



Queensland University of Technology
Brisbane Australia

This is the author's version of a work that was submitted/accepted for publication in the following source:

Al Olama, Ali Amin, Kote-Jarai, Zsofia, Berndt, Sonja I., Conti, David V., Schumacher, Fredrick, Han, Ying, Benlloch, Sara, Hazelett, Dennis J., Wang, Zhaoming, Saunders, Ed, Leongamornlert, Daniel, Lindstrom, Sara, Jugurnauth-Little, Sara, Dadaev, Tokhir, Tymrakiewicz, Malgorzata, Stram, Daniel O., Rand, Kristin, Wan, Peggy, Stram, Alex, Sheng, Xin, Pooler, Loreall C., Park, Karen, Xia, Lucy, Tyrer, Jonathan, Kolonel, Laurence N., Le Marchand, Loic, Hoover, Robert N., Machiela, Mitchell J., Yeager, Merideth, Burdette, Laurie, Chung, Charles C., Hutchinson, Amy, Yu, Kai, Goh, Chee, Ahmed, Mahbubl, Govindasami, Koveela, Guy, Michelle, Tammela, Teuvo L.J., Auvinen, Anssi, Wahlfors, Tiina, Schleutker, Johanna, Visakorpi, Tapio, Leinonen, Katri A., Xu, Jianfeng, Aly, Markus, Donovan, Jenny, Travis, Ruth C., Key, Tim J., Siddiq, Afshan, Canzian, Federico, Khaw, Kay-Tee, Takahashi, Atsushi, Kubo, Michiaki, Pharoah, Paul, Pashayan, Nora, Weischer, Maren, Nordestgaard, Borge G., Nielsen, Sune F., Klarskov, Peter, Røder, Martin Andreas, Iversen, Peter, Thibodeau, Stephen N., McDonnell, Shannon K., Schaid, Daniel J., Stanford, Janet L., Kolb, Suzanne, Holt, Sarah, Knudsen, Beatrice, Coll, Antonio Hurtado, Gapstur, Susan M., Diver, W. Ryan, Stevens, Victoria L., Maier, Christiane, Luedeke, Manuel, Herkommer, Kathleen, Rinckleb, Antje E., Strom, Sara S., Pettaway, Curtis, Yeboah, Edward D., Tettey, Yao, Biritwum, Richard B., Adjei, Andrew A., Tay, Evelyn, Truelove, Ann, Niwa, Shelley, Chokkalingam, Anand P., Cannon-Albright, Lisa, Cybulski, Cezary, Wokołarczyk, Dominika, Kluźniak, Wojciech, Park, Jong, Sellers, Thomas, Lin, Hui-Yi, Isaacs, William B., Partin, Alan W., Brenner, Hermann, Dieffenbach, Aida Karina, Stegmaier, Christa, Chen, Constance, Giovannucci, Edward L., Ma, Jing, Stampfer, Meir, Penney, Kathryn L., Mucci, Lorelei, John, Esther M., Ingles, Sue A., Kittles, Rick A., Murphy, Adam B., Pandha, Hardev, Michael, Agnieszka, Kierzek, Andrzej M., Blot, William, Signorello, Lisa B., Zheng, Wei, Albanes, Demetrius, Virtamo, Jarmo, Weinstein, Stephanie, Nemesure, Barbara, Carpten, John, Leske, Cristina, Wu, Suh-Yuh, Hennis, Anselm, Kibel, Adam S., Rybicki, Benjamin A., Neslund-Dudas, Christine, Hsing, Ann W., Chu, Lisa, Goodman, Phyllis J., Klein, Eric A., Zheng, S. Lilly, [Batra, Jyotsna](#), [Clements, Judith](#), [Spurdle, Amanda](#), Teixeira, Manuel R., Paulo, Paula, Maia, Sofia, Slavov, Chavdar, Kaneva, Radka, Mitev, Vanio, Witte, John S., Casey, Graham, Gillanders, Elizabeth M., Seminara, Daniella, Riboli, Elio, Hamdy, Freddie C., Coetzee, Gerhard A., Li, Qiyuan, Freedman, Matthew L., Hunter, David J., Muir, Kenneth, Gronberg, Henrik, Neal, David E., Southey, Melissa, Giles, Graham G., Severi, Gianluca, Cook, Michael B., Nakagawa, Hidewaki, Wiklund, Fredrik, Kraft, Peter, Chanock, Stephen J., Henderson, Brian E., Easton, Douglas F., Eeles, Rosalind A., & Haiman, Christopher A.
(2014)

A meta-analysis of 87 040 individuals identifies 23 new susceptibility loci



Published in final edited form as:

Nat Genet. 2014 October ; 46(10): 1103–1109. doi:10.1038/ng.3094.

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

A full list of authors and affiliations appears at the end of the article.

Abstract

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 novel susceptibility loci were revealed at $P < 5 \times 10^{-8}$; 15 variants were identified among men of European ancestry, 7 from multiethnic analyses and one was associated with early-onset prostate cancer. These 23 variants, in combination with the known prostate cancer risk variants, explain 33% of the familial risk of the disease in European ancestry populations. These findings provide new regions for investigation into the pathogenesis of prostate cancer and demonstrate the utility of combining ancestrally diverse populations to discover risk loci for disease.

Correspondence should be addressed to C.A.H (Haiman@usc.edu) and R.E. (Rosalind.eeles@icr.ac.uk).

*These authors contributed equally to this work.

**Co-senior authors who jointly directed this work.

Author Contributions: C.A.H, R.A.E., Z.K.-J., D.F.E., B.E.H., S.J.C., S.I.B., P.K., 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.C., E.S., D.L., T.D. S.J.L. performed the statistical analysis. D.O.S. and D.C. provided statistical support. D.J.H., A.S., K.P., X.S., G.A.C, Q.L., M.L.F. provided bioinformatic support as well as functional annotation and QTL data. L.C.P, K.P., L.X., L.B., M.T. conducted the genotyping and sequencing. S.B., C.G. and M.A. managed the PRACTICAL and COGS database. K.G., M.G managed the UKGPCS database. The following authors provided samples and data to the study and commented on the manuscript. 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.T., T.K., A.S., F.C. are EPIC investigators. E.R. is the principal investigator of EPIC. A.T., 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 R.W.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.K., L.M., E.L.G., J.M., M.S. are co-investigators of the Harvard cohorts and BPC3. H.G. is principal investigator of CAPS and STHLM1. 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 the 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 study coordinator of ATBC. B.N., J.C., C.L. S.-Y. W. and A.H. are principal investigators of PCBP. B.A.R. and C.N.-D. are principal investigators of GECAP. J.W. and G.C. are principal investigators of CaP Genes. D.S. is the program officer of GAME-ON. P.G., E.A.K., A.H and L.C are investigators of SELECT. F.C.H, J.L.D. and D.E.N. are principal investigators of ProtecT. E.D.Y., Y.T., R.B.B., A.A.A., E.T., A.T., S.N., are investigators of the Ghana Prostate Study. S.J.C., S.I.B., R.N.H., M.M., M.Y., C.C.C., A.H. and K.Y. are investigators of PLCO. M.R.T. is the principal investigator and P.P. and S.M. are investigators of IPO-Porto. J.B., J.C., A.S. are principal investigator of QLD. R.K. and C.S. are the principal investigators, and V.M. is an investigator of PCMUS. J.P. and 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.C. is the principal investigator of the Poland study. S.T. is the principal investigator of the Mayo study. P.P. 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., C.K., A.R. and P.I. are the principal investigators of CPCS1 and CPCS2. T.W, A.A. and T.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.S. coordinated the data collection of the ESTHER study. G.G.G. and G.S. are the principal investigators of MCCS; M.S. is an investigator. H.P, A.M and A.K are principal investigators of the PPF-UNIS study.

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. Genome-wide association studies (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 discovered also found to be associated with prostate cancer risk in other racial/ethnic 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/Australia⁴, Cancer of the Prostate in Sweden (CAPS)¹⁰, Breast and Prostate Cancer Cohort Consortium (BPC3)⁶, PEGASUS, and iCOGS/PRACTICAL²]; African ancestry[5,327 cases and 5,136 controls from the African Ancestry Prostate Cancer GWAS Consortium (AAPC)¹¹ and the Ghana Prostate Study¹²]; Japanese ancestry[2,563 cases and 4,391 controls from a GWAS in Japanese in the Multiethnic Cohort (MEC)⁸, and Biobank Japan^{13,14}]; and, Latino ancestry[1,034 cases and 1,046 controls from the MEC⁸]. Imputation was performed in each study using a cosmopolitan reference panel from the 1000 Genomes Project (1KGP; March, 2012). Across the various studies, 5.8-16.8M genotyped and imputed SNPs, as well as insertion/deletion variants 1% frequency were examined in association with prostate cancer risk (**Online Methods**, Supplementary Tables 1-3, Supplementary Information).

We first conducted ethnic-specific meta-analyses, with the large European ancestry sample providing the strongest statistical power for discovery of novel loci, followed by a multiethnic meta-analysis of all populations to identify additional loci with pan-ethnic 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 a) aggressive disease in the large European ancestry sample; b) aggressive disease in the combined multiethnic sample; and c) prostate cancer diagnosed at 55 years of age in the European ancestry sample only. Aggressive prostate cancer was defined as a Gleason score 8, disease stage as 'distant', a prostate-specific antigen (PSA) level >100 ng/ml, or death from prostate cancer. For these two secondary phenotypes, we utilized 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-d.f. 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 (λ/λ_{1000} : European, 1.14/1.00; African, 1.03/1.01; Japanese, 1.06/1.02; Hispanic, 1.00/1.00) or in the multiethnic analysis ($\lambda=1.08, \lambda_{1000}=1.00$; **Online Methods**, Supplementary Table 4, Supplementary Figure 1).

In the meta-analysis of the European ancestry studies, 20 novel signals in 18 regions ± 500 kb outside of previously associated loci were observed to be associated with prostate cancer

risk at $P < 5 \times 10^{-8}$ (Figure 1; Supplementary Figure 2 Supplementary Figure 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 which had high imputation information scores (r^2 range, 0.76-1) through direct genotyping or sequencing across multiple studies (Table 1; **Online Methods**, Supplementary Tables 5-8). Two of the variants were located within 370kb of each other on chromosome Xq13 and are independent signals based on conditional analyses (rs6625711, $P = 6.1 \times 10^{-10}$ and rs4844289, $P = 2.0 \times 10^{-8}$; $r^2 < 0.01$ in EUR 1KGP; Supplementary Table 9). All 15 variants were common, with minor allele frequencies (MAFs) 0.09, in the European ancestry population, and all but three (rs80130819/12q13, rs76939039/10q11 and rs17694493/9p21) were also common (MAF 0.05) in African, Japanese and Latino populations. Evidence of heterogeneity in the per-allele OR was noted with 4 variants ($P_{\text{het}} = 0.01 - 8.4 \times 10^{-6}$; rs17599629/1q21, rs115306967/6p21, rs17694493/9p21 and rs6625711/Xq13). Four of the 15 variants (rs10009409/4q13, rs4713266/6p24, rs80130819/12q13 and rs2807031/Xp11) had directional effects that were consistent with men of European ancestry and were nominally statistically significant ($P < 0.05$) in at least one other population (Table 1) and for 3 SNPs, combining data across populations strengthened the statistical significance of the association (Table 1). In this large European ancestry sample we also confirmed the reported signal at 22q13 with variant rs58133635 ($P = 5.8 \times 10^{-9}$; $r^2 = 0.74$ with rs9623117 in 1KGP European ancestry populations (EUR); Supplemental Figure 2; Supplementary Figure 3).¹⁵

No novel risk loci were revealed in ethnic-specific analyses within the African, Japanese or Latino ancestry populations possibly due to lack of power (Supplemental Figure 2). However, in combining results across populations in a multiethnic meta-analysis (43,303 cases, 43,737 controls), 11 additional variants were identified in association with prostate cancer risk in novel risk regions at $P < 5 \times 10^{-8}$ (Table 1; Supplemental Table 5; Figure 2). We confirmed the imputed genotypes for 7 variants which had high imputation information scores (r^2 range, 0.81-1) through additional genotyping and sequencing (**Online Methods**, Supplementary Tables 6-8). All 7 variants were nominally associated with risk ($P < 0.05$) in at least one of the non-European ancestry populations and the per-allele effects were directionally consistent across all 4 populations for 6 of the 7 variants. All variants had MAFs 0.05 in all four populations, and no significant evidence of population heterogeneity was noted with any of these 7 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 and all controls, n=27,212). The association with this variant was weaker for cases diagnosed >55 years of age (23,564 cases versus all controls, n=27,212: OR=1.04; p=0.004; $P_{\text{het}} = 2.2 \times 10^{-4}$; Supplementary Table 10). We did not detect any genome-wide significant associations with aggressive disease in the European population (n=7,903 cases) or in the combined multiethnic sample (n=10,209 cases; Supplemental Figure 4).

For the 23 novel risk variants (15 in European, 7 in multiethnic and 1 in the early onset analysis), the per-allele effects ranged from 1.06-1.14 and were consistent with log-additive effects (Supplemental Table 11). The association of each variant was noted for both

aggressive and non-aggressive prostate cancer (Supplemental Table 12); for only one variant, rs7153648 at 14q23, there was suggestive evidence of a difference by disease severity (OR=1.17 for aggressive and OR=1.09 for non-aggressive disease; $P_{\text{het}}=0.03$). These results confirm what has been observed in prostate cancer GWAS to date; risk loci appear to confer risk for prostate cancer overall and not discriminate between the 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 ($p<0.05$) (Supplemental Table 9). Only two of the 23 variants was modestly associated with PSA levels among controls (rs9287719 at 2p25, $P=0.03$ and rs115306967 at 6p21, $P=0.05$; Supplemental Table 13).

Of the 23 novel risk variants, 13 are located in intronic regions of genes and 2 are correlated with non-synonymous variants in adjacent genes (rs12051443/16q22, $r^2=0.98$ with rs4788821/*E60K* in *MARVELD3*; rs2238776/22q11, $r^2=0.67$ with rs72646967/*N397H* in *TBX1*). Based on functional annotations of transcription factor (TF) occupancy, response element disruption, histone marks and DNaseI sensitive regions in prostate cancer cell lines (**Online Methods**), 12 of the risk variants are either directly located within putative functional elements or are correlated (at $r^2>0.9$ in 1KGP EUR) 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 SNP and expression of gene transcripts within a 1Mb region. Among the 23 loci, 5 *cis*-associations were observed, albeit the associations were modest (Supplemental Table 14; **Online Methods**).

A number of the novel susceptibility regions are located in close proximity to genes which have either an established role, or have been directly implicated, in cancer (Table 1). The most notable is rs1041449 on chromosome 21q22, which is situated 20kb 5' of 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 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 risk rs1041449 is located within a number of histone marks and TF occupancy sites in the predicted enhancer region of *TMPRSS2* (Figure 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 region of notable importance is on chromosome 9p21. The risk variant, rs17694493, is intronic in *CDKN2B-AS1*, which encodes a long non-coding RNA – ANRIL, and is part of the *CDKN2B-CDKN2A* gene cluster (Figure 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, falls within chromatin bio features and is predicted to disrupt two TF motifs (*STAT1* and *RUNX1*) suggesting that it may have a functional effect on the regulation of the *CDKN2B-AS1* or *CDNK* genes (Figure 3, Supplementary Table 14), however, the variant

was not found to be strongly associated with expression of either *CDKN2A* ($P=0.19$) or *CDKN2B* ($P=0.40$) in the 145 TCGA prostate tumors.

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

The identification of novel risk loci for prostate cancer through a multiethnic analysis demonstrates the value of combining genetic data across populations to increase statistical power for discovery. As further support for conducting multiethnic analyses, we examined the genome-wide evidence for consistency in the direction of the allelic associations between populations. Excluding SNPs $\pm 500\text{kb}$ 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 $<10^{-2}$ - 10^{-5} . For the sets of SNPs defined for men of European ancestry, 53-64% had ORs that are directionally concordant for African ($p=0.04$ - 0.003 , dependent on the p -value threshold bin), Asian ($p=0.31$ - 0.02) or Hispanic men ($p=0.04$ - 0.002) with the ORs in Europeans. This same observation remained once we removed the 23 risk loci identified by the current study (Supplementary Figure 5). The excess of directionally consistent associations between populations implies that additional common risk loci for prostate may be revealed through discovery efforts in multiethnic studies.

These 23 novel 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 these 100 risk loci account for $\sim 33\%$ 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 $\sim 3.1\%$ of the familial risk (**Online Methods**). Based on 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% CI 2.8-3.1) relative risk of prostate cancer and the top 1% of men have a 5.7 fold (95% CI 4.8-6.6) relative risk compared with the population average (Supplemental Table 17). The top 10% is at a RR compared with the average of the population where it will be important to examine whether targeted screening based on family history genetic risk may reduce the over-diagnosis of indolent disease, which is a main limitation of PSA screening. Our findings demonstrate the importance of conducting large-scale genetic studies in diverse populations for the discovery of novel risk loci which continue to provide novel insights into disease mechanisms for complex traits.

Online Methods

Primary genotype data were used from four prostate cancer GWAS in men of European ancestry (UK/Australia Stages 1 and 2; CAPS 1 and 2; BPC3 and Pegasus), and a ~200K custom replication array (iCOGS), two GWAS in men of African ancestry (AAPC and Ghana Prostate Study), two GWAS in Japanese men (JAPC and BBJ) and a single scan in Latinos (LABC).^{2,4-8,10-14} (Supplementary Tables 1-3; Supplementary Information). Genotypes in all scans were imputed for ~17 M SNPs/indels using the 1000 Genome 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 V2.³⁰ BPC3, BBJ and Pegasus were imputed using Minimac. Betas and standard errors for each SNP were estimated stratified 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 ≤ 55). Aggressive prostate cancer was defined as a Gleason score ≥ 8 , disease stage as 'distant', a prostate-specific antigen (PSA) level >100 ng/ml, or death from prostate cancer. We included imputed data for SNPs with quality information scores >0.3 (IMPUTE V2) or with estimated correlation between the genotype scores and the true genotypes (r^2) >0.3 (Minimac). We limited the analysis to SNPs/indels on chromosomes 1-22 as well as the X with minor allele frequency greater than 1%, except in iCOGS and Pegasus, which utilized 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 study were assessed using likelihood ratio tests. Risk heterogeneity by disease aggressiveness and age was assessed using a case-only analysis. The associations between SNP genotypes and PSA level were assessed using linear regression, after log-transformation of PSA level to correct for skewness. Analyses were performed using SNPTEST, ProbABEL³¹, PLINK, Stata and an in-house C++ program (Supplementary Table 2). METAL was used to perform fixed effect ethnic-specific and multi-ethnic meta-analyses for overall prostate cancer, as well as secondary meta-analyses of aggressive and early-onset disease.³²

Inflation

We excluded SNPs with ± 500 kb distance of any previously known prostate cancer risk locus and estimated the inflation for each study based on the 45th percentile of the test statistic. The inflation was estimated to be 1.00 in the Latino, 1.03 in the African, 1.06 in the Japanese and 1.14 in the European ancestry studies, and, 1.07 in the European ancestry studies when SNPs at known risk loci and the iCOGS and UK2 studies were removed (see Supplementary Table 4). The inflation was converted to an equivalent inflation for a study with 1000 cases and 1000 controls (λ_{1000}) by adjusting by effective study size, namely

$$\lambda_{1000} = 1 + \frac{500(\lambda - 1)}{\left(\frac{1}{n_k} + \frac{1}{m_k}\right)^{-1}}$$

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

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 1847 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 (Supplemental Table 6).

Functional Annotation

We used a number of publicly available prostate epithelia and prostate cancer ENCODE datasets of chromatin features to identify putative enhancer/regulatory regions at each risk locus.^{33,34} The integration of chromatin bio feature annotations with the index SNPs and correlated markers ($r^2 > 0.9$) from 1KGP EUR populations was performed using FunciSNP.³⁵ These datasets included LNCaP and RWPEI DNaseI HS sites (GSE32970) ENCODE; PrEC DNaseI HS sites (GSE29692) ENCODE; LNCaP CTCF ChIP-seq peaks (GSE33213) ENCODE; LNCaP H3K27ac and TCF7L2 (GSE51621)³³, H3K4me3 and H3K4me1 histone modification ChIP-seq peaks GSE27823³⁶; FoxA1 ChIP-seq peaks (GSE28264)³⁷; Androgen Receptor (AR) ChIP-seq peaks³⁸ and AR binding sites (GSE28219)³⁹; NKX3-1 ChIP-seq peaks (GSE28264).³⁷ We also used the highly conserved set of predicted targets of microRNA targeting (miRcode 11, June 2012 release)⁴⁰. To determine whether any of the putative functional SNPs potentially affect the binding of known transcription factors, position-specific frequency matrices were employed from Factorbook.^{33,41}

cis-eQTL analysis

Each risk locus is represented by an index SNP. For each index SNP, we retrieved all the correlated ($r^2 > 0.9$) variants EUR populations from 1KGP. The genotypes of the correlated variants in 145 prostate tumor samples and 33 normal tissue samples were downloaded from TCGA database (Feb 2013). If a variant was not represented in the TCGA data, the genotypes were imputed using IMPUTE2.³⁰ A cis-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 (ref. ⁴²). Each risk variant was corrected for the number of transcripts in the interval. Significant associations were defined as a nominal p-value < 0.05 and a false discovery rate < 0.05 based on Benjamini-Hochberg method.

For the TMPRSS2 locus, we also used gene expression data generated from formalin-fixed paraffin embedded (FFPE) tissue in the Physicians' Health Study cohort.⁴³ RNA was extracted with the Agencourt Form a Pure FFPE kit (Beckman Coulter, Indianapolis, IN) and amplified using the WT-Ovation FFPE System V2 (NuGEN, San Carlos, CA). cDNA was hybridization on the GeneChip Human Exon 1.0 ST microarray (Affymetrics, Santa Clara, CA). The residuals were shifted to have the original mean expression values and normalized using the RMA method.^{44,45} The SNP (rs1041449) was available in the BPC3

GWAS samples⁶; 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 FFPE 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 qRT-PCR on RNA from fresh-frozen tumor tissues using a TaqMan gene expression assay (Hs03063375_ft, Life Technologies, Carlsbad, CA) for the fusion transcript T1G4, which is present in approximately 90% of all *TMPRSS2-ERG* positive prostate cancer.

Comparison of 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 ($\pm 500\text{kb}$ of index SNP) and compared the direction of association of SNPs defined in the European ancestry population with the other populations for several p-value thresholds. The p-values provided are based on a Chi-square binomial test for comparing proportions versus 50% chance to be in the same direction for each p-value cut-off.

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

$$\frac{\sum_k (\log \lambda_k)}{(\log \lambda_0)}$$

where λ_0 is the observed familial risk to 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 odds ratio.²

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

$$Score_j = \sum_{i=1}^N \beta_i g_{ij}$$

Where:

N : Number of SNPs

g_{ij} : Allele dose at SNP i (0, 1, 2) for individual j

β_i : Per-allele log-odds ratio 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%, >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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Ali Amin Al Olama^{1,*}, Zsofia Kote-Jarai^{2,*}, Sonja I. Berndt³, David V. Conti^{4,5}, Fredrick Schumacher^{4,5}, Ying Han⁴, Sara Benlloch¹, Dennis J. Hazelett^{4,5}, Zhaoming Wang^{3,6}, Ed Saunders², Daniel Leongamornlert², Sara Lindstrom⁷, Sara Jugurnauth-Little², Tokhir Dadaev², Malgorzata Tymrakiewicz², Daniel O. Stram^{4,5}, Kristin Rand⁴, Peggy Wan⁴, Alex Stram⁴, Xin Sheng⁴, Loreall C. Pooler⁴, Karen Park⁴, Lucy Xia⁴, Jonathan Tyrer¹, Laurence N. Kolonel⁸, Loic Le Marchand⁸, Robert N. Hoover³, Mitchell J. Machiela³, Merideth Yeager³, Laurie Burdette³, Charles C. Chung³, Amy Hutchinson³, Kai Yu³, Chee Goh², Mahbub Ahmed², Koveela Govindasami², Michelle Guy², Teuvo L.J. Tammela⁹, Anssi Auvinen¹⁰, Tiina Wahlfors¹¹, Johanna Schleutker^{11,12}, Tapio Visakorpi¹³, Katri A. Leinonen¹³, Jianfeng Xu¹⁴, Markus Aly^{15,16}, Jenny Donovan¹⁷, Ruth C. Travis¹⁸, Tim J. Key¹⁸, Afshan Siddiq¹⁹, Federico Canzian²⁰, Kay-Tee Khaw²¹, Atsushi Takahashi²², Michiaki Kubo²³, Paul Pharoah²⁴, Nora Pashayan²⁴, Maren Weischer²⁵, Borge G. Nordestgaard^{25,26}, Sune F. Nielsen^{25,26}, Peter Klarskov²⁷, Martin Andreas Røder²⁸, Peter Iversen²⁸, Stephen N. Thibodeau²⁹, Shannon K McDonnell²⁹, Daniel J Schaid²⁹, Janet L. Stanford^{30,31}, Suzanne Kolb³⁰, Sarah Holt³², Beatrice Knudsen³³, Antonio Hurtado Coll³⁴, Susan M. Gapstur³⁵, W. Ryan Diver³⁵, Victoria L. Stevens³⁵, Christiane Maier³⁶, Manuel Luedeke³⁶, Kathleen Herkommer³⁷, Antje E. Rinckleb³⁶, Sara S. Strom³⁸, Curtis Pettaway³⁹, Edward D. Yeboah^{40,41}, Yao Tettey^{40,41}, Richard B. Biritwum^{40,41}, Andrew A. Adjei^{40,41}, Evelyn Tay^{40,41}, Ann Truelove⁴², Shelley Niwa⁴², Anand P. Chokkalingam⁴³, Lisa Cannon-Albright^{44,45}, Cezary Cybulski⁴⁶, Dominika Wokołarczyk⁴⁶, Wojciech Klu niak⁴⁶, Jong Park⁴⁷, Thomas Sellers⁴⁷, Hui-Yi Lin⁴⁸, William B. Isaacs⁴⁹, Alan W. Partin⁴⁹, Hermann

Brenner^{50,51}, Aida Karina Dieffenbach^{50,51}, Christa Stegmaier⁵², Constance Chen⁷, Edward L. Giovannucci^{53,54}, Jing Ma⁵⁵, Meir Stampfer^{53,54,55}, Kathryn L. Penney^{53,55}, Lorelei Mucci^{53,55}, Esther M. John^{56,57}, Sue A. Ingles^{4,5}, Rick A. Kittles⁵⁸, Adam B. Murphy⁵⁹, Hardev Pandha⁶⁰, Agnieszka Michael⁶⁰, Andrzej M. Kierzek⁶⁰, William Blot^{61,62}, Lisa B. Signorello^{53,55}, Wei Zheng⁶², Demetrius Albanes⁶³, Jarmo Virtamo⁶⁴, Stephanie Weinstein⁶³, Barbara Nemesure⁶⁵, John Carpten⁶⁶, Cristina Leske⁶⁵, Suh-Yuh Wu⁶⁵, Anselm Hennis^{65,67}, Adam S. Kibel⁶⁸, Benjamin A. Rybicki⁶⁹, Christine Neslund-Dudas⁶⁹, Ann W. Hsing^{56,57}, Lisa Chu^{56,57}, Phyllis J. Goodman⁷⁰, Eric A Klein⁷¹, S. Lilly Zheng¹⁴, Jyotsna Batra⁷², Judith Clements⁷², Amanda Spurdle⁷³, Manuel R. Teixeira^{74,75}, Paula Paulo⁷⁴, Sofia Maia⁷⁴, Chavdar Slavov⁷⁶, Radka Kaneva⁷⁷, Vanio Mitev⁷⁷, John S. Witte^{78,79}, Graham Casey^{4,5}, Elizabeth M. Gillanders⁸⁰, Daniella Seminara⁸⁰, Elio Riboli⁸¹, Freddie C. Hamdy⁸², Gerhard A. Coetzee^{4,5}, Qiyuan Li⁸³, Matthew L. Freedman⁸³, David J. Hunter⁷, Kenneth Muir^{84,85}, Henrik Gronberg¹⁵, David E. Neal^{86,87}, Melissa Southey⁸⁸, Graham G. Giles^{89,90}, Gianluca Severi^{89,90,91}, The Breast and Prostate Cancer Cohort Consortium (BPC3)⁹², The PRACTICAL (Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome) Consortium⁹², The COGS (Collaborative Oncological Gene-environment Study) Consortium⁹², The GAME-ON/ELLIPSE Consortium⁹², Michael B. Cook^{3,**}, Hidewaki Nakagawa^{93,**}, Fredrik Wiklund^{15,**}, Peter Kraft^{7,94,**}, Stephen J. Chanock^{3,**}, Brian E. Henderson^{4,5,**}, Douglas F. Easton^{1,**}, Rosalind A. Eeles^{2,96,**}, and Christopher A. Haiman^{4,5,**}

Affiliations

¹Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK ²The Institute of Cancer Research, London, UK ³Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institute of Health, Bethesda, MD ⁴Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA ⁵Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, USA ⁶Cancer Genomics Research Laboratory, NCI-DCEG, SAIC-Frederick Inc., Frederick, MD, USA ⁷Program in Genetic Epidemiology and Statistical Genetics, Department of Epidemiology, Harvard School of Public Health, Boston, MA ⁸Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI, USA ⁹Department of Urology, Tampere University Hospital and Medical School, University of Tampere, Finland ¹⁰Department of Epidemiology, School of Health Sciences, University of Tampere, Tampere, Finland ¹¹BioMediTech, University of Tampere and FimLab Laboratories, Tampere, Finland ¹²Department of Medical Biochemistry, Institute of Biomedicine, University of Turku, Finland ¹³Institute of Biomedical Technology/BioMediTech, University of Tampere and Tampere University Hospital, Tampere, Finland ¹⁴Center for Cancer Genomics, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA ¹⁵Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden ¹⁶Department of Clinical Sciences at Danderyds Hospital, Stockholm, Sweden ¹⁷School of Social and Community Medicine, University of Bristol, Bristol, UK

¹⁸Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK ¹⁹Department of Genomics of Common Disease, School of Public Health, Imperial College London, London, UK ²⁰Genomic Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany ²¹Clinical Gerontology Unit, University of Cambridge, Cambridge, UK ²²Laboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan ²³Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan ²⁴Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, UK ²⁵Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark ²⁶Faculty of Healthy and Medical Sciences, University of Copenhagen, Herlev, Denmark ²⁷Department of Urology, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark ²⁸Copenhagen Prostate Cancer Center, Department of Urology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark ²⁹Mayo Clinic, Rochester, MN, USA ³⁰Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA ³¹Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington, USA ³²Fred Hutchinson Cancer Research Center, Seattle, WA, USA ³³Translational Pathology, Cedars-Sinai, Los Angeles, CA, USA ³⁴The Prostate Center, Vancouver, BC, Canada ³⁵Epidemiology Research Program, American Cancer Society, Atlanta, GA ³⁶Department of Urology, University Hospital Ulm, Germany ³⁷Department of Urology, Klinikum rechts der Isar der Technischen Universitaet Muenchen, Munich, Germany ³⁸Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA ³⁹Department of Urology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA ⁴⁰University of Ghana Medical School, Accra, Ghana ⁴¹Korle Bu Teaching Hospital, Accra, Ghana ⁴²Westat, Rockville, MD, USA ⁴³School of Public Health, University of California, Berkeley, Berkeley, CA, USA ⁴⁴Division of Genetic Epidemiology, Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah, USA ⁴⁵George E. Wahlen Department of Veterans Affairs Medical Center, Salt Lake City, Utah, USA ⁴⁶International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland ⁴⁷Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL, USA ⁴⁸Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, FL, USA ⁴⁹James Buchanan Brady Urological Institute, Johns Hopkins Hospital and Medical Institution, Baltimore, MD, USA ⁵⁰Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany ⁵¹German Cancer Consortium, Heidelberg, Germany ⁵²Saarland Cancer Registry, Saarbrücken, Germany ⁵³Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA ⁵⁴Department of Nutrition, Harvard School of Public Health, Boston, MA, USA ⁵⁵Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA ⁵⁶Cancer Prevention Institute of California, Fremont, CA, USA ⁵⁷Stanford Cancer Institute, Stanford University School of

Medicine, Stanford, CA, USA ⁵⁸Department of Medicine, University of Illinois at Chicago, Chicago, IL, USA ⁵⁹Department of Urology, Northwestern University, Chicago, IL ⁶⁰Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK ⁶¹International Epidemiology Institute, Rockville, MD 20850, USA ⁶²Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, Tennessee, US ⁶³Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institute of Health, Bethesda, MD, USA ⁶⁴Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland ⁶⁵Department of Preventive Medicine, Stony Brook University, Stony Brook, NY, USA ⁶⁶The Translational Genomics Research Institute, Phoenix, AZ, USA ⁶⁷Chronic Disease Research Centre, University of the West Indies, Bridgetown, Barbados ⁶⁸Division of Urologic Surgery, Brigham and Women's Hospital, Dana-Farber Cancer Institute, Boston, MA, USA ⁶⁹Department of Public Health Sciences, Henry Ford Hospital, Detroit, MI ⁷⁰SWOG Statistical Center, Fred Hutchinson Cancer Research Center, Seattle, WA ⁷¹Department of Urology, Glickman Urological & Kidney Institute, Cleveland Clinic, Cleveland, OH, USA ⁷²Australian Prostate Cancer Research Centre-Qld, Institute of Health and Biomedical Innovation and School of Biomedical Science, Queensland University of Technology, Translational Research Institute, Brisbane, Australia ⁷³Molecular Cancer Epidemiology Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia ⁷⁴Department of Genetics, Portuguese Oncology Institute, Porto, Portugal ⁷⁵Biomedical Sciences Institute, University of Porto, Porto, Portugal ⁷⁶Department of Urology, Medical University - Sofia, Bulgaria ⁷⁷Department of Medical Chemistry and Biochemistry, Molecular Medicine Center, Medical University - Sofia, Bulgaria ⁷⁸Institute for Human Genetics, University of California, San Francisco, San Francisco, CA, USA ⁷⁹Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA ⁸⁰Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, Maryland, USA ⁸¹Department of Epidemiology & Biostatistics, School of Public Health, Imperial College, London, UK ⁸²Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK ⁸³Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA ⁸⁴Institute of Population Health, University of Manchester, Manchester, UK ⁸⁵Warwick Medical School, University of Warwick, Coventry, UK ⁸⁶Cancer Research UK, Cambridge Research Institute, Li Ka Shing Centre, Cambridge, UK ⁸⁷University of Cambridge, Department of Oncology, Addenbrooke's Hospital, Cambridge, UK ⁸⁸Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia ⁸⁹Cancer Epidemiology Centre, The Cancer Council Victoria, Melbourne, Victoria, Australia ⁹⁰Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Victoria, Australia ⁹¹Human Genetics Foundation, Torino, Italy ⁹²Full lists of members and affiliations appear in the Supplementary Note ⁹³Laboratory for Genome Sequencing Analysis, RIKEN Center for Integrative Medical Sciences,

Tokyo, Japan ⁹⁴Department of Biostatistics, Harvard School of Public Health, Boston, MA ⁹⁵Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA ⁹⁶Royal Marsden National Health Services (NHS) Foundation Trust, London and Sutton, UK

Acknowledgments

A full listing of acknowledgements is detailed in the Supplementary Note.

References

1. Eeles R, et al. The genetic epidemiology of prostate cancer and its clinical implications. *Nat Rev Urol*. 2014; 11:18–31. [PubMed: 24296704]
2. Eeles RA, et al. Identification of 23 new prostate cancer susceptibility loci using the iCOGS custom genotyping array. *Nat Genet*. 2013; 45:385–91. 391e1–2. [PubMed: 23535732]
3. Park JH, et al. Estimation of effect size distribution from genome-wide association studies and implications for future discoveries. *Nat Genet*. 2010; 42:570–5. [PubMed: 20562874]
4. Eeles RA, et al. Multiple newly identified loci associated with prostate cancer susceptibility. *Nat Genet*. 2008; 40:316–21. [PubMed: 18264097]
5. Gudmundsson J, et al. Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. *Nat Genet*. 2009; 41:1122–6. [PubMed: 19767754]
6. Schumacher FR, et al. Genome-wide association study identifies new prostate cancer susceptibility loci. *Hum Mol Genet*. 2011; 20:3867–75. [PubMed: 21743057]
7. Thomas G, et al. Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet*. 2008; 40:310–5. [PubMed: 18264096]
8. Cheng I, et al. Evaluating genetic risk for prostate cancer among Japanese and Latinos. *Cancer Epidemiol Biomarkers Prev*. 2012; 21:2048–58. [PubMed: 22923026]
9. Haiman CA, et al. Characterizing genetic risk at known prostate cancer susceptibility loci in African Americans. *PLoS Genet*. 2011; 7:e1001387. [PubMed: 21637779]
10. Duggan D, 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. [PubMed: 18073375]
11. Haiman CA, et al. Genome-wide association study of prostate cancer in men of African ancestry identifies a susceptibility locus at 17q21. *Nat Genet*. 2011; 43:570–3. [PubMed: 21602798]
12. Cook MB, et al. A genome-wide association study of prostate cancer in West African men. *Hum Genet*. 2013
13. Akamatsu S, et al. Common variants at 11q12, 10q26 and 3p11.2 are associated with prostate cancer susceptibility in Japanese. *Nat Genet*. 2012; 44:426–9. S1. [PubMed: 22366784]
14. Takata R, et al. Genome-wide association study identifies five new susceptibility loci for prostate cancer in the Japanese population. *Nat Genet*. 2010; 42:751–4. [PubMed: 20676098]
15. Sun J, et al. Sequence variants at 22q13 are associated with prostate cancer risk. *Cancer Res*. 2009; 69:10–5. [PubMed: 19117981]
16. Hedstrom L. Serine protease mechanism and specificity. *Chem Rev*. 2002; 102:4501–24. [PubMed: 12475199]
17. Morris DS, Tomlins SA, Montie JE, Chinnaiyan AM. The discovery and application of gene fusions in prostate cancer. *BJU Int*. 2008; 102:276–82. [PubMed: 18422767]
18. Falchi M, et al. Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. *Nat Genet*. 2009; 41:915–9. [PubMed: 19578365]
19. Hussussian CJ, et al. Germline p16 mutations in familial melanoma. *Nat Genet*. 1994; 8:15–21. [PubMed: 7987387]

20. Shete S, et al. Genome-wide association study identifies five susceptibility loci for glioma. *Nat Genet.* 2009; 41:899–904. [PubMed: 19578367]
21. Stacey SN, et al. New common variants affecting susceptibility to basal cell carcinoma. *Nat Genet.* 2009; 41:909–14. [PubMed: 19578363]
22. Turnbull C, et al. Genome-wide association study identifies five new breast cancer susceptibility loci. *Nat Genet.* 2010; 42:504–7. [PubMed: 20453838]
23. Wrensch M, et al. Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. *Nat Genet.* 2009; 41:905–8. [PubMed: 19578366]
24. Timofeeva MN, et al. Influence of common genetic variation on lung cancer risk: meta-analysis of 14 900 cases and 29 485 controls. *Hum Mol Genet.* 2012; 21:4980–95. [PubMed: 22899653]
25. Tikhmyanova N, Little JL, Golemis EA. CAS proteins in normal and pathological cell growth control. *Cell Mol Life Sci.* 2010; 67:1025–48. [PubMed: 19937461]
26. Loikkanen I, et al. Myosin VI is a modulator of androgen-dependent gene expression. *Oncol Rep.* 2009; 22:991–5. [PubMed: 19787211]
27. Puri C, et al. Overexpression of myosin VI in prostate cancer cells enhances PSA and VEGF secretion, but has no effect on endocytosis. *Oncogene.* 2010; 29:188–200. [PubMed: 19855435]
28. Wei S, Dunn TA, Isaacs WB, De Marzo AM, Luo J. GOLPH2 and MYO6: putative prostate cancer markers localized to the Golgi apparatus. *Prostate.* 2008; 68:1387–95. [PubMed: 18543251]
29. Michailidou K, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat Genet.* 2013; 45:353–61. 361e1–2. [PubMed: 23535729]
30. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* 2009; 5:e1000529. [PubMed: 19543373]
31. Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics.* 2010; 11:134. [PubMed: 20233392]
32. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics.* 2010; 26:2190–1. [PubMed: 20616382]
33. Hazelett DJ, et al. Comprehensive functional annotation of 77 prostate cancer risk loci. *PLoS Genet.* 2014; 10:e1004102. [PubMed: 24497837]
34. Thurman RE, et al. The accessible chromatin landscape of the human genome. *Nature.* 2012; 489:75–82. [PubMed: 22955617]
35. Coetzee SG, Rhie SK, Berman BP, Coetzee GA, Noushmehr H. FunciSNP: an R/bioconductor tool integrating functional non-coding data sets with genetic association studies to identify candidate regulatory SNPs. *Nucleic Acids Res.* 2012; 40:e139. [PubMed: 22684628]
36. Wang D, et al. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. *Nature.* 2011; 474:390–4. [PubMed: 21572438]
37. Tan PY, et al. Integration of regulatory networks by NKX3-1 promotes androgen-dependent prostate cancer survival. *Mol Cell Biol.* 2012; 32:399–414. [PubMed: 22083957]
38. Andreu-Vieyra C, et al. Dynamic nucleosome-depleted regions at androgen receptor enhancers in the absence of ligand in prostate cancer cells. *Mol Cell Biol.* 2011; 31:4648–62. [PubMed: 21969603]
39. Sharma NL, et al. The androgen receptor induces a distinct transcriptional program in castration-resistant prostate cancer in man. *Cancer Cell.* 2013; 23:35–47. [PubMed: 23260764]
40. Jeggari A, Marks DS, Larsson E. miRcode: a map of putative microRNA target sites in the long non-coding transcriptome. *Bioinformatics.* 2012; 28:2062–3. [PubMed: 22718787]
41. Wang J, et al. Sequence features and chromatin structure around the genomic regions bound by 119 human transcription factors. *Genome Res.* 2012; 22:1798–812. [PubMed: 22955990]
42. Li Q, et al. Integrative eQTL-based analyses reveal the biology of breast cancer risk loci. *Cell.* 2013; 152:633–41. [PubMed: 23374354]
43. Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. *N Engl J Med.* 1989; 321:129–35. [PubMed: 2664509]

44. Irizarry RA, et al. Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res.* 2003; 31:e15. [PubMed: 12582260]
45. Irizarry RA, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics.* 2003; 4:249–64. [PubMed: 12925520]
46. Summersgill B, Clark J, Shipley J. Fluorescence and chromogenic in situ hybridization to detect genetic aberrations in formalin-fixed paraffin embedded material, including tissue microarrays. *Nat Protoc.* 2008; 3:220–34. [PubMed: 18274524]
47. Perner S, et al. TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. *Am J Surg Pathol.* 2007; 31:882–8. [PubMed: 17527075]
48. Saramaki OR, et al. TMPRSS2:ERG fusion identifies a subgroup of prostate cancers with a favorable prognosis. *Clin Cancer Res.* 2008; 14:3395–400. [PubMed: 18519769]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

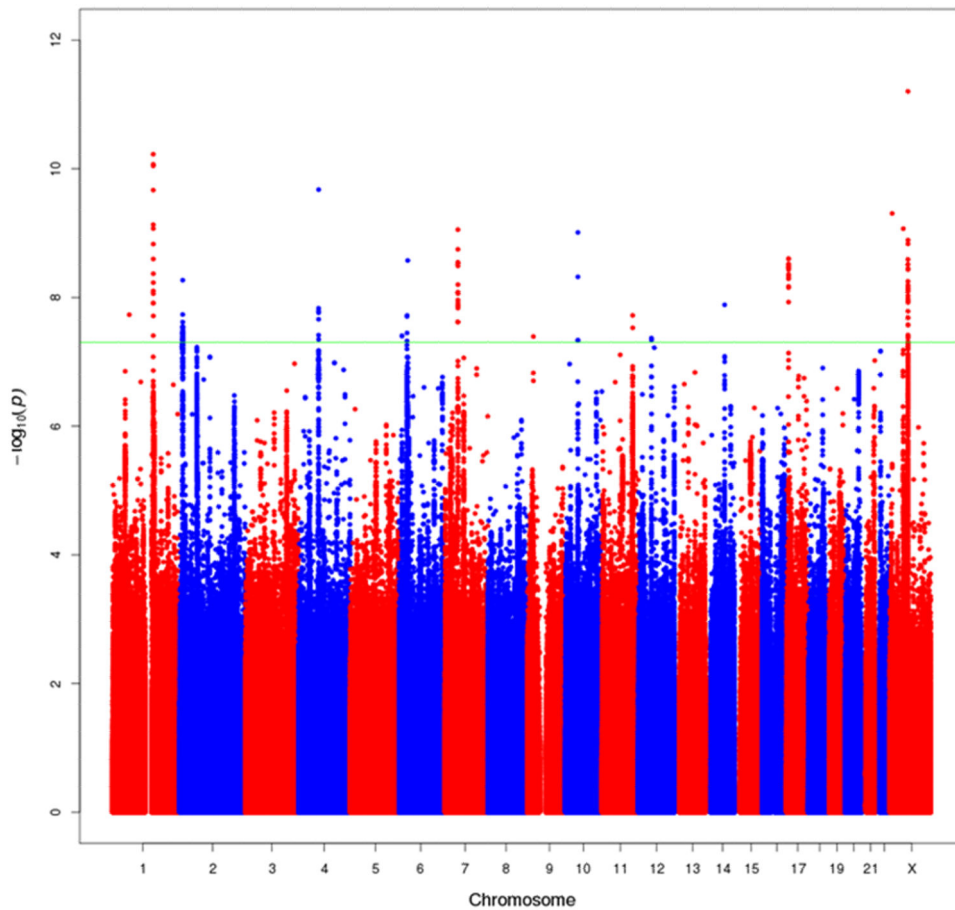


Figure 1. Manhattan Plot of genotyped and imputed results from the European ancestry meta-analysis of overall prostate cancer risk. All SNPs within 500kb of known GWAS SNPs are omitted. The green line represents $P=5 \times 10^{-8}$. This figure shows all new variants with $P < 5 \times 10^{-8}$, regardless of the confirmation results (one signal on chr1, one on chr4, one on chr17, and 2 on chr X were not confirmed). Many of the new signals are in close proximity to one another on the same chromosome (see Supplementary Table 6).

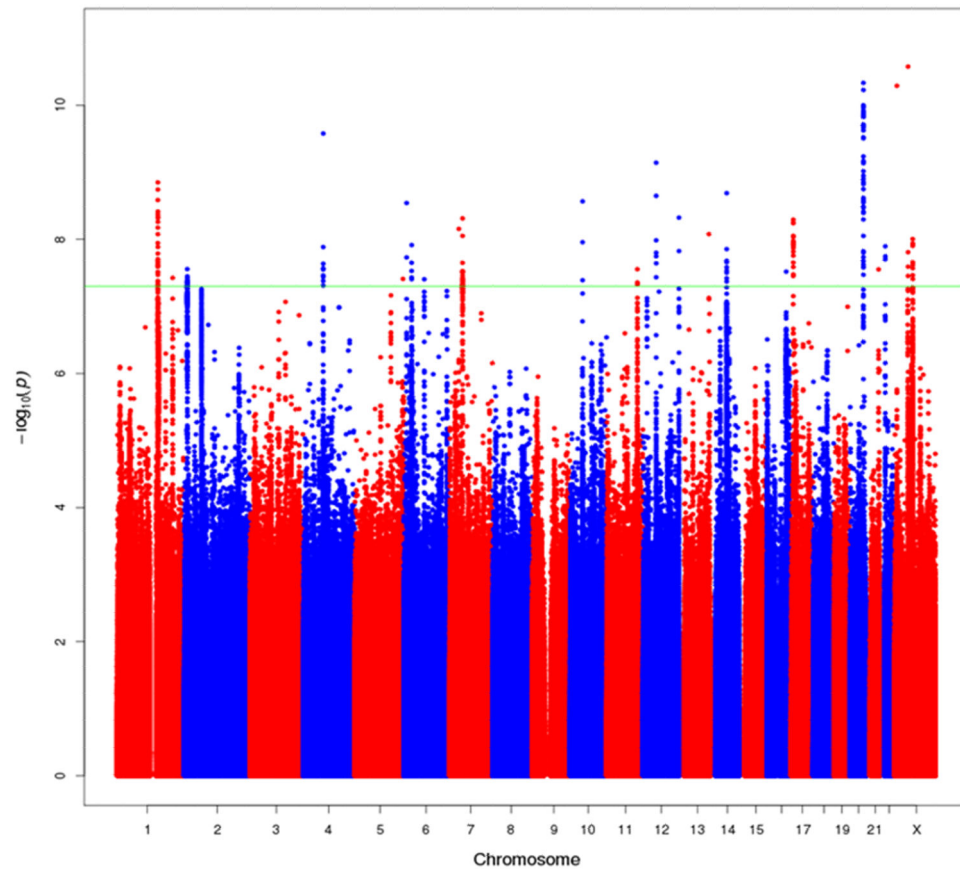


Figure 2. Manhattan Plot of results from the multiethnic meta-analysis of overall prostate cancer risk. All SNPs within 500kb of known GWAS SNPs are omitted. The green line represents $P=5 \times 10^{-8}$. This figure shows all new variants with $P < 5 \times 10^{-8}$, regardless of the confirmation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

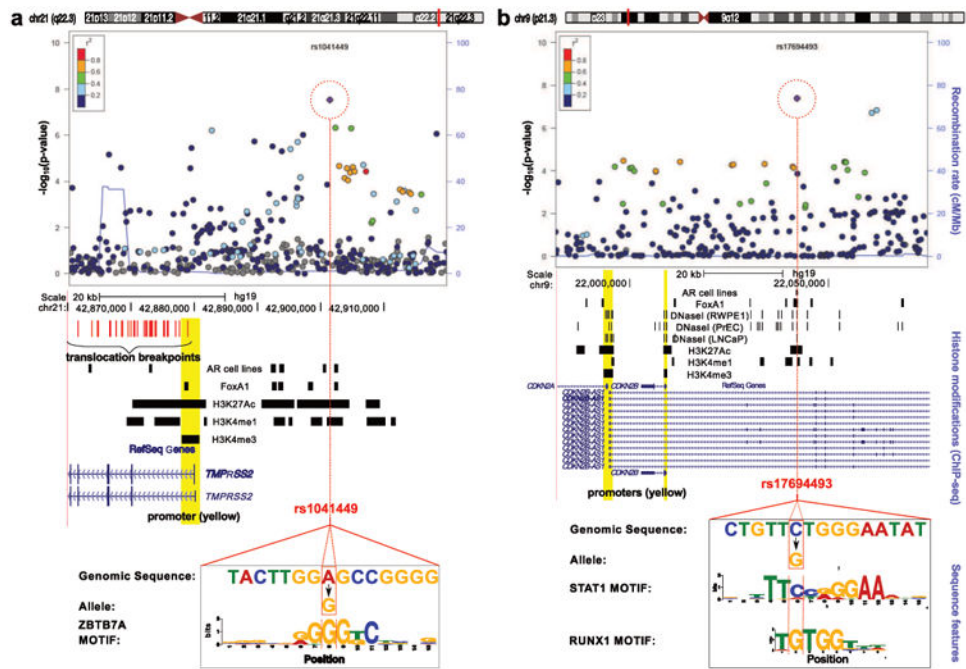


Figure 3.

Regional plots of two novel genome-wide significant loci associated with prostate cancer risk. rs1041449/21q22 (*TMPRSS2* region, left) and rs17694493/9p21 (*CDKN2B-AS1* region, right). Top: SNPs are plotted by their position 500kb on either side of the index SNP (purple diamond) on the chromosome against their association ($-\log_{10} P$) with prostate cancer from the multiethnic meta-analysis (rs1041449) and European 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 (March 2012). MIDDLE: Significant peaks from TF and histone modification ChIP-seq experiments in the same genomic window (see **Online Methods**). All ChIP-seq in LNCaP unless otherwise indicated. BOTTOM: Genomic sequence (enclosed in black box) surrounding the SNP (red box) aligned to a LOGO graphic representing the proposed motif disruption.

Table 1

Association results for 23 novel risk variants for prostate cancer.

SNP ID	Chromosome, position ^b	Nearby Genes	Alleles ^c	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			Multiethnic 44,107 cases 45,172 controls			P _{Het} value ^d	
				OR	P	RAF ^a	OR	P	RAF ^a	OR	P	RAF ^a	OR	P	RAF ^a	OR	P	RAF ^a		
Risk Loci Revealed in European Ancestry Meta-Analysis																				
rs17599629	1q21, 150658287	<i>GOLPH3L</i>	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	0.26	1.08	8.6×10 ⁻³
rs9287719	2p25, 10710730	<i>NOL10</i>	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	0.45	1.06	0.21
rs10009409	4q13, 73855253	<i>COX18</i>	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	0.50	1.08	0.12
rs4713266	6p24, 11219030	<i>NEDD9</i>	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	0.40	1.06	0.89
rs115457135	6p22, 30073776	<i>TRIM31</i>	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	0.26	1.07	0.25
rs115306967	6p21, 32400939	<i>HLA-DRB6</i>	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	0.76	1.06	5.2×10 ⁻⁴
rs56232506	7p12, 47437244	<i>TNS3</i>	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	0.52	1.06	0.13
rs17694493	9p21, 22041998	<i>CDKN2B-AS1</i>	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	0.08	1.08	0.01
rs76934034	10q11, 46082985	<i>MARCH8</i>	T/C	1.14	4.8×10 ⁻⁹	0.91	0.98	0.88	0.98				1.06	0.64	0.92	1.13	1.1×10 ⁻⁸	0.92	1.13	0.39
rs11214775	11q23, 113807181	<i>HTR3B</i>	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	0.81	1.07	0.39
rs80130819	12q13, 48419618	<i>RPI-228P16.4</i>	A/C	1.13	4.3×10 ⁻⁸	0.91	1.28	0.02	0.98				1.22	0.17	0.94	1.14	2.2×10 ⁻⁹	0.94	1.14	0.44
rs8014671	14q24, 71092256	<i>TTC9</i>	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	0.60	1.06	0.09
rs2807031	Xp11, 52896949	<i>XAGE3</i>	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	0.09	1.07	0.77
rs6625711	Xq13, 70139850	<i>SLC7A</i>	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	0.61	1.04	8.4×10 ⁻⁶
rs4844289	Xq13, 70407983	<i>NLGN3/BCYRN1</i>	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	0.59	1.04	0.04
Risk Loci Revealed in Multiethnic Meta-Analysis																				
rs1775148	1q32, 205757824	<i>SLC41A1</i>	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	0.66	1.06	0.40
rs9443189	6q14, 76495882	<i>MYO6</i>	A/G	1.07	5.2×10 ⁻⁵	0.86	1.11	4.5×10 ⁻⁴	0.47	1.07	0.08	0.68	1.01	0.93	0.86	1.08	3.9×10 ⁻⁸	0.86	1.08	0.64
rs7153648	14q23, 61122526	<i>SIX1</i>	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	0.10	1.11	0.50
rs12051443	16q22, 71691329	<i>PHLPP2</i>	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	0.50	1.06	0.69
rs12480328	20q13, 49527922	<i>ADNP</i>	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	0.93	1.13	0.18
rs1041449	21q22, 42901421	<i>TMPRSS2</i>	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	0.44	1.06	0.84
rs2238776	22q11, 19757892	<i>TBX1</i>	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	0.73	1.08	0.60

SNP ID	Chromosome, position ^b	Nearby Genes	Alleles ^c	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			Multiethnic 44,107 cases 45,172 controls		P _{het} value ^d
<i>Risk Loci Revealed in European Ancestry Meta-Analysis</i>																		
<i>Risk Loci Revealed in Early-Onset Meta-Analysis^f</i>																		
rs636291	1p35, 10556097	PEX14	A/G	OR	P	RAF ^a	OR	P	RAF ^a	OR	P	RAF ^a	OR	P	RAF ^a	OR	P	
				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 Minor allele frequency <1%

^f Analysis limited to European ancestry populations as only small numbers of early onset cases (<55 years) were available in the other populations.