijn K, et al. Three genome-wide association studies and a linkage analysis identify HERC2 as a human iris color gene. *Am J Hum Genet* 2008;82:411–23.
45. Sturm RA, Duffy DL, Zhao ZZ, Leite FP, Stark MS, Hayward NK, et al. A single SNP in an evolutionary conserved region within intron 86 of the HERC2 gene determines human blue-brown eye color. *Am J Hum Genet* 2008;82:424–31.
46. Bekker-Jensen S, Rendtlew Danielsen J, Fugger K, Gromova I, Nerstedt A, Lukas C, et al. HERC2 coordinates ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes. *Nat Cell Biol* 2010;12:80–6.
47. Kang TH, Lindsey-Boltz LA, Reardon JT, Sancar A. Circadian control of XPA and excision repair of cisplatin-DNA damage by cryptochrome and HERC2 ubiquitin ligase. *Proc Natl Acad Sci U S A* 2010;107:4890–5.
48. Wu W, Sato K, Koike A, Nishikawa H, Koizumi H, Venkitaraman AR, et al. HERC2 is an E3 ligase that targets BRCA1 for degradation. *Cancer Res* 2010;70:6384–92.
49. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science* 1998;281:1305–8.
50. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 2006;38:209–13.