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A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer

Genome-wide association studies (GWAS) have identified 76 variants associated with prostate cancer risk predominantly in populations of European ancestry. To identify additional susceptibility loci for this common cancer, we conducted a meta-analysis of >10 million SNPs in 43,303 prostate cancer cases and 43,737 controls from studies in populations of European, African, Japanese and Latino ancestry. Twenty-three new susceptibility loci were identified at association $P < 5 \times 10^{-8}$; 15 variants were identified among men of European ancestry, 7 were identified in multi-ancestry analyses and 1 was associated with early-onset prostate cancer. These 23 variants, in combination with known prostate cancer risk variants, explain 33% of the familial risk for this disease in European-ancestry populations. These findings provide new regions for investigation into the pathogenesis of prostate cancer and demonstrate the usefulness of combining ancestrally diverse populations to discover risk loci for disease.

Prostate cancer is the most common non-skin cancer in men in the Western world, and epidemiological studies have shown strong evidence for genetic predisposition to prostate cancer, based on two of the most important factors—ancestry and family history. GWAS have identified 76 common risk loci (reviewed in ref. 1); however, over 1,000 additional common SNPs are estimated to contribute prostate cancer risk[2,3]. Previous prostate cancer GWAS have been conducted primarily in populations of European ancestry[2,4–7], with the majority of risk loci that have been discovered also found to be associated with prostate cancer risk in other populations[8,9]. The generalizability of risk associations for a large fraction of loci suggests that combining GWAS across ancestral populations could increase power to detect risk loci that are shared among diverse populations.

To search for additional genetic risk factors for prostate cancer, we combined data from studies with existing high-density SNP genotyping in prostate cancer GWAS discovery or replication efforts in the following populations: European ancestry (34,379 cases and 33,164 controls from UK/Australia[4], Cancer of the Prostate in Sweden (CAPS)[10], the Breast and Prostate Cancer Cohort Consortium (BPC3)[6], PEGASUS and iCOGS/PRACTICAL[2]); African ancestry (5,327 cases and 5,136 controls from the African Ancestry Prostate Cancer GWAS Consortium (AAPC)[11] and the Ghana Prostate Study[12]); Japanese ancestry (2,563 cases and 4,391 controls from a GWAS in the Japanese in the Multiethnic Cohort (MEC)[8] and Biobank Japan[13,14]); and Latino ancestry (1,034 cases and 1,046 controls from the MEC[8]). Imputation was performed in each study using a cosmopolitan reference panel from the 1000 Genomes Project (March 2012). Across the various studies, 5.8–16.8 million genotyped and imputed SNPs, as well as insertion-deletion variants with a frequency of ≥1%, were examined in association with prostate cancer risk (Online Methods, **Supplementary Tables 1–3** and **Supplementary Note**).

We first conducted ancestry-specific meta-analyses, with the large European-ancestry sample providing the strongest statistical power for the discovery of new loci, followed by a multi-ancestry meta-analysis of all populations, to identify additional loci with pan-ancestry effects. For these primary analyses, we employed a *P*-value threshold of 5×10^{-8} to define genome-wide significance. Secondary meta-analyses focused on (i) aggressive disease in the large European-ancestry sample; (ii) aggressive disease in the combined multi-ancestry sample; and (iii) prostate cancer diagnosed at ≤55 years of age in the European-ancestry sample only. Aggressive prostate cancer was defined by a Gleason score of ≥8, a disease stage of 'distant', a prostate-specific antigen (PSA) level of >100 ng/ml or death from prostate cancer. For these two secondary phenotypes, we used a more stringent *P*-value threshold of $5 \times 10^{-8}/2 = 2.5 \times 10^{-8}$ for genome-wide significance. In each study, we tested for gene dosage effects via a 1-degree-of-freedom test for trend from logistic regression models adjusted for genetic ancestry (principal components). We observed little evidence of inflation in the test statistics in any single study or population ($\lambda/\lambda_{1,000}$ values: European ancestry, 1.14/1.00; African ancestry, 1.03/1.01; Japanese ancestry, 1.06/1.02; Latino ancestry, 1.00/1.00) or in the multi-ancestry analysis ($\lambda = 1.08$ and $\lambda_{1,000} = 1.00$; Online Methods, **Supplementary Fig. 1** and **Supplementary Table 4**).

In the meta-analysis of the European-ancestry studies, 20 new signals in 18 regions at least 500 kb away from previously associated loci were observed to be associated with prostate cancer risk at $P < 5 \times 10^{-8}$ (**Fig. 1** and **Supplementary Figs. 2** and **3**). The most significant associations in each region were observed with imputed variants, and we were able to confirm the imputed genotypes for 15 variants that had high imputation information scores (r^2 range of 0.76–1) through direct genotyping or sequencing across multiple studies (**Table 1**, Online Methods and **Supplementary Tables 5–8**). Two of the variants were located within 370 kb of each other on chromosome Xq13 and were determined to be independent signals on the basis of conditional analyses (rs6625711, $P = 6.1 \times 10^{-10}$ and rs4844289, $P = 2.0 \times 10^{-8}$; linkage disequilibrium (LD) $r^2 < 0.01$ in the European-ancestry (EUR) 1000 Genomes Project populations; **Supplementary Table 9**).

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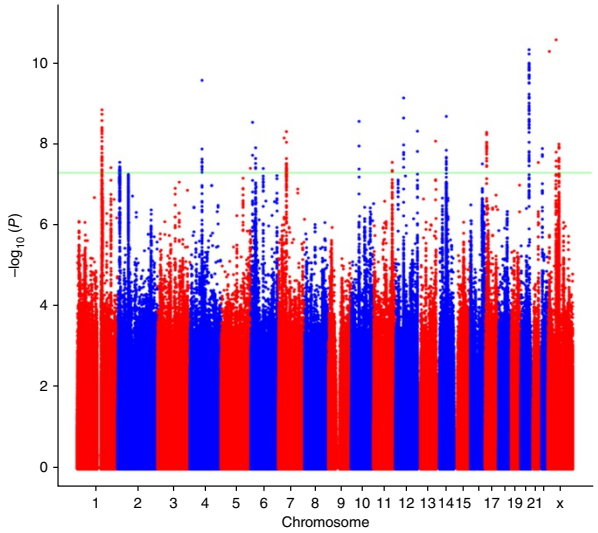
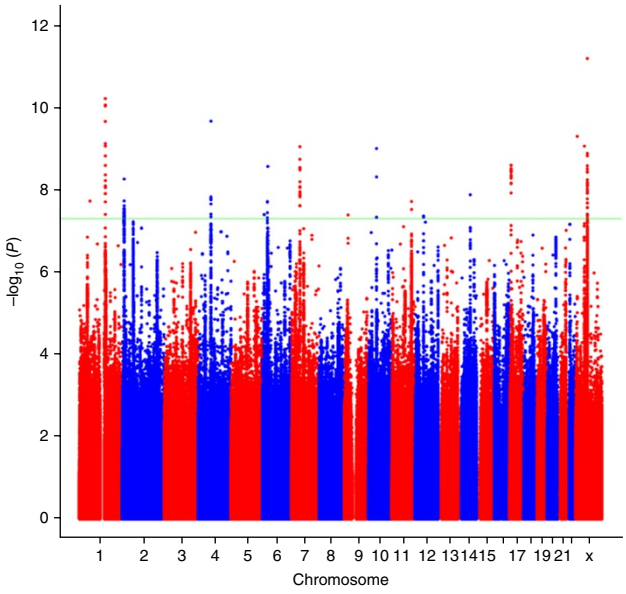
Figure 1 Manhattan plot of genotyped and imputed results from the European-ancestry meta-analysis of overall prostate cancer risk. All SNPs within 500 kb of known GWAS SNPs are omitted. The green line represents $P = 5 \times 10^{-8}$. This figure shows all new variants with $P < 5 \times 10^{-8}$, regardless of confirmation results (one signal on chromosome 1, one signal on chromosome 4, one signal on chromosome 17 and two signals on the X chromosome were not confirmed). Many of the new signals are in close proximity to one another on the same chromosome (**Supplementary Table 6**).

All 15 variants were common, with minor allele frequencies (MAFs) of ≥0.09 in the European-ancestry population, and all but 3 (rs80130819 at 12q13, rs76939039 at 10q11 and rs17694493 at 9p21) were also common (MAF ≥ 0.05) in populations of African, Japanese and Latino ancestry. Evidence of heterogeneity in the per-allele odds ratio (OR) was noted with four variants (P_{het} = 0.01 to 8.4 × 10^{-6}; rs17599629 at 1q21, rs115306967 at 6p21, rs17694493 at 9p21 and rs6625711 at Xq13). Four of the 15 variants (rs10009409 at 4q13, rs4713266 at 6p24, rs80130819 at 12q13 and rs2807031 at Xp11) had directional effects that were consistent with those for men of European ancestry and were nominally statistically significant ($P < 0.05$) in at least one other population (**Table 1**), and, for three SNPs, combining data across populations strengthened the statistical significance of the association (**Table 1**). In the large European-ancestry sample, we also confirmed the reported signal at 22q13 represented by variant rs58133635 ($P = 5.8 \times 10^{-9}$; $r^2 = 0.74$ with rs9623117 in 1000 Genomes Project EUR populations; **Supplementary Figs. 2** and **3**)[15].

No new risk loci were identified in ancestry-specific analyses within the populations of African, Japanese or Latino ancestry, possibly owing to a lack of power (**Supplementary Fig. 2**). However, in combining results across populations in a multi-ancestry meta-analysis (43,303 cases and 43,737 controls), 11 additional variants were identified in association with prostate cancer risk in new risk regions at $P < 5 \times 10^{-8}$ (**Fig. 2**, **Table 1** and **Supplementary Table 5**). We confirmed the imputed genotypes for seven variants that had high imputation information scores (r^2 range of 0.81–1) through additional genotyping and sequencing (Online Methods and **Supplementary Tables 6–8**). All seven variants were nominally associated with risk ($P < 0.05$) in at least one of the non-European-ancestry populations, and per-allele effects were directionally consistent across all four populations for six of the seven variants. All variants had MAF ≥ 0.05 in all four populations, and no significant evidence of population heterogeneity was noted with any of these seven variants (**Table 1**).





In secondary GWAS analyses, we detected an association with variant rs636291 at 1p36 (risk allele frequency = 0.16; OR = 1.18; $P = 2.1 \times 10^{-8}$; **Table 1**) and early-onset disease among men of European ancestry (4,147 cases ≤55 years of age versus all controls, $n = 27{,}212$). The association with this variant was weaker for cases diagnosed at >55 years of age (23,564 cases versus all controls, $n = 27{,}212$: OR = 1.04; $P = 0.004$; $P_{het} = 2.2 \times 10^{-4}$; **Supplementary Table 10**). We did not detect any genome-wide significant associations with aggressive disease in the European-ancestry population ($n = 7{,}903$ cases) or in the combined multi-ancestry sample ($n = 10{,}209$ cases; **Supplementary Fig. 4**).

For the 23 new risk variants (15 from the European-ancestry analysis, 7 from the multi-ancestry analysis and 1 from the early-onset analysis), the per-allele effects ranged from 1.06 to 1.14 and were consistent with log-additive effects (**Supplementary Table 11**). The association of each variant was noted for both aggressive and non-aggressive prostate cancer (**Supplementary Table 12**); for only one variant, rs7153648 at 14q23, there was suggestive evidence of a difference by disease severity (OR = 1.17 for aggressive disease and OR = 1.09 for non-aggressive disease; $P_{het} = 0.03$). These results confirm what has been observed in prostate cancer GWAS thus far; risk loci seem to confer risk for prostate cancer overall and do not discriminate between aggressive and indolent disease. In analyses stratified by age, 17 of the 23 variants demonstrated larger effects at younger ages (≤55 versus >55 years), although only 6 had evidence of a significant difference ($P < 0.05$) (**Supplementary Table 10**). Only 2 of the 23 variants were modestly associated with PSA levels among controls (rs9287719 at 2p25, $P = 0.03$ and rs115306967 at 6p21, $P = 0.05$; **Supplementary Table 13**).

Of the 23 new risk variants, 13 were located in intronic regions of genes and 2 were correlated with nonsynonymous variants in adjacent genes (rs12051443 at 16q22, $r^2 = 0.98$ with rs4788821 (encoding p.Glu60Lys) in *MARVELD3* and rs2238776 at 22q11, $r^2 = 0.67$

Figure 2 Manhattan plot of the results from the multi-ancestry meta-analysis of overall prostate cancer risk. All SNPs within 500 kb of known GWAS SNPs are omitted. The green line represents $P = 5 \times 10^{-8}$. This figure shows all new variants with $P < 5 \times 10^{-8}$, regardless of the confirmation results, as well as signals that were reported in the European-ancestry meta-analysis that also reached $P = 5 \times 10^{-8}$ in the multi-ancestry meta-analysis (**Table 1** and **Supplementary Table 6**).

Table 1 Association results for 23 new risk variants for prostate cancer

				European (35,093 cases, 34,599 controls)			African (5,327 cases, 5,136 controls)			Japanese (2,563 cases, 4,391 controls)			Latino (1,034 cases, 1,046 controls)			Multi-ancestry (44,107 cases, 45,172 controls)		
SNP ID	Chromosome, position[b]	Nearby gene	Alleles[c]	OR	*P*	RAF[a]	OR	*P*	RAF[a]	OR	*P*	RAF[a]	OR	*P*	RAF[a]	OR	*P*	P_{het}[d]
Risk loci identified in European-ancestry meta-analysis																		
rs17599629	1q21, 150,658,287	*GOLPH3L*	G/A	1.10	5.9×10^{-11}	0.22	1.09	0.13	0.08	0.97	0.48	0.18	0.92	0.23	0.26	1.08	2.6×10^{-9}	8.6×10^{-3}
rs9287719	2p25, 10,710,730	*NOL10*	C/T	1.07	1.8×10^{-8}	0.46	1.00	0.98	0.26	1.07	0.06	0.42	1.00	0.99	0.45	1.06	2.8×10^{-8}	0.21
rs10009409	4q13, 73,855,253	*COX18*	T/C	1.09	2.1×10^{-10}	0.32	1.02	0.56	0.35	1.10	0.02	0.56	1.00	0.96	0.50	1.08	2.3×10^{-10}	0.12
rs4713266	6p24, 11,219,030	*NEDD9*	C/T	1.07	3.9×10^{-8}	0.52	1.07	0.03	0.78	1.06	0.21	0.23	1.02	0.81	0.40	1.06	2.9×10^{-9}	0.89
rs115457135	6p22, 30,073,776	*TRIM31*	A/G	1.08	1.9×10^{-8}	0.22	1.01	0.91	0.15	1.01	0.87	0.27	1.03	0.69	0.26	1.07	1.4×10^{-7}	0.25
rs115306967	6p21, 32,400,939	*HLA-DRB6*	G/C	1.08	2.7×10^{-9}	0.65	0.92	0.02	0.81	1.09	0.29	0.81	1.01	0.86	0.76	1.06	8.7×10^{-7}	5.2×10^{-4}
rs56232506	7p12, 47,437,244	*TNS3*	A/G	1.07	1.8×10^{-9}	0.45	0.99	0.76	0.13	1.00	0.99	0.31	1.11	0.12	0.52	1.06	8.9×10^{-9}	0.13
rs17694493	9p21, 22,041,998	*CDKN2B-AS1*	G/C	1.10	4.0×10^{-8}	0.14	1.00	0.97	0.11	1.04	0.78	0.02	0.78	0.04	0.08	1.08	1.1×10^{-6}	0.01
rs76934034	10q11, 46,082,985	*MARCH8*	T/C	1.14	4.8×10^{-9}	0.91	0.98	0.88	0.98			[e]	1.06	0.64	0.92	1.13	1.1×10^{-8}	0.39
rs11214775	11q23, 113,807,181	*HTR3B*	G/A	1.08	3.0×10^{-8}	0.71	1.04	0.22	0.71	1.02	0.70	0.71	1.06	0.47	0.81	1.07	4.5×10^{-8}	0.39
rs80130819	12q13, 48,419,618	*RP1-228P16.4*	A/C	1.13	4.3×10^{-8}	0.91	1.28	0.02	0.98			[e]	1.22	0.17	0.94	1.14	2.2×10^{-9}	0.44
rs8014671	14q24, 71,092,256	*TTC9*	G/A	1.07	1.3×10^{-8}	0.59	1.00	0.85	0.46	1.03	0.40	0.36	0.98	0.75	0.60	1.06	2.5×10^{-7}	0.09
rs2807031	Xp11, 52,896,949	*XAGE3*	C/T	1.07	8.5×10^{-10}	0.18	1.06	0.02	0.22	1.17	0.16	0.05	1.02	0.82	0.09	1.07	2.7×10^{-11}	0.77
rs6625711	Xq13, 70,139,850	*SLC7A*	A/T	1.07	6.3×10^{-12}	0.41	0.92	0.004	0.83	0.99	0.86	0.48	0.97	0.52	0.61	1.04	6.4×10^{-7}	8.4×10^{-6}
rs4844289	Xq13, 70,407,983	*NLGN3-BCYRN1*	G/A	1.05	1.3×10^{-9}	0.39	0.99	0.58	0.68	1.00	0.99	0.72	1.09	0.05	0.59	1.04	8.9×10^{-8}	0.04
Risk loci identified in multi-ancestry meta-analysis																		
rs1775148	1q32, 205,757,824	*SLC41A1*	C/T	1.06	1.0×10^{-5}	0.27	1.06	0.04	0.63	1.12	2.0×10^{-3}	0.52	1.02	0.82	0.66	1.06	3.8×10^{-8}	0.40
rs9443189	6q14, 76,495,882	*MYO6*	G/A	1.07	5.2×10^{-5}	0.14	1.11	4.5×10^{-4}	0.53	1.07	0.08	0.32	1.01	0.93	0.14	1.08	3.9×10^{-8}	0.64
rs7153648	14q23, 61,122,526	*SIX1*	C/G	1.09	6.8×10^{-4}	0.06	1.11	8.8×10^{-4}	0.34	1.17	1.4×10^{-4}	0.30	1.12	0.27	0.10	1.11	2.0×10^{-9}	0.50
rs12051443	16q22, 71,691,329	*PHLPP2*	A/G	1.06	1.1×10^{-5}	0.34	1.09	0.01	0.25	1.10	0.02	0.65	1.06	0.34	0.50	1.06	3.0×10^{-8}	0.69
rs12480328	20q13, 49,527,922	*ADNP*	T/C	1.13	1.6×10^{-7}	0.93	1.14	2.3×10^{-3}	0.87	1.30	7.7×10^{-4}	0.94	0.97	0.81	0.93	1.13	4.6×10^{-11}	0.18
rs1041449	21q22, 42,901,421	*TMPRSS2*	G/A	1.06	2.6×10^{-7}	0.44	1.07	0.03	0.39	1.02	0.79	0.12	1.03	0.65	0.44	1.06	2.8×10^{-8}	0.84
rs2238776	22q11, 19,757,892	*TBX1*	G/A	1.09	1.6×10^{-7}	0.80	0.98	0.81	0.95	1.08	0.03	0.60	1.09	0.22	0.73	1.08	1.8×10^{-8}	0.60
Risk loci identified in early-onset meta-analysis[f]																		
rs636291	1p35, 10,556,097	*PEX14*	A/G	1.18	2.1×10^{-8}	0.16												

[a]Risk allele frequency. [b]Genome Build 37. [c]Risk allele/other allele. [d]*P* value for effect heterogeneity across populations. [e]MAF of <1%. [f]Analysis was limited to European-ancestry populations as only small numbers of early-onset cases (≤55 years of age) were available in the other populations.

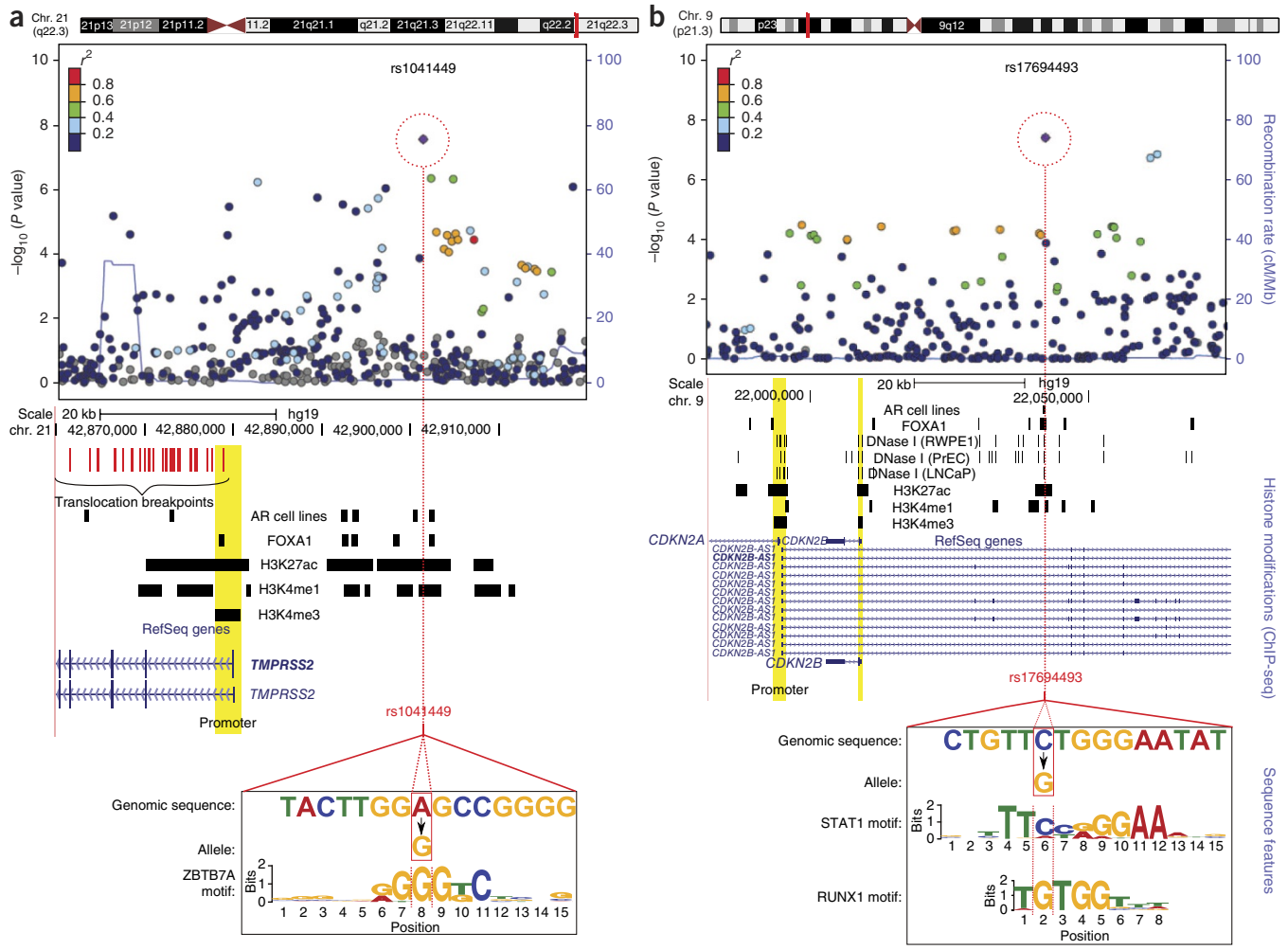


Figure 3 Regional plots of two new genome-wide significant loci associated with prostate cancer risk. (**a**,**b**) Plots are shown for rs1041449 at 21q22 (*TMPRSS2* region) (**a**) and rs17694493 at 9p21 (*CDKN2B-AS1* region) (**b**). Top, SNPs are plotted by their position within 500 kb of the index SNP (purple diamond) on the chromosome against their association ($-\log_{10} P$) with prostate cancer from the multi-ancestry meta-analysis (rs1041449) and European-ancestry meta-analysis (rs17694493). SNPs surrounding the index SNP are colored to indicate the local LD structure using pairwise r^2 data from the EUR panel of the 1000 Genomes Project (March 2012). Middle, significant peaks from transcription factor and histone modification chromatin immunoprecipitation and sequencing (ChIP-seq) experiments in the same genomic windows (Online Methods). All ChIP-seq analysis was performed in LNCaP cells unless otherwise indicated. H3K27ac, acetylation of histone H3 at lysine 27; H3K4me1, monomethylation of histone H3 at lysine 4; H3K4me3; trimethylation of histone H3 at lysine 4. Promoter regions are highlighted in yellow. Bottom, the genomic sequence (outlined by the black box) surrounding each SNP (red box) aligned to a LOGO graphic representing the proposed motif disruption.

with rs72646967 (encoding p.Asn397His) in *TBX1*). On the basis of functional annotations of transcription factor occupancy, response element disruption, histone marks and DNase I–sensitive regions in prostate cancer cell lines (Online Methods), 12 of the risk variants were found to be either directly located within putative functional elements or were correlated (at $r^2 > 0.9$ in 1000 Genomes Project EUR populations) with such variants (**Supplementary Table 14**). Using gene expression data for 145 prostate cancer tumor samples from The Cancer Genome Atlas (TCGA) (Online Methods), we also examined the *cis* associations between the index SNPs and the expression of gene transcripts within the 1-Mb region centered on each SNP. Among the 23 loci, 5 *cis* associations were observed, albeit the associations were modest (Online Methods and **Supplementary Table 14**).

A number of the new susceptibility regions were located in close proximity to genes that either have an established role or have been directly implicated in cancer (**Table 1**). The most notable was rs1041449 on chromosome 21q22, which was situated 20 kb 5′ to the *TMPRSS2* gene, which encodes a member of a serine protease family[16]. Expression of *TMPRSS2* is highly specific to prostate tissue, and chromosomal translocation resulting in fusion of the *TMPRSS2* promoter-enhancer region with the ETS transcription factors *ERG* and *ETV1* are frequently observed in prostate cancer[17]. In analyzing the data of 552 tumors characterized for the *TMPRSS2-ERG* fusion (46% positive) (Online Methods), we found no evidence of an association between the risk allele and fusion status ($P = 0.53$; **Supplementary Table 15**). The variant rs1041449 was located within a number of histone marks and transcription factor occupancy sites in the predicted enhancer region of *TMPRSS2* (**Fig. 3**); however, we found little evidence that this variant influences *TMPRSS2* expression in prostate tumors ($n = 244$; $P = 0.60$) or in normal prostate tissue ($n = 87$; $P = 0.62$) (Online Methods).

Another notable region was on chromosome 9p21. The risk variant, rs17694493, was intronic in *CDKN2B-AS1*, which encodes a long noncoding RNA, *ANRIL*, and is part of the *CDKN2B-CDKN2A*

gene cluster (**Fig. 3**). The region contains highly penetrant alleles for familial melanoma and common susceptibility alleles for melanoma, breast cancer, basal cell carcinoma, lung cancer and glioma[18–24]. The index SNP, rs17694493, fell within chromatin biofeatures and was predicted to disrupt two transcription factor motifs (STAT1 and RUNX1), suggesting that it might have a functional effect on the regulation of the *CDKN2B-AS1* or *CDKN2B-CDKN2A* genes (**Fig. 3** and **Supplementary Table 14**); however, the variant was not found to be strongly associated with the expression of either *CDKN2A* ($P = 0.19$) or *CDKN2B* ($P = 0.40$) in the 145 TCGA prostate tumors.

Variant rs4713266 on chromosome 6p25 was located in intron 1 of *NEDD9*, a gene that participates in cell adhesion, motility, the cell cycle and apoptosis and has been implicated in the progression and metastasis of several cancer types[25]. Variant rs9443189 on chromosome 6q14 was intronic in *MYO6*, a modulator of androgen-dependent gene expression that has been found to be overexpressed in prostate cancer tumors and to enhance prostate tumor growth and metastasis[26–28]. Variant rs636291 on chromosome 1p36, which we found in association with early-onset prostate cancer, was located in intron 2 of *PEX14* and was correlated with rs616488 ($r^2 = 0.66$ in 1000 Genomes Project EUR populations), a variant reported in a GWAS of breast cancer[29].

The identification of new risk loci for prostate cancer through a multi-ancestry analysis demonstrates the value of combining genetic data across populations to increase statistical power for discovery. As further support for conducting multi-ancestry analyses, we examined the genome-wide evidence for consistency in the direction of the allelic associations between populations. Excluding SNPs within 500 kb of index signals at known loci ($n = 77$), we defined independent signals ($r^2 < 0.2$) for the European-ancestry population of nominal significance at various *P*-value thresholds between $<1 \times 10^{-2}$ and 1×10^{-5}. For the sets of SNPs defined for men of European ancestry, 53–64% had ORs that were directionally concordant for African ($P = 0.04$–0.003, dependent on the *P*-value threshold bin), Asian ($P = 0.31$–0.02) or Latino ($P = 0.04$–0.002) men with the ORs in Europeans. This same observation remained once we removed the 23 risk loci identified by the current study (**Supplementary Fig. 5**). The excess of directionally consistent associations between populations implies that additional common risk loci for prostate cancer may be identified through discovery efforts in multi-ancestry studies.

These 23 new loci (including rs58133635 at 22q13)[15] bring the total number of susceptibility variants for prostate cancer to 100 (**Supplementary Table 16**). In total, we estimate that these 100 risk loci account for ~33% of the familial risk of prostate cancer in populations of European ancestry, with these additional 23 loci, with effect sizes ranging from 1.06 to 1.14, explaining ~3.1% of the familial risk (Online Methods). On the basis of a polygenic risk score comprising these 100 variants for men of European ancestry (Online Methods), the top 10% of men in the highest risk stratum have a 2.9-fold (95% confidence interval (CI) = 2.8- to 3.1-fold) increase in relative risk of prostate cancer and the top 1% of men have a 5.7-fold (95% CI = 4.8- to 6.6-fold) increase in relative risk in comparison with the population average (**Supplementary Table 17**). The top 10% of men are at a relative risk compared with the average of the population where it will be important to examine whether targeted screening based on family history genetic risk might reduce the overdiagnosis of indolent disease, which is a main limitation of screening by PSA. Our findings demonstrate the importance of conducting large-scale genetic studies in diverse populations for the discovery of new risk loci that continue to provide new insights into disease mechanisms for complex traits.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

A full list of acknowledgments is detailed in the **Supplementary Note**.

AUTHOR CONTRIBUTIONS

A.H., R.A.E., Z.K.-J., D.F.E., B.E.H., S.J.C., S.I.B., P. Kraft, F.W., H.N. and M.B.C. designed the study. C.A.H., Z.K.-J., A.A.A.O. and R.A.E. wrote the manuscript. A.A.A.O., F.S., Y.H., Z.W., P.W., C. Chen, E.S., D.L., K.R., T.D., S.J.-L. and K.L.P. performed the statistical analysis. D.O.S. and D.V.C. provided statistical support. D.J. Hazelett, A. Stram, K.P., X.S., G.A.C., Q.L. and M.L.F. provided bioinformatics support as well as functional annotation and QTL data. L.C.P., K.P., L.X., L.B. and M.T. conducted the genotyping and sequencing. S.B., C.G. and M. Ahmed managed the PRACTICAL and COGS database. K.G. and M.G. managed the UKGPCS database. The following authors provided samples and/or data to the study and commented on the manuscript: J.T., T.V., K.A.L., K.-T.K., S.K.M., D.J.S., S.H., B.K., A.H.C., A.P.C., D.W., W.K., A.W.P. and E.M.G. L.N.K., L.L.M. and B.E.H. are principal investigators of the MEC. J.X. and S.L.Z. are principal investigators of NCPCS. R.C.T., T.J.K., A. Siddiq and F.C. are EPIC investigators. E.R. is the principal investigator of EPIC. A. Takahashi, M.K. and H.N. are principal investigators of BBJ. J.L.S. is the principal investigator of KCPCS; S.K. coordinated data collection. V.L.S. and W.R.D. are investigators and S.M.G. is the principal investigator of CPSII. S.S.S. and C.P. are principal investigators of the MDA prostate cancer studies. S.L., D.J. Hunter, P. Kraft, L.M., E.L.G., J.M. and M. Stampfer are coinvestigators of the Harvard cohorts and BPC3. H.G. is principal investigator of CAPS and STHLM1. M. Aly and F.W. are investigators of CAPS. W.B.I. is the principal investigator of the IPCG study. A.S.K. is the principal investigator of WUGS. E.M.J. is the principal investigator of SFPCS. S.A.I. is the principal investigator of LAAPC. R.A.K. and A.B.M. are investigators of DCPC. W.B., L.B.S. and W.Z. are principal investigators of SCCS. D.A. and J.V. are principal investigators and S.W. is the study coordinator of ATBC. B.N., J. Carpten, C.L., S.-Y.W. and A. Hennis are principal investigators of PCBP. B.A.R. and C.N.-D. are principal investigators of GECAP. J.S.W. and G.C. are principal investigators of CaP Genes. D.S. is the program officer of GAME-ON. P.J.G., E.A.K., A.W.H. and L.C. are investigators of SELECT. F.C.H., J.D. and D.E.N. are principal investigators of ProtecT. E.D.Y., Y.T., R.B.B., A.A.A., E.T., A. Truelove, S.N. and A.W.H. are investigators of the Ghana Prostate Study. S.J.C., S.I.B., R.N.H., M.J.M., M.Y., C.C.C., A. Hutchinson and K.Y. are investigators of PLCO. M.R.T. is the principal investigator and P. Paulo and S.M. are investigators of IPO-Porto. J.B., J. Clements and A. Spurdle are principal investigators of QLD. R.K. and C. Slavov are the principal investigators and V.M. is an investigator of PCMUS. J.P., T.S. and H.-Y.L. are the investigators of the MOFFITT study. L.C.-A. is the principal investigator of the Utah study. C. Cybulski is the principal investigator of the Poland study. S.N.T. is the principal investigator of the Mayo study. P. Pharoah and N.P. are investigators of SEARCH. C.M. is the principal investigator of ULM; M.L., K.H. and A.E.R. are investigators of ULM. M.W., S.F.N., B.G.N., P. Klarskov, M.A.R. and P.I. are the principal investigators of CPCS1 and CPCS2. T.W., A.A. and T.L.J.T. are investigators and J.S. is the principal investigator of TAMPERE. K.M. is a UKGPCS investigator. H.B. is the principal investigator, A.K.D. prepared the data and C. Stegmaier coordinated the data collection of the ESTHER study. G.G.G. and G.S. are the principal investigators of MCCS; M. Southey is an investigator and H.P., A.M. and A.M.K. are principal investigators of the PPF-UNIS study.

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The authors declare no competing financial interests.

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ONLINE METHODS

Primary genotype data were used from 4 prostate cancer GWAS in men of European ancestry (UK/Australia stages 1 and 2; CAPS 1 and 2; BPC3; and Pegasus) and a custom replication array with ~200,000 markers (iCOGS), 2 GWAS in men of African ancestry (AAPC and Ghana Prostate Study), 2 GWAS in Japanese men (JAPC and BBJ) and a single scan in Latinos (LABC)[2,4–8,10–14] (**Supplementary Tables 1–3** and **Supplementary Note**). Genotypes in all scans were imputed for ~17 million SNPs/indels using the 1000 Genomes Project (March 2012 release) as a reference panel. UK/Australia stages 1 and 2, CAPS 1 and 2, PEGASUS, iCOGS, AAPC, Ghana Prostate Study, LABC and JAPC were imputed using IMPUTE V2 (ref. 30). BPC3, BBJ and PEGASUS were imputed using Minimac. β values and standard errors for each SNP were estimated with stratification by study adjusting for principal components. In addition to analyses of overall prostate cancer risk, we performed secondary analyses of aggressive and early-onset disease (age at diagnosis of ≤55). Aggressive prostate cancer was defined by a Gleason score of ≥8, a disease stage of 'distant', a PSA level of >100 ng/ml or death from prostate cancer. We included imputed data for SNPs with quality information scores of >0.3 (IMPUTE V2) or with estimated correlation between the genotype scores and the true genotypes (r^2) of >0.3 (Minimac). We limited the analysis to SNPs/indels on chromosomes 1–22 as well as the X chromosome with MAF greater than 1%, except in iCOGS and PEGASUS, which used arrays with coverage of less common alleles, where the MAF threshold was reduced to 0.5%.

Tests of homogeneity of the ORs across populations and studies were assessed using likelihood ratio tests. Risk heterogeneity by disease aggressiveness and age was assessed using a case-only analysis. Associations between SNP genotypes and PSA levels were assessed using linear regression, after log transformation of PSA levels to correct for skewness. Analyses were performed using SNPTEST, ProbABEL[31], PLINK, Stata and an in-house C++ program (**Supplementary Table 2**). METAL was used to perform fixed-effect ancestry-specific and multi-ancestry meta-analyses for overall prostate cancer, as well as secondary meta-analyses of aggressive and early-onset disease[32].

Inflation. We excluded SNPs within 500 kb of any previously known prostate cancer risk locus and estimated the inflation for each study on the basis of the 45th percentile of the test statistic. Inflation was estimated to be 1.00 in the Latino-ancestry study, 1.03 in the African-ancestry study, 1.06 in the Japanese-ancestry study and 1.14 in the European-ancestry study and was estimated to be 1.07 in the European-ancestry studies when SNPs at known risk loci and the iCOGS and UK2 studies were removed (**Supplementary Table 4**). The inflation factor was converted to an equivalent inflation factor for a study with 1,000 cases and 1,000 controls ($\lambda_{1,000}$) by adjusting by effective study size, namely:

$$\lambda_{1,000} = 1 + \frac{500(\lambda - 1)}{\sum_k \left(\frac{1}{n_k} + \frac{1}{m_k}\right)^{-1}}$$

where n_k and m_k were the number of cases and controls, respectively, for study k. Following the conversion, the study-specific λ values ranged from 0.995 to 1.083.

Genotyping and concordance. The most significant associations in the meta-analyses were observed with imputed SNPs. To validate the accuracy of the imputed genotypes, we genotyped each variant in ≥1,847 samples (except rs9443189 and rs12051443, which were sequenced in 183 and 265 samples, respectively) that were included in the meta-analysis, and estimated the correlation between imputed and genotyped alleles. A correlation of ≥0.75 was used as the confidence threshold for imputation quality (**Supplementary Table 6**).

Functional annotation. We used a number of publicly available prostate epithelium and prostate cancer Encyclopedia of DNA Elements (ENCODE) data sets of chromatin features to identify putative enhancer and regulatory regions at each risk locus[33,34]. Integration of chromatin biofeature annotations with the index SNPs and correlated markers ($r^2 > 0.9$) from the 1000 Genomes Project EUR populations was performed using FunciSNP[35]. These data sets included LNCaP and RWPEI DNase I–hypersensitive sites (Gene Expression Omnibus (GEO), GSE32970) from ENCODE; PrEC DNase I–hypersensitive sites (GSE29692) from ENCODE; LNCaP CTCF ChIP-seq peaks (GSE33213) from ENCODE; LNCaP H3K27ac and TCF7L2 ChIP-seq peaks (GSE51621)[33]; LNCaP H3K4me3 and H3K4me1 histone modification ChIP-seq peaks (GSE27823)[36]; FOXA1 ChIP-seq peaks (GSE28264)[37]; androgen receptor (AR) ChIP-seq peaks[38] and AR-binding sites (GSE28219)[39]; and NKX3-1 ChIP-seq peaks (GSE28264)[37]. We also used the highly conserved set of predicted microRNA targets (miRcode 11, June 2012 release)[40]. To determine whether any of the putative functional SNPs potentially affected the binding of known transcription factors, position-specific frequency matrices were employed from Factorbook[33,41].

***Cis* expression quantitative trait locus analysis.** Each risk locus was represented by an index SNP. For each index SNP, we retrieved all the correlated ($r^2 \geq 0.9$) variants from the EUR populations of the 1000 Genomes Project. The genotypes of the correlated variants in 145 prostate tumor samples and 33 normal tissue samples were downloaded from the TCGA database (February 2013). If a variant was not represented in the TCGA data, the genotypes were imputed using IMPUTE2 (ref. 30). A *cis* expression quantitative trait locus (eQTL) analysis was performed for these variants and any transcript within a 1-Mb interval (500 kb on either side). Gene expression values were adjusted for somatic copy number and CpG methylation as previously described[42]. Each risk variant was corrected for the number of transcripts in the interval. Significant associations were defined as having a nominal P value of <0.05 and a false discovery rate of <0.05 on the basis of the Benjamini-Hochberg method.

For the *TMPRSS2* locus, we also used gene expression data generated from formalin-fixed, paraffin-embedded tissue in the Physicians' Health Study cohort[43]. RNA was extracted with the Agencourt FormaPure FFPE kit (Beckman Coulter) and amplified using the WT-Ovation FFPE System V2 (NuGEN). cDNA was hybridized to the GeneChip Human Exon 1.0 ST microarray (Affymetrix). The residuals were shifted to have the original mean expression values and were normalized using the RMA method[44,45]. The SNP (rs1041449) was available in the BPC3 GWAS samples[6]; 99 participants had both tumor expression and genotype data; 54 had both normal prostate expression and genotype data.

Determination of *TMPRSS2-ERG* fusion status. The *TMPRSS2-ERG* fusion was assessed in a subset of 552 cases from study samples of FHCRC, UKGPCS, TAMPERE, ULM and IPO-PORTO. The majority of cases were typed for *TMPRSS2-ERG* rearrangements on formalin-fixed, paraffin-embedded tumor materials using FISH techniques according to Summersgill *et al.*[46] (for UKGPCS and FHCRC), Perner *et al.*[47] (for ULM) or Saramaki *et al.*[48] (for TAMPERE). The IPO-PORTO group applied quantitative RT-PCR to RNA from fresh-frozen tumor tissues using a TaqMan gene expression assay (Hs03063375_ft, Life Technologies) for the fusion transcript T1G4, which is present in approximately 90% of all *TMPRSS2-ERG*–positive prostate cancers.

Comparison of the number of associated loci among populations. We used the meta-analysis results from each population to evaluate the excess fraction of directionally consistent effect estimates (ORs) across populations, as evidence for additional shared susceptibility loci. We excluded the previously known prostate cancer risk regions as well as those identified in the current study (within 500 kb of index SNP) and compared the direction of association of SNPs defined in the European-ancestry population with those in the other populations for several P-value thresholds. The P values provided are based on a χ^2 binomial test for comparing proportions versus the 50% chance that the effects are in the same direction for each P-value cutoff.

Contribution to familial risk and risk stratification. The contribution of the known SNPs to the familial risk of prostate cancer, under a multiplicative model, was computed using the formula:

$$\sum_k (\log \lambda_k) \Big/ (\log \lambda_0)$$

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where λ_0 is the observed familial risk to the first-degree relatives of prostate cancer cases, assumed to be 2, and λ_k is the familial relative risk due to locus k, given by:

$$\lambda_k = \frac{p_k r_k^2 + q_k}{(p_k r_k + q_k)^2}$$

where p_k is the frequency of the risk allele for locus k, $q_k = 1 - p_k$ and r_k is the estimated per-allele OR (ref. 2).

On the basis of the assumption of a log-additive model, we constructed a polygenic risk score (PRS) from the summed genotypes weighted by the per-allele log OR (ref. 3). Thus, for each individual j we derived:

$$\text{Score}_j = \sum_{i=1}^{N} \beta_i g_{ij}$$

where N is the number of SNPs, g_{ij} is the allele dosage at SNP i (0, 1 or 2) for individual j and β_i is the per-allele log OR of SNP i.

The risk of prostate cancer was estimated for percentiles of the distribution of the PRS (<1%, 1–10%, 10–25%, 25–57%, 75–90%, 90–99% and >99%). We used effect sizes obtained from the meta-analysis of the European-ancestry population and used the data from the iCOGS study for this estimation.

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