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Two-stage Study of Familial Prostate Cancer by Whole-exome Sequencing and Custom Capture Identifies 10 Novel Genes Associated with the Risk of Prostate Cancer

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Abstract

Background: Family history of prostate cancer (PCa) is a well-known risk factor, and both common and rare genetic variants are associated with the disease.

Objective: To detect new genetic variants associated with PCa, capitalizing on the role of family history and more aggressive PCa.

Design, setting, and participants: A two-stage design was used. In stage one, whole-exome sequencing was used to identify potential risk alleles among affected men with a strong family history of disease or with more aggressive disease (491 cases and 429 controls). Aggressive disease was based on a sum of scores for Gleason score, node status, metastasis, tumor stage, prostate-specific antigen at diagnosis, systemic recurrence, and time to PCa death. Genes identified in stage one were screened in stage two using a custom-capture design in an independent set of 2917 cases and 1899 controls.

Outcome measurements and statistical analysis: Frequencies of genetic variants (singly or jointly in a gene) were compared between cases and controls.

Results and limitations: Eleven genes previously reported to be associated with PCa were detected (*ATM*, *BRCA2*, *HOXB13*, *FAM111A*, *EMSY*, *HNFB1B*, *KLK3*, *MSMB*, *PCAT1*, *PRSS3*, and *TERT*), as well as an additional 10 novel genes (*PABPC1*, *QK1*, *FAM114A1*, *MUC6*, *MYCBP2*, *RAPGEF4*, *RNASEH2B*, *ULK4*, *XPO7*, and *THAP3*). Of these 10 novel genes, all but *PABPC1* and *ULK4* were primarily associated with the risk of aggressive PCa.

Conclusions: Our approach demonstrates the advantage of gene sequencing in the search for genetic variants associated with PCa and the benefits of sampling patients with a strong family history of disease or an aggressive form of disease.

Patient summary: Multiple genes are associated with prostate cancer (PCa) among men with a strong family history of this disease or among men with an aggressive form of PCa.

Keywords

Whole-exome sequencing; Custom-capture sequencing; Familial prostate cancer; Genetic risk variants

1. Introduction

It is estimated that there were 1 276 106 new cases of prostate cancer (PCa) worldwide in 2018 [1]. In the USA, 174 650 new cases of PCa and 31 620 deaths due to PCa were predicted to occur during 2019 [2]. The well-established risk factors for PCa are older age, African-American ancestry [3,4], and a family history of the disease. Variants in the genes *BRCA2* and *HOXB13* are well established as having a strong association with PCa risk. *ATM* and the genes involved in mismatch repair (*BRCA1*, *MLH1*, *PMS2*, *MSH2*, and *MSH6*) have also been implicated in PCa risk. In addition, genome-wide association studies have identified approximately 170 common genetic variants spread across all autosomes and the X chromosome that account for an estimated 28.4% of the familial PCa (FPC) risk [5]. As much of the hereditary risk remains unexplained, identification of additional genes and genetic variants associated with PCa risk can potentially aid in early detection, discernment of aggressive disease, and possibly provide targets for therapy.

To search for genes involved in hereditary or more aggressive PCa, we capitalized on pedigrees and samples available from the International Consortium for Prostate Cancer Genetics (ICPCG), an international collaboration that has conducted numerous family-based studies for almost 20 yr [6–15].

2. Patients and methods

We used a two-stage design in which the first stage was used to screen for genes with suggestive evidence of association with PCa, followed by a second-stage case-control study that conducted a more rigorous evaluation of the candidate genes suggested by the first stage. Study participants for both stages were recruited from the 15 ICPCG groups (see the Supplementary material). Analyses focused on men with European ancestry because of a

limited number of samples with other ancestries. As a guide, Figure 1 provides details on the samples used in the two stages and why some samples were excluded. All participants gave informed consent to utilize samples for research purposes, and the study was approved by the respective institutional review boards.

2.1. Stage-one samples

Families with FPC, defined as families with three or more affected first-degree relatives with PCa, were reviewed to select 539 PCa cases from 366 pedigrees for whole-exome sequencing (WES) at the Mayo Clinic. Controls were 494 samples from noncancer studies with WES conducted at the Mayo Clinic. To increase power, we obtained auxiliary WES data for 140 unrelated cases and 592 unrelated controls. Secondary analyses included the cases and controls used in the primary analyses, pooled with the auxiliary data (see the Supplementary material for details).

2.2. Stage-one WES

For all cases and controls sequenced at the Mayo Clinic, exome capture was performed using Agilent SureSelect Human All Exon 50Mb or V4 + UTR capture kits. Samples were pooled after the capture and sequenced three to a lane using the Illumina HiSeq. All cases sequenced at the Mayo Clinic were also genotyped on the Illumina Infinium OmniExpress-12 array. Externally sequenced samples were sequenced using a variety of capture kits, including Illumina TruSeq and Nimblegen SeqCap (see Supplementary Table 1 and the Supplementary material).

2.3. Stage-one statistical analyses

The association of PCa with genetic variants, either as a single variant or as a group of variants in a gene, was assessed by association analyses (comparing cases with controls) and cosegregation of genetic variants within pedigrees (eg, excess sharing of alleles among relatives). All variants with minor allele frequency (MAF) >0.001 were included in single-variant association analyses. To screen for less common variants, all variants with MAF $\geq 5\%$ were included in gene-based tests. Single-variant and gene-level associations were evaluated using burden (ie, sum of alternate alleles for variants within a gene) and kernel statistics that allow for known pedigree relationships [16]. As variants are likely to differ in terms of functional effect on traits, a variety of weighting schemes were used to evaluate simultaneously all variants within a gene (see the Supplementary material). For single-variant tests, we considered only tier-1 (likely to cause protein truncation) and tier-2 (nonsynonymous coding variants and in-frame indels) variants when selecting genes to carry forward to stage two. For primary analyses, five principal components and capture kits (V3 vs V4 + UTR) were included as covariates. For secondary analyses, 12 principal components were included. We used a liberal significance threshold of $p < 0.01$ to select genes that showed some minimal association with PCa to carry forward to stage two.

2.4. Stage-two samples

In stage two, 3105 unrelated PCa cases were selected for targeted sequencing. Of these cases, 2145 were selected from the remaining FPC pedigrees that were not included in stage

one, with only one individual selected per family. Cases with DNA available were selected based on the strongest family history and the most aggressive PCa. In pedigrees with multiple aggressive cases, the individual with the youngest age at diagnosis was chosen. Beyond these cases with a family history of PCa, 960 men with more aggressive disease were selected because of the clinical importance of this phenotype and because more aggressive cancer might be more enriched for causative genetic factors. These aggressive cases were unrelated to the other stage-one or stage-two patients, and some lacked a family history of PCa. The initial criteria for selecting aggressive cases were based on the ICPCG criteria used in stage one (see the Supplementary material). We subsequently refined a score for aggressive disease based on the sum of scores for the following clinical factors: Gleason score 8–10 (+2 points), node status N1+ (+2 points), metastasis M1 (+2 points), tumor stage T4 (+2 points), prostate-specific antigen (PSA) at diagnosis (PSA >20, +1 point; PSA >50, +2 points), systemic recurrence (+2 points), and time to PCa death (<5 yr, +2 points; 5–10 yr, +1 point). For our analyses, cases with a score of at least 2 were categorized as aggressive. Owing to missing clinical data, some cases could have a score of 0–1 and were categorized as nonaggressive.

A set of 2156 stage-two controls was identified from 12 ICPCG member groups and was restricted to unrelated men with no personal history of cancer, preferably those with no family history of PCa, who were unrelated to cases used in stages one and two, and were of European descent. Each of the ICPCG groups selected controls by frequency matching with their contributed cases based on birth year. Controls selected from the Mayo Biobank were required to be free of PCa and at least 70 yr old.

2.5. Stage-two custom-capture resequencing

A custom-capture sequencing array was designed to characterize all genes meeting stage-one significance criteria. The custom array was designed by Agilent to target 1202 genes (5.9 Mb) at an average coverage of 100×. Coverage of the targeted genes included the exons, ±30 bp surrounding each exon, and 1 kb of the 5' UTR. In addition, we created a custom genotyping panel consisting of 29 highly polymorphic single nucleotide polymorphisms to provide sample identity verification.

2.6. Stage-two statistical analyses

Gene-level association analyses were conducted by burden tests and kernel statistics as implemented in SKAT-O [17]. Single-variant associations were performed using PLINK [18]. Covariates associated with PCa status included as adjusting covariates were a factor representing the ICPCG member group, log(missing call rate), and log(Qubit concentration); see the Supplementary material for details.

3. Results

3.1. Stage one: screening by WES

Stage-one primary analyses included 491 cases and 429 controls (see Table 1 for clinical characteristics of cases). In total, 720 genes met the significance threshold of $p < 0.01$ and were selected for targeted sequencing in stage two. An additional 482 candidate genes were

nominated by ICPCG collaborating members, resulting in 1202 genes for targeted sequencing in stage two (see the Supplementary material for details of stage-one results and Supplementary Table 5 for genes sequenced in stage two).

3.2. Stage-two association results

After extensive quality control (QC; see Fig. 1, and Supplementary Table 3) and restriction to European ancestry, 2917 unrelated cases and 1899 unrelated controls were used in statistical analyses. Clinical characteristics of the cases are presented in Table 1 (see Supplementary Table 4 for more details). Of the 1202 sequenced genes, 1188 had at least one variant that passed QC (total of 29 838 variants for single-variant analyses), and 1116 genes had at least one tier-1 or tier-2 variant that passed QC for gene-level analyses.

3.3. Single-variant associations

The results of analyzing 29 838 single variants are summarized in Figure 2 using Manhattan plots for the four types of comparisons: all cases versus controls (Fig. 2A), familial cases versus controls (Fig. 2B), aggressive cases versus controls (Fig. 2C), and aggressive cases versus nonaggressive cases (Fig. 2D). We used a Bonferroni correction for multiple testing based on a p -value threshold of $1e-6$ (red horizontal line in Fig. 2). For the comparisons of all cases and familial cases with controls, 15 variants were statistically significant (summarized in Table 2). The missense variant in *HOXB13* (rs138213197) showed the greatest risk when comparing all cases with controls (odds ratio [OR] = 21.01; 95% confidence interval [CI]: 6.58, 67.15; Table 2) and familial cases with controls (OR = 22.44, 95% CI: 6.99, 71.97; Table 2). Two variants in the *MSMB* and *TERT* genes were also associated with an increase in PCa risk, where the alternate allele was more frequent in cases than in controls (Table 2). In contrast, the remaining variants in the *HNF1B*, *KLK3*, *MSMB*, *PCAT1*, and *ULK4* genes were associated with reduced PCa risk, where the alternate alleles were significantly less frequent in cases than in controls. To place our results in context of the findings from a recent large-scale genome-wide association study, we compared our results with those from the PRACTICAL consortium, which analyzed >140 000 cases and controls and had 20 370 946 genotyped or high-quality imputed variants [19]. Our results from comparing all cases or familial cases with controls were consistent with those from PRACTICAL, in terms of direction of OR and significant p values, for 14 of the 15 variants (Table 2) corresponding to seven genes: *HNF1B*, *HOXB13*, *KLK3*, *MSMB*, *PCAT1*, *TERT*, and *ULK4*.

When comparing the aggressive cases with unaffected controls (Table 3), the variant rs138213197 in *HOXB13* showed the largest risk (OR = 23.36; 95% CI: 6.75, 80.87; Table 3), followed by the variant rs764430438 in the *PRSS3* gene (OR = 2.53; 95% CI: 1.88, 3.42) and the variant rs374242789 in the *MYCBP2* gene (OR = 1.73; 95% CI: 1.40, 2.13). The remaining variants in Table 3 showed significantly less frequent alternate alleles among aggressive cases compared with controls or nonaggressive cases. These included variants in the following genes: *EMSY*, *FAM114A1*, *MUC6*, *MYCBP2*, *RAPGEF4*, *RNASEH2B*, *THAP3*, *ULK4*, and *XPO7*.

3.4. Gene-level associations

The results from 1116 SKAT-O gene-level analyses are summarized in Figure 3 using Manhattan plots for each of the four comparisons. Genes detected by burden analyses were a subset of those detected by SKAT-O, except for the *ATM* gene that was borderline significant in the SKAT-O analyses yet crossed the significance threshold in the burden analyses. When considering all analyses, there were six genes that achieved statistical significance based on Bonferroni threshold $p < 5e-5$: *ATM* (156 variants), *BRCA2* (172 variants), *FAM111A* (35 variants), *HOXB13* (15 variants), *PABPC1* (29 variants), and *QK1* (11 variants). Details on the gene-level and single-variant analyses for the variants that had an allele frequency of at least 0.001 are provided in Supplementary Tables 6–8.

4. Discussion

Although multiple studies have demonstrated that PCa risk has a significant genetic component, it has been difficult to identify the contributing genes. Large-scale genome-wide association studies have suggested as many as 170 distinct risk loci, with many contributing a very small amount of risk [5]. To enrich for genetic causes of PCa, our study focused on cases with a strong family history of the disease selected from the ICPCG resource, which has collected high-risk families for several decades as well as cases with a more aggressive disease.

We successfully identified a genetic variant (rs138213197) in the *HOXB13* gene—our strongest association with PCa. This rare nonconservative substitution (G84E) has previously been reported and validated in multiple studies and different populations [20]. In addition, we found evidence for 10 genes previously reported to be associated with PCa: *ATM*, *BRCA2*, and *FAM111A* (detected by gene-level analyses), and *EMSY*, *HNFB1B*, *KLK3*, *MSMB*, *PCAT1*, *PRSS3*, and *TERT* (detected by single-variant analyses). Background information on these genes is provided in Supplementary Table 8. Further, we detected 10 genes whose association with PCa has not been reported previously: *PABPC1* and *QK1* (identified by gene-level analyses), and *FAM114A1*, *MUC6*, *MYCBP2*, *RAPGEF4*, *RNASEH2B*, *ULK4*, *XPO7*, and *THAP3* (identified by single-variant analyses). Background information on these genes is provided in Supplementary Table 9. Of these 10 novel genes, all but *PABPC1* and *ULK4* were primarily associated with the risk of aggressive PCa.

A major strength of our study is the number of PCa cases with a strong family history of disease or those with an aggressive phenotype. Our two-stage approach was a cost-efficient way to screen for less common variants that might be associated with PCa risk. Some limitations of our study include limited power to detect rare variants due to sample size restrictions [21] and the mixture of different capture kits in our stage-one samples. Although the controls we used for stage one were a mix of deidentified samples from prior WES studies conducted at the Mayo Clinic, with no information on PCa screening or family history of cancers, using them would not likely increase the chance of false associations, albeit power to detect genetic associations at stage one could have been diminished. For stage two, ICPCG groups selected controls by frequency matching age with their contributed cases, thereby adjusting for age, although missing detailed family history of some controls

limited control for family history. Despite these limitations, we detected 11 genes previously known to be associated with PCa, thus validating our study approach, as well as 10 novel genes that have not been reported previously. It will be important to replicate our novel findings, to assure that they are not false positives, as well as conduct functional studies to evaluate thoroughly the clinical relevance of our detected genes and their potential as therapeutic targets.

It is intriguing that many of our discovered genes have variants whose alternate allele is less frequent among aggressive PCa cases compared with controls, giving the impression that the alternate allele is protective and also implying that the common reference allele is a risk allele. However, it is possible that the analyzed variant with the common risk allele is negatively correlated with a high-risk unmeasured variant, in which case the unmeasured less common allele is the actual risk allele. We recognize that our definition of aggressive disease was pragmatic based on available clinical data and included some variables dependent on the type of treatment administered (ie, recurrence and time to death), which could introduce hidden biases. Additional studies linking factors related to aging and lifespan may aid in better understanding of our findings.

5. Conclusions

We identified 11 genes previously reported to be associated with PCa (*ATM*, *BRCA2*, *HOXB13*, *FAM111A*, *EMSY*, *HNFB*, *KLK3*, *MSMB*, *PCAT1*, *PRSS3*, and *TERT*) and an additional 10 novel genes (*PABPC1*, *QK1*, *FAM114A1*, *MUC6*, *MYCBP2*, *RAPGEF4*, *RNASEH2B*, *ULK4*, *XPO7*, and *THAP3*). Of these 10 novel genes, all but *PABPC1* and *ULK4* were primarily associated with the risk of aggressive PCa. Our results are consistent with other large-scale genomic studies that find multiple genes associated with the risk of PCa. Replication of our findings is needed to determine the value of these genes for screening purposes, and functional studies are needed to determine the therapeutic potential of these genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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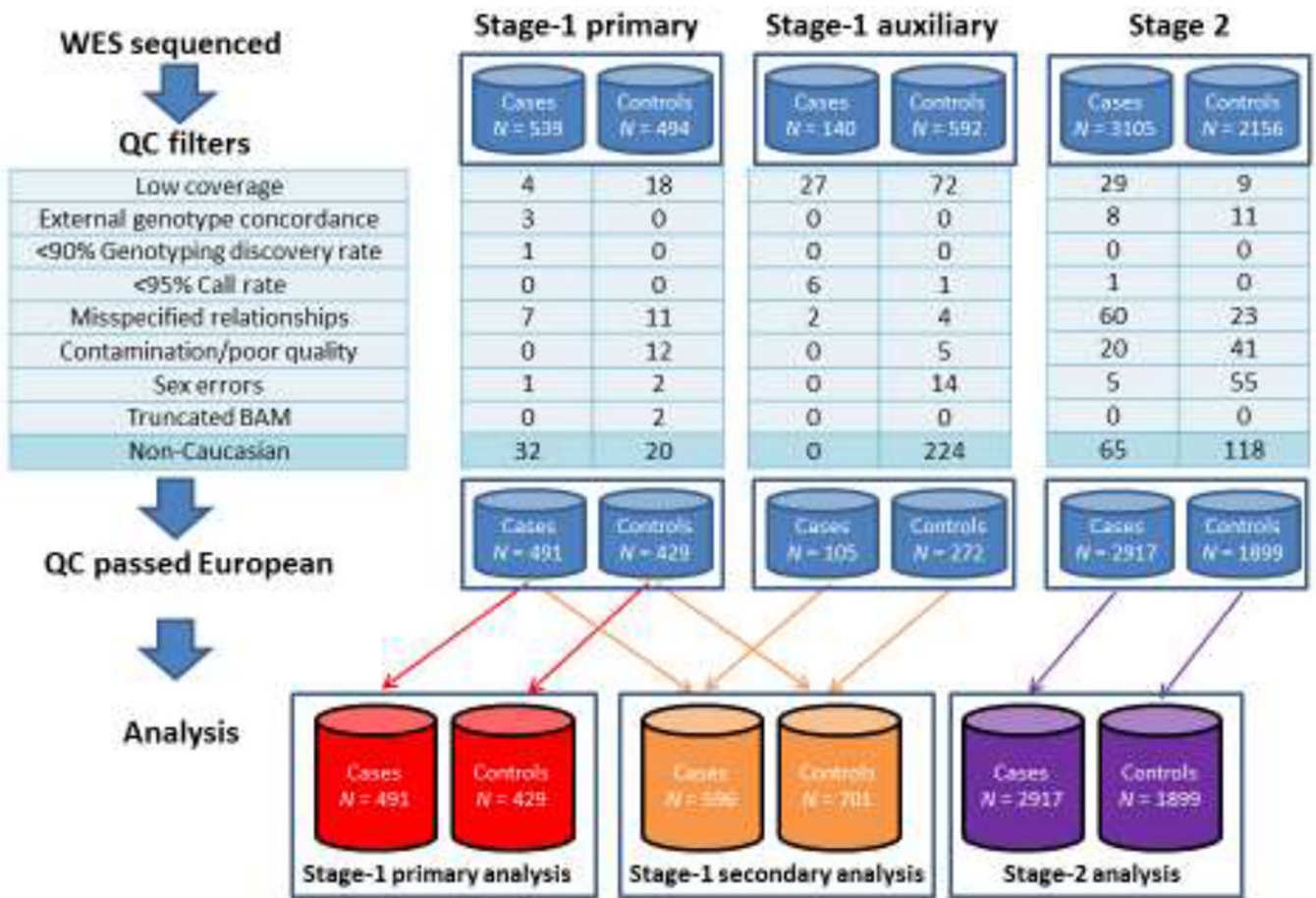


Fig. 1 – Flow of samples excluded from stage one (primary and auxiliary samples) and stage two, resulting in sample sizes used in analyses at the bottom of the figure. QC = quality control; WES = whole-exome sequencing.

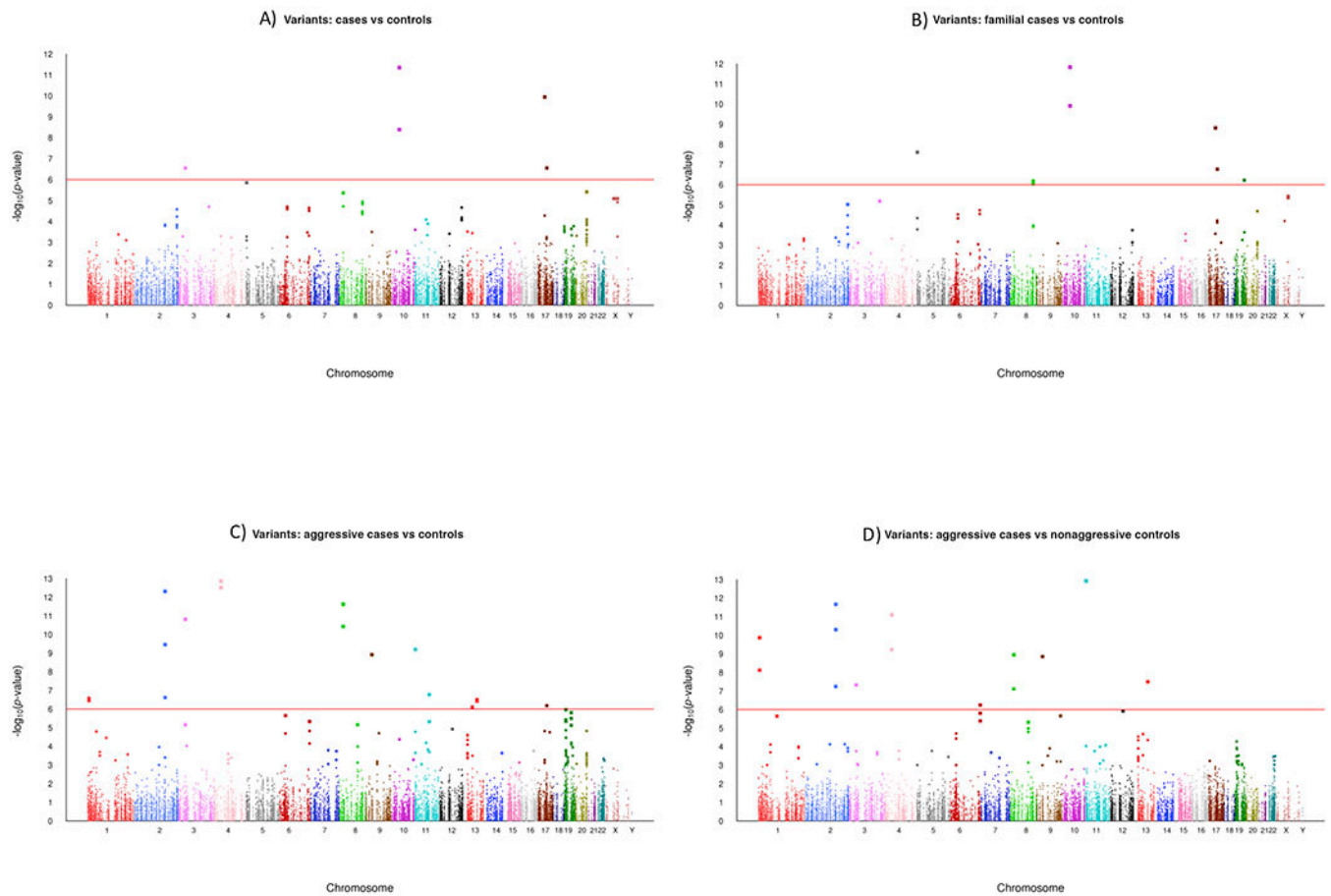


Fig. 2 –.

Manhattan plots of stage-two associations of single variants with PCa. The x axes show the chromosomal positions and the y axes show the $-\log_{10} p$ value. (A) All PCa cases versus controls. (B) PCa cases with a positive family history versus controls. (C) Aggressive PCa cases versus controls; (D) Aggressive PCa cases versus nonaggressive PCa cases. Statistical analyses based on log-additive effects of alternate alleles. PCa = prostate cancer.

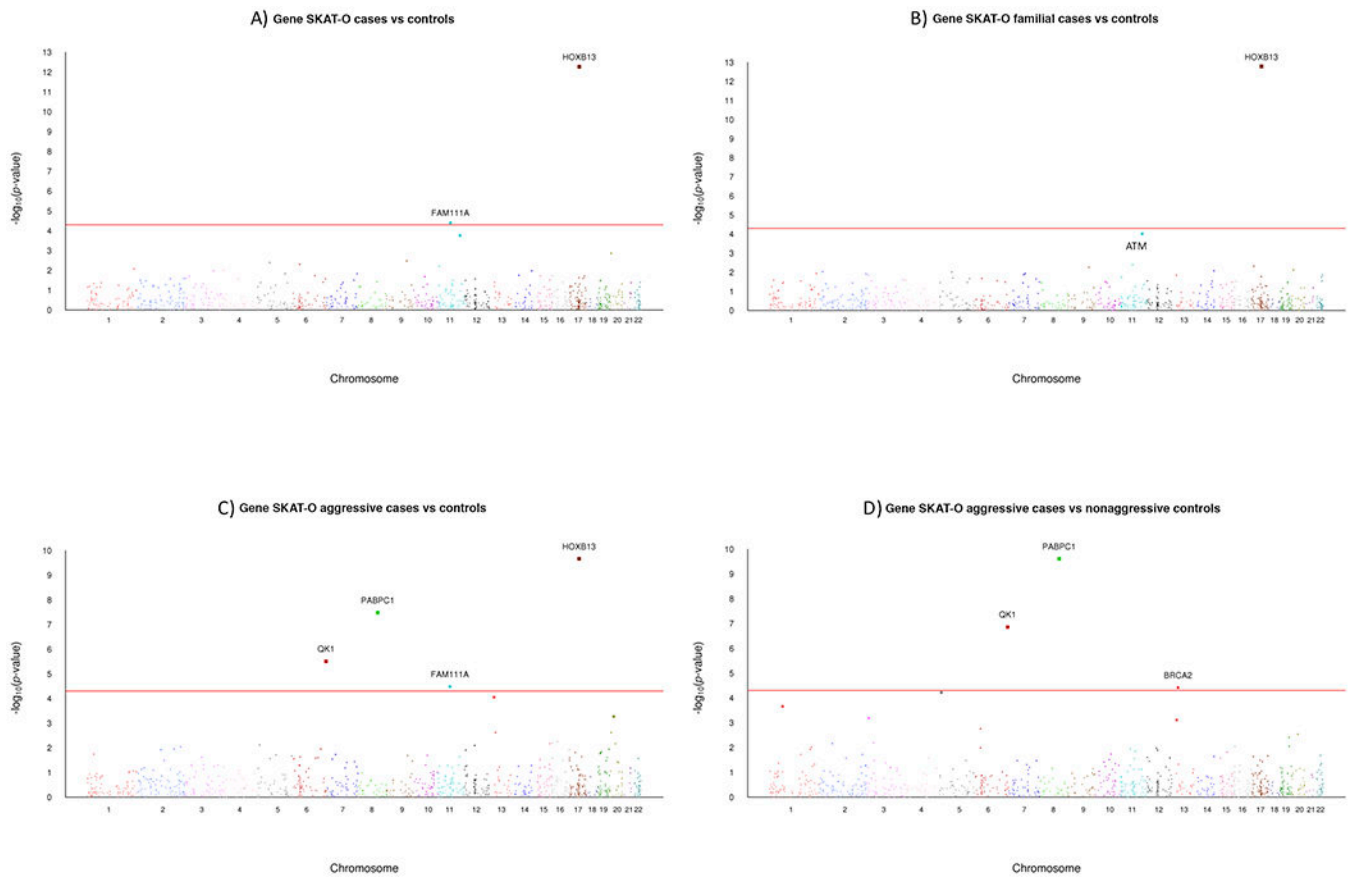


Fig. 3 –. Manhattan plots of stage-two SKAT-O gene-level associations with PCa. The x axes show the chromosomal positions and the y axes show the $-\log_{10} p$ value. (A) All PCa cases versus controls. (B) PCa cases with a positive family history versus controls. (C) Aggressive PCa cases versus controls. (D) Aggressive PCa cases versus nonaggressive PCa cases. PCa = prostate cancer.

Table 1 –
Clinical characteristics of stage-one and stage-two cases and controls

	Stage-one cases		Stage-two cases		Stage-two controls	
	491	%	2917	%	1899	%
Family history	491	100.0	1993	68.3	0	0
Positive						
Negative	0	0	924	31.7	1218	64.1
Unknown	0	0	0	0	681	35.9
Age at diagnosis (yr)						
50	26	5.3	233	8.0	170	9
51–65	264	53.8	1683	57.7	772	40.7
66–75	165	33.6	826	28.3	509	26.8
>75	30	6.1	157	5.4	374	19.7
Unknown	6	1.2	18	0.6	74	3.9
Gleason score						
<7	129	26.3	840	28.8		
7	108	22.0	695	23.8		
>8	59	12.0	698	23.9		
Unknown	195	39.7	684	23.4		
Stage						
T1	21	4.3	368	12.6		
T2	79	16.1	836	28.7		
T3	56	11.4	722	24.8		
T4	6	1.2	101	3.5		
Unknown	318	64.8	890	30.5		
Metastasis						
N0	52	10.6	1018	34.9		
N1	7	1.4	329	11.3		
N2	0	0.0	4	0.1		
NX	432	88.0	1566	53.7		
M0	68	13.8	1096	37.6		
M1	12	2.4	306	10.5		
MX	411	83.7	1515	51.9		
PSA						
<4	25	5.1	233	8.0		
4–19	157	32.0	979	33.6		
20–99	60	12.2	403	13.8		
100	11	2.2	194	6.7		
Unknown	238	48.5	1108	38.0		
ICPCG aggressiveness						
Insignificant	2	0.4	45	1.5		
Moderate	155	31.6	704	24.1		

	Stage-one cases		Stage-two cases		Stage-two controls	
	491	%	2917	%	1899	%
Aggressive	252	51.3	1905	65.3		
Unknown	82	16.7	263	9.0		
Aggressiveness score ^a						
Missing all clinical data	138	28.1	153	5.2		
0	223	45.4	1390	47.7		
1	36	7.3	116	4.0		
2	55	11.2	457	15.7		
3	11	2.2	176	6.0		
4	17	3.5	295	10.1		
5	3	0.6	89	3.1		
6	7	1.4	130	4.5		
7	1	0.2	26	0.9		
8	0	0.0	60	2.1		
9	0	0.0	10	0.3		
10	0	0.0	12	0.4		
12	0	0.0	3	0.1		

ICPCG = International Consortium for Prostate Cancer Genetics; PCa = prostate cancer; PSA = prostate-specific antigen.

^aAggressiveness score is the sum of scores for the following clinical factors: Gleason score 8–10 (+2 points), node status N1+ (+2 points), metastasis M1 (+2 points), tumor stage T4 (+2 points), PSA at diagnosis (PSA >20, +1 point; PSA >50, +2 points), systemic recurrence (+2 points), and time to PCa death (<5 yr, +2 points; 5–10 yr, +1 point).

Table 2 –

All cases and familial cases: stage-two single-variant analyses that detected significant associations, illustrating the gene and summary statistics for variants

Gene ^a	rsID	Allele	All cases (N = 2817) vs controls (N = 1899)			PRACTICAL ^b			Familial cases (N = 1993) vs controls (N = 1899)					
			REF	ALT	OR	95% CI	p value	OR	p value	OR	95% CI	p value	All cases	Familial cases
<i>HNF1B</i>	rs3216929	G	GCAGA	0.74	0.68, 0.81	1.13E-10	0.85	1.55E-81	0.74	0.67, 0.82	1.52E-09	0.3145	0.3084	0.3775
<i>HOXB13</i>	rs138213197	C	T	21.01	6.58, 67.15	2.78E-07	3.85	9.17E-63	22.44	6.99, 71.97	1.69E-07	0.0135	0.0161	0.0008
<i>KLK3</i>	rs17632542	T	C	0.72	0.61, 0.86	1.70E-04	0.74	6.69E-81	0.61	0.50, 0.74	5.96E-07	0.0591	0.0489	0.0777
<i>MSMB</i> (56 bp)	rs10993994	A	G	0.74	0.68, 0.80	4.41E-12	0.81	2.29E-147	0.71	0.65, 0.78	1.46E-12	0.5329	0.5258	0.6124
<i>MSMB</i> (238 bp)	rs12770171	G	A	1.38	1.24, 1.54	4.05E-09	1.18	3.71E-58	1.46	1.30, 1.63	1.23E-10	0.2384	0.2481	0.1854
<i>PCAT1</i>	rs1551515	T	A	0.82	0.75, 0.90	3.93E-05	0.88	1.55E-48	0.77	0.70, 0.86	7.67E-07	0.2691	0.2561	0.3099
	rs1551513	T	C	0.82	0.75, 0.90	3.31E-05	0.88	1.17E-48	0.77	0.70, 0.85	6.53E-07	0.2691	0.2561	0.3104
	rs4473999	C	T	0.82	0.75, 0.90	3.93E-05	0.88	2.16E-48	0.77	0.70, 0.86	7.67E-07	0.2691	0.2561	0.3099
	rs9656964	C	G	0.82	0.75, 0.90	3.93E-05	0.88	2.80E-48	0.77	0.70, 0.86	7.67E-07	0.2691	0.2561	0.3099
	rs17762938	T	C	0.82	0.75, 0.90	3.31E-05	0.88	3.42E-48	0.77	0.70, 0.85	6.53E-07	0.2691	0.2561	0.3104
	rs7823297	C	T	0.82	0.75, 0.90	3.31E-05	0.88	2.12E-48	0.77	0.70, 0.85	6.53E-07	0.2691	0.2561	0.3104
<i>PCAT1</i> (135 bp)	rs6651240	A	T	0.82	0.75, 0.90	4.44E-05	0.88	1.62E-48	0.77	0.70, 0.86	9.02E-07	0.2691	0.2561	0.3096
	rs4573233	G	A	0.82	0.75, 0.90	3.93E-05	0.88	1.65E-48	0.77	0.70, 0.86	7.67E-07	0.2691	0.2561	0.3099
<i>TERT</i> (909 bp)	rs7712562	A	G	1.35	1.20, 1.53	1.40E-06	1.18	2.24E-48	1.47	1.29, 1.69	2.45E-08	0.8768	0.8861	0.8420
<i>ULK4</i>	rs202114865	G	A	0.62	0.52, 0.75	2.81E-07	NA	NA	0.80	0.66, 0.96	0.02	0.0502	0.0686	0.0796

CI = confidence interval; NA = genetic variants from this current study were not genotyped in PRACTICAL; OR = odds ratio.

^a Variants outside a gene have their base-pair distance to the nearest gene in parentheses, based on Genome Reference Consortium Human Build 38.

^b PRACTICAL OR and p value provided by the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium, based on genome-wide analyses of >140 000 men [19].

