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Multiple Novel Prostate Cancer Predisposition Loci Confirmed by an International Study: The PRACTICAL Consortium

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Abstract

A recent genome-wide association study found that genetic variants on chromosomes 3, 6, 7, 10, 11, 19 and X were associated with prostate cancer risk. We evaluated the most significant single-nucleotide polymorphisms (SNP) in these loci using a worldwide consortium of 13 groups (PRACTICAL). Blood DNA from 7,370 prostate cancer cases and 5,742 male controls was analyzed by genotyping assays. Odds ratios (OR) associated with each genotype were estimated using unconditional logistic regression. Six of the seven SNPs showed clear evidence of association with prostate cancer ($P = 0.0007$ - $P = 10^{-17}$). For each of these six SNPs, the estimated per-allele OR was similar to those previously reported and ranged from 1.12 to 1.29. One SNP on 3p12 (rs2660753) showed a weaker association than previously reported [per-allele OR, 1.08 (95% confidence interval, 1.00-1.16; $P = 0.06$) versus 1.18 (95% confidence interval, 1.06-1.31)]. The combined risks associated with each pair of SNPs were consistent with a multiplicative risk model. Under this model, and in combination with previously reported SNPs on 8q and 17q, these loci explain 16% of the familial risk of the disease, and men in the top 10% of the risk distribution have a 2.1-fold increased risk relative to general population rates. This study provides strong confirmation of these susceptibility loci in multiple populations and shows that they make an important contribution to prostate cancer risk prediction.

Introduction

Prostate cancer is now the most common cancer in men in the Western world (Cancer Research UK Factsheets 2008). However, its etiology remains poorly understood. Incidence rates vary substantially worldwide, and have done so for many decades, indicating that lifestyle risk factors, perhaps in combination with genetic background, are important. To date, however, no lifestyle risk factors have been definitely identified.

Aside from demographic factors, the only well-established risk factors for prostate cancer are family history and racial/ethnic background. The risk of the disease in first-degree relatives of cases is approximately twice that in the general population (1,2) and the disease is more common in men of African descent (3). High-penetrance susceptibility loci, identified following genome-wide scans of high-risk families, have been reported by several research groups. However, the relative failure of linkage studies to replicate findings of prostate cancer loci (4) suggests that the disease is genetically complex, with substantial locus heterogeneity. If variant alleles confer a small or moderate risk for prostate cancer, even large-scale family-based, linkage studies including hundreds of families will lack sufficient power to detect deleterious variants.

Genome-wide association studies have emerged as a powerful approach to identify common genetic variants associated with disease risk (5-8). Eeles et al. (9) recently conducted such a study based on 1,854 prostate cancer cases with clinically significant disease who were diagnosed at age ≤ 60 years or with a positive family history. The 1,894 controls were aged ≥ 50 years and had a low prostate-specific antigen (PSA; < 0.5 ng/mL), which predicts a low lifetime prostate cancer risk. DNA samples were evaluated for 541,129 single-nucleotide polymorphisms (SNP) using the Illumina Infinium platform. Associations were replicated in a second stage that included 3,245 cases and 3,329 controls from studies conducted in the United Kingdom and Australia. By this process, we identified SNPs associated with prostate cancer risk at “genome-wide” levels of significance ($P_{\text{trend}} < 10^{-7}$) in seven novel regions: 3p, 6q, 7q, 10q, 11q, 19q, and Xp (9). In each region, a single SNP was sufficient to explain the association with all other tested SNPs (although none is necessarily causally associated with disease risk and the associations may reflect the effects of other as yet untested variants). The associations in three regions on 8q and two on 17q were also confirmed.

To provide accurate estimates of the risks associated with genetic variants and to evaluate the combined associations of these variants, it is necessary to evaluate the variants in a large series of cases and controls. To this end, we have established a new international consortium, PRACTICAL (*Prostate cancer association group to investigate cancer associated alterations in the genome*). This consortium currently includes studies from 13 centers. Here we report the first results from PRACTICAL for the seven SNPs reported in the U.K./Australian study.

Materials and Methods

Subjects were included from 13 studies comprising prostate cancer cases and controls: 6 from Europe, 5 from North America, and 2 from Australia. These comprised 7,623 prostate cancer cases and 5,913 male controls. Three of the studies used a hospital-based ascertainment of prostate cancer cases, whereas the remainder were population-based studies. The Mayo Clinic study oversampled cases from multiple case families. For two of the studies (San Francisco and University of Southern California), a substantial fraction of subjects were of African American ancestry (19% and 29%, respectively). The University of Southern California study also included a substantial fraction of Hispanic men (26%). The remaining studies were predominantly of men of European ancestry. The Montreal study ascertained men of Ashkenazi Jewish ancestry. All studies have the relevant Institutional Review Board approval in each country in accordance with the principles embodied in the Declaration of Helsinki. Details of each study set are given below, and a summary of the studies is given in Table 1.

Fred Hutchinson Cancer Research Center, Seattle, Washington

The study population consists of participants from two population-based case-control studies in Caucasian and African American residents of King County, Washington (Study I and Study II), which have previously been described. Incident cases with histologically confirmed prostate cancer were ascertained from the Seattle-Puget Sound Surveillance, Epidemiology, and End Results cancer registry. In Study I, cases were diagnosed between January 1, 1993, and December 31, 1996 and were 40 to 64 y of age at diagnosis. In Study II, cases were diagnosed between January 1, 2002, and December 31, 2005 and were 35 to 74 y of age at diagnosis. Overall, 2,244 eligible prostate cancer patients were identified and 1,754 (78%) were interviewed. Blood samples yielding sufficient DNA for genotyping were drawn from 1,457 (83%) cases who completed the study interview.

A comparison group of controls without a self-reported history of prostate cancer, residing in King County, Washington, was identified for each study using random digit telephone dialing. Controls were frequency matched to cases by 5-y age groups and recruited evenly throughout each ascertainment period for cases. A total of 2,448 men were identified who met the eligibility

criteria and 1,754 (72%) completed a study interview. Blood samples were drawn and DNA prepared from 1,358 (77%) interviewed controls.

Hannover Prostate Cancer Study, Hannover, Germany

A hospital-based series of 499 unselected Caucasian patients with prostate cancer, who were treated with brachytherapy between October 2000 and September 2007 at Hannover Medical School, were enrolled for this study. All patients had biopsy-proven adenocarcinoma of the prostate. Indication for permanent brachytherapy was clinically localized low-risk early prostate cancer (cT_{2a} or less with a PSA serum level <10 ng/mL and a Gleason score <7) following the European Society for Therapeutic Radiology and Oncology/European Association of Urology/European Organization for Research and Treatment of Cancer recommendations. The median age at diagnosis was 67 y in this patient series (range, 42-82 y). For comparison, a series of 504 genomic DNA samples was established from ethnically matched adult male blood donors at Hannover Medical School in the period from 2006 to 2007.

Mayo Clinic, Rochester, Minnesota

The Mayo Clinic study (10) consisted of hospital-based cases, including 432 affected men from 177 families with prostate cancer, 489 men with sporadic prostate cancer, 193 with aggressive (Gleason score >7) prostate cancer, and 492 population-based controls. The controls (all males) were randomly selected from a sampling frame of Olmsted County, Minnesota, provided by the Rochester Epidemiology Project. The methods used to ascertain familial and sporadic prostate cancer patients, as well as controls, have previously been described (10). All individuals from the Mayo Clinic study included in this report were of self-reported European descent.

Melbourne Collaborative Cohort Study, Melbourne, Australia

This is a prospective cohort of 17,154 men of ages 40 to 69 y at recruitment in 1990 to 1994 (Melbourne Collaborative Cohort Study; $n = 190$). Cases ($n = 322$) were participants diagnosed with prostate cancer during follow-up in early 2005 and were ascertained through linkage with the Victorian Cancer Registry and the National Cancer Statistics Clearing House that includes diagnoses from other states in Australia. Controls ($n = 280$) were a random sample of the Melbourne Collaborative Cohort Study participants that were not diagnosed with prostate cancer during follow-up. All study subjects were of Caucasian origin.

Montreal Canada

Prostate cancer cases were identified using a hospital-based tumor registry. They included self-reported Ashkenazi Jewish men with prevalent prostate cancer diagnosed between 1991 and 2002 and diagnosed or treated in one of three large McGill University affiliated hospitals in metropolitan Montreal. Patients were considered to be Ashkenazi Jewish if both parents were reported as Ashkenazi Jewish, with no Sephardic heritage. The diagnosis of invasive prostate carcinoma was confirmed by examining the pathology reports present in the medical charts of all eligible participants.

The control group is derived from Ashkenazi Jewish donors who contributed a DNA sample to the National Laboratory for the Genetics of Israeli Populations.³³ All participants are adult Israeli citizens (>18 y old). Available information on these controls is limited to gender and ethnic origin.

³³<http://www.tau.ac.il/medicine/NLGIP/nlgip.htm>

ProtecT, United Kingdom

ProtecT (11) is a national study of community-based PSA testing and a randomized trial of subsequent prostate cancer treatment. Approximately 200,000 men between the ages of 50 and 69 y, ascertained through general practices in nine regions in the United Kingdom, are being recruited. Men known to be non-White were excluded. For this study, 449 cases identified by PSA screening within the ProtecT study were analyzed. Controls with normal PSA levels (<3 ng/mL) were selected from the same GP register and 5-y age band as the cases.

Queensland, Australia

The Queensland (12) cases were ascertained from two studies: (a) a series of men recruited within 2 y of their diagnosis of prostate cancer (Retrospective Queensland Study) and identified through physician referrals from three hospitals in Brisbane, Queensland ($n = 173$; age range, 51-87 y); (b) a longitudinal cohort study (Prostate Cancer Supportive Care and Patient Outcomes Project: ProsCan) being conducted in Queensland, through which men newly diagnosed with prostate cancer from 26 private practices and 10 public hospitals were directly referred to ProsCan at the time of diagnosis by their treating clinician ($n = 353$; age range, 43-87 y). All cases had histopathologically confirmed prostate cancer, following presentation with an abnormal serum PSA and/or lower urinary tract symptoms. Controls comprised healthy male blood donors with no personal history of any cancer, recruited through the Australian Red Cross Blood Services in Brisbane ($n = 176$; age range, 19-76 y).

San Francisco, California

This population-based case-control study of advanced prostate cancer in non-Hispanic White and African American men was conducted in the San Francisco Bay Area (13). Newly diagnosed cases of ages 40 to 79 y were identified through the regional cancer registry, which is part of the Surveillance, Epidemiology, and End Results Cancer Registry Program. Non-Hispanic White cases were diagnosed between July 1, 1997 and February 28, 2000, and African American cases were diagnosed between July 1, 1997 and December 31, 2000. Overall, 1,015 patients with a first primary advanced prostate cancer were identified. Of these, 106 were deceased at the time of contact; 33 were enrolled in another study and thus not available; 12 were declined contact by their physician; and 76 no longer lived in the San Francisco Bay area or did not meet other eligibility criteria. Of 788 eligible cases contacted, 568 (72%) completed the interview and 533 (68%) provided a biospecimen sample. DNA from blood samples was available for 389 cases.

Non-Hispanic White and African American population controls of ages 40 to 79 y were identified through random-digit dialing. In addition, controls of ages 65 to 79 y were randomly selected from the rosters of beneficiaries of the Health Care Financing Administration. Controls were frequency matched to cases by 5-y age group and race. Of 1,081 controls selected into the study, 16 were deceased at the time of contact; 41 had a history of prostate cancer; and 156 did not meet other eligibility criteria. Of 868 eligible controls contacted, 545 (63%) completed the interview and 525 (60%) provided a biospecimen sample. DNA from blood samples was available for 256 controls.

Tampere, Finland

All samples collected in Tampere are of Finnish origin (14). The mean age of diagnosis for the 838 unselected consecutive prostate cancer patients was 61 y (range, 43-77 y). The patients were diagnosed with prostate cancer in 1991 to 2006 in the Tampere University Hospital, Department of Urology. Tampere University Hospital is a regional referral center in the area for all patients with prostate cancer, which results in an unselected, population-based collection of patients. The 919 Finnish population controls consisted of DNA samples from anonymous,

voluntary, and healthy male blood donors obtained from the Blood Center of the Finnish Red Cross in Tampere.

U.K. Genetic Prostate Cancer Study, United Kingdom

Samples were ascertained through the U.K. Genetic Prostate Cancer Study and through a systematically collected series from prostate cancer clinics in the Urology Unit at The Royal Marsden NHS Foundation Trust over a 14-y period, as described by Eeles et al. (9).

Controls were collected as part of the “Gene-Environment Interactions in Prostate Cancer” or The Prostate Cancer Research Foundation studies run from the University of Nottingham from GP practices participating in the U.K. Genetic Prostate Cancer Study. They had no personal or family history of prostate cancer.

Ulm, Germany

Cases were recruited in two different ways (15). Familial prostate cancer probands (index cases) were ascertained from all over Germany. They were advised by their attending physicians to contact the Clinic of Urology of Ulm. The positive family history was then verified by reviewing medical records or death certificates of family members. In each case, only one member of each family (e.g., the index proband) was enrolled in the present study. Sporadic cases, who reported no relatives affected with prostate cancer, were almost exclusively collected at Ulm in the course of treatment (e.g., radical prostatectomy) in our Clinic of Urology. Controls were age-matched men from Ulm who reported no evidence of the disease and no relatives affected with prostate cancer. Of the controls, 83.2% were confirmed by digital rectal examination, which had to be negative, and/or PSA <4 ng/mL. In the remaining 16.8% of the controls, no further verification of their healthy status was possible.

University of Southern California, Los Angeles, Southern California

Subjects were participants in a population-based case-control study of aggressive prostate cancer conducted in Los Angeles County. Cases were identified through the Los Angeles County Cancer Surveillance Program rapid case ascertainment system. Large hospitals are screened at least weekly, and other sites at least monthly. Eligible cases included African American, Hispanic, and non-Hispanic White men diagnosed with a first primary prostate cancer between January 1, 1999 and December 31, 2003. Eligible cases also had (a) prostatectomy with documented tumor extension outside the prostate, (b) metastatic prostate cancer in sites other than prostate, (c) needle biopsy of the prostate with Gleason grade ≥ 8 , or (d) needle biopsy with Gleason grade 7 and tumor in more than two thirds of the biopsy cores.

Eligible controls were men never diagnosed with prostate cancer, living in the same neighborhood as a case, and were frequency matched to cases on age (± 5 y) and race/ethnicity. Controls were identified by a neighborhood walk algorithm, which proceeds through an obligatory sequence of adjacent houses or residential units beginning at a specific residence that has a specific geographic relationship to the residence where the case lived at diagnosis.

Valais, Switzerland

Between December 1, 2002 and January 31, 2007, all urologists in a relatively isolated alpine region of Switzerland (canton du Valais) invited their patients diagnosed with invasive prostate cancer (all stages) to participate to a research project on genetic factors involved in prostate cancer. Both parents were required to originate from the canton du Valais. A detailed family history on at least three generations was collected by a trained research nurse, as well as a blood sampling. A series of healthy men, without a self-reported family history of prostate cancer,

originating from the same region, participated as controls (blood donors and elderly patients seen in private practice). Most of these men had regular PSA screening.

Genotyping

We tested the seven SNPs (one from each genomic region) that were independently associated with prostate cancer in the study by Eeles et al. (Table 2; ref. 9). One SNP (rs5945619) failed for the Queensland study and this study is not therefore included in the analysis of this SNP. For all but two centers, the genotyping was done with the 5' nuclease assay (TaqMan™) using the ABI Prism 7900HT sequence detection system according to the manufacturer's instructions. Primers and probes were supplied by Applied Biosystems as Assay-by-Design™. For the genotyping done in Montreal (Valais and Montreal sample sets), the Applied Biosystems 7500 fast Real-time PCR system, using 96-well plates, was used according to the manufacturer's instructions for TaqMan. The Australian center in Queensland used the iPLEX Sequenom MassArray system. For genotyping of The Fred Hutchinson Cancer Research Center samples (the Fred Hutchinson Cancer Research Center/National Human Genome Research Institute group), National Human Genome Research Institute investigators used the Applied Biosystems SNPlex Genotyping System and proprietary GeneMapper software for allele assignment.³⁴ Discrimination of the specific SNP allele was carried out with the ABI 3730xl DNA Analyzer, based on the presence of a unique sequence assigned to the original allele-specific oligonucleotide.

Assays at all sites included at least two negative controls and two duplicates (in Queensland there were 140 duplicates). Quality control guidelines were followed by all the participating groups (see Supplementary data). We excluded any subjects that provided genotype data for less than six of the seven SNPs. All markers passed the Hardy-Weinberg threshold of $P < 0.00001$ and the call rate threshold of 95%. All centers typed at least 2% of samples as blind duplicates; in all instances, fewer than 2% of duplicate samples were discordant. Before the publication by Eeles et al. (9), the SNP hits were disclosed to the PRACTICAL collaborating groups under confidentiality release agreements. The Mayo Clinic group had, before the establishment of PRACTICAL, independently typed several SNPs in collaboration with deCODE Genetics (16). Although not identical, one of these was in the vicinity of the X-chromosome marker reported in the present PRACTICAL study. Of the total Mayo samples genotyped for this region, 90% are in common between the two studies (16).

Statistical Methods

ORs and 95% confidence intervals (95% CI) associated with each genotype were estimated using unconditional logistic regression, stratified by study and racial/ethnic group. We assessed the association between each SNP and prostate cancer using a stratified 1-degree-of-freedom Cochran-Armitage trend test and a general 2-degree-of-freedom χ^2 test. For all studies, except the San Francisco and University of Southern California studies, minority racial/ethnic minority groups were excluded. We also excluded 148 female controls typed by the Montreal study. The Mayo Clinic study included an additional 675 affected individuals from 177 multiple case families. For the test statistics, only one affected individual from each family, selected at random, was included. For the OR estimates, cases from these multiple case families were excluded entirely to avoid inflation of the risk estimates due to selection for family history. After these exclusions, 7,370 prostate cancer cases and 5,742 control subjects were included in the analysis.

The combined associations of multiple SNPs were first evaluated by testing for departures from a multiplicative model of interaction. This was done in two ways: first, using a case-only

³⁴<http://www.appliedbiosystems.com>

analysis in which the genotypes at one SNP were regressed against the genotypes of another SNP in cases using polytomous regression; second, by a case-control analysis, which included an interaction term in multiple logistic regression. We also assessed the combined effects of the SNPs by deriving a risk score based on the estimated ORs for each SNP (without interactions) and then estimating the OR based on the quantiles of the distribution of the risk score. Modification of the OR by age at diagnosis was tested using a case-only analysis, which assessed the association of age at onset on SNP genotypes in the cases using polytomous regression. The association of family history on the ORs was also assessed by a case-only analysis, treating a positive history as the outcome in a logistic regression. The contribution of each locus to the overall familial risk was estimated by $\log(\lambda_k)/\log(\lambda_0)$, where λ_k is the predicted relative risk to offspring of affected individuals due to locus k , given the allele frequency and per-allele OR, and λ_0 is the observed familial relative risk, assumed to be 2 (1, 2, 17). The combined effect of all loci was computed by summing the individual effects (i.e., assuming a multiplicative model). All analyses were done using Stata version 9.0 (18).

Results and Discussion

Six of the seven SNPs tested in this study showed clear evidence of association with prostate cancer (Table 2; Fig. 1). For each SNP, the estimated per-allele OR was very similar to that estimated by the second stage of the original genome-wide association study (9). For the 3p marker, rs2660753, the estimated OR (OR, 1.08; 95% CI, 1.00-1.16) was lower than found by the original study (OR, 1.18) and of borderline statistical significance, although the confidence intervals still overlapped. None of the SNPs showed significant heterogeneity in the per-allele OR across studies.

Three of the six autosomal markers showed a clear allele dosage association, with an intermediate risk for heterozygotes, consistent with the associations reported previously. For two markers, rs6465657 (7q21) and rs10993994 (10q11), the homozygote OR was greater than predicted under a multiplicative model, but not significantly so. For rs9364554 (6q25), we saw no apparent difference in risk between heterozygotes and homozygotes (i.e., a dominant model; $P = 0.06$ for departure from multiplicativity), consistent with the pattern seen in stage 2 of the genome-wide association study. rs2660753 (3p12) and rs2735839 (19q13) also seemed to be more consistent with a dominant model, but the minor allele for these markers was relatively rare in men of European and Australian and American ancestry (11% and 15%, respectively), and the ORs did not differ significantly from a multiplicative model. This apparent dominant association was also seen in stage 2 of the genome-wide association study for rs2735839 (19q13), but not for rs2660753 (3p12) where the homozygote OR was markedly higher (OR, 2.09; 95% CI, 1.39-3.15) than observed here (OR, 1.06; 95% CI, 0.84-1.34). For six of the seven SNPs, the minor allele in men of European ancestry was associated with an increased prostate cancer risk [for rs7931342 (11q13), the alleles were of approximately equal frequency]. There were, however, substantial differences in allele frequencies between racial/ethnic groups for many of the SNPs. In particular, for rs6465657 (7q21) and rs10993994 (10q11), the risk allele was the major allele in the African American controls. The number of African American samples analyzed here was too small to estimate the ORs separately for this group. However, there was no evidence for a difference in the per-allele OR between this racial/ethnic group and the men of European ancestry for any of the tested SNPs. In particular, for the most strongly associated SNP (rs10993994 in *MSMB*) the estimated OR did not differ between European and African American men [OR, 1.24 (95% CI, 0.97-1.59; $P = 0.09$) in African Americans and 1.25 (95% CI, 1.18-1.31; $P = 5 \times 10^{-17}$) in men of European/European American ancestry].

The per-allele ORs for each SNP by family history are summarized in Table 3. For rs10993994 (10q11), the frequency of the risk allele was somewhat higher in men with a family history

($P = 0.06$), as would be expected under a simple polygenic model (Table 3). None of the other markers showed a significantly stronger association for men with a family history. However, only 20% of cases reported a positive family history in any relative, so the confidence limits of the ORs associated with a family history are wide. There was some suggestion of an increase in the OR with age at diagnosis for rs2660753 (3p12; $P = 0.07$), but there was no evidence for a similar trend in OR for any other SNP (Table 4).

Gleason score was available for 3,125 of the cases used in the analyses; of these, 190 had a score of <5 ; 2,454 were in the range of 5 to 7; and 481 men had a score of ≥ 8 . We found some evidence that the association with the chromosome 6 marker, rs9364554, was more pronounced for high-grade tumors ($P = 0.06$; Table 5). There was no clear pattern of association with tumor grade for any of the other SNPs.

We investigated the joint association of each pair of SNPs, using both case-only and case-control analyses, to examine departures from a multiplicative model. None of the 21 pairwise analyses showed significant evidence for interaction on this scale: the strongest evidence found was for rs2735839 (19q13) and rs5945619 (Xp11; $P = 0.07$ in the case-only analysis), rs9364554 (6q25) and rs10993994 (10q11; $P = 0.08$ in the case-control analysis), and rs7931342 (11q13) and rs2735839 (19q13; $P = 0.08$ in the case-control analysis).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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We next ranked each man based on his predicted risk, assuming a simple multiplicative model [i.e., the sum of the number of high-risk alleles, weighted by the estimated log (per-allele OR)], and computed empirical ORs for categories of risk. As expected, the estimated ORs increased consistently with increasing risk score, the OR being 3.52-fold for men in the top 1% of the distribution compared with men in the lowest 1% (Table 6). These ORs are close to those predicted under a simple multiplicative model.

The primary purpose of this study was to test a large number of prostate cancer cases and controls collected from diverse populations for association with seven loci previously identified by a genome-wide association study. All seven loci showed at least some evidence of association consistent with that found by the original study. Five of the SNPs were significant at $P = 7 \times 10^{-6}$ or better in the replication study, with per-allele ORs close to those found in stage 2 of the genome-wide association study (9), which was based on case-control studies conducted in Australia and the United Kingdom. For the chromosome 19 SNP (rs2735839), which lies between *KLK2* and *KLK3*, the association was more modest ($P = 0.0007$) but the

per-allele OR was still close to that observed in the original genome-wide association study. rs2660753, on chromosome 3, showed a somewhat weaker association. This may simply be a chance finding because the OR did not differ significantly from that previously found, but it may reflect, in part, “winner's curse,” whereby the magnitudes of the most significant associations tend to be exaggerated in the initial study compared with those in the replication studies. Although rs2660753 reached genome-wide levels of significance, this was the weakest of the associations found by the genome-wide association study ($P = 2.7 \times 10^{-8}$) and was therefore the most susceptible to this phenomenon.

Of the autosomal loci, the strongest association was with rs10993994 on 10q11, for which the estimated per-allele OR was 1.25 and the estimated OR for TT homozygotes, relative to CC homozygotes, was 1.57. This SNP lies only 2 bp upstream of the transcription initiation site of the *MSMB* gene, a gene that encodes PSP94, an immunoglobulin binding factor that is secreted into the seminal plasma (19). Thomas et al. (20) independently reported an association with this SNP, with a very similar OR, from their recent genome-wide association study. The same group resequenced across *MSMB* in 80 individuals and found no other closely correlated SNPs, suggesting that this variant may be causally implicated in the observed association. It is interesting to note that this SNP seems to confer a similar relative risk in African American men to that in men of European ancestry, indicating that this association is independent of racial/ethnic background. Thomas et al. (20) also found strong evidence of association with a marker on chromosome 11q13, rs10896449, which is closely correlated with rs7931342 at 11q13.

The highest per-allele OR for any marker was found on the X chromosome with marker rs5945619; the estimated per-allele OR in the present study (OR, 1.29; 95% CI, 1.20-1.39) was actually slightly higher than that found in the genome-wide association study. This marker lies in a block spanning >2 Mb that includes the X centromere. Gudmundsson et al. (16) reported an association with another SNP in the same region, rs5945572, which is closely correlated with this SNP, further supporting this association.

The combined associations of these loci on prostate cancer risk seem to be consistent with a simple multiplicative model, as judged both by examining pairwise interactions between loci and by estimating the ORs using quantiles of the risk distribution. If this model is assumed to be correct, the estimated risk to men in the top 1% of the risk distribution is ~3.8-fold greater than the risk to men in the bottom 1%, which translates into an ~2-fold risk relative to the population average. In addition to the loci considered here, eight additional common prostate cancer susceptibility loci have been reported: three on 8q, two on 17q, and the recent loci on 2q reported by Gudmundsson et al. (16) and on 10q and 7p by Thomas et al. (20). If one assumes that all these loci combine multiplicatively, the combined risk distribution defined by all these SNPs would imply a risk of ~3.2-fold for the top 1% of the risk distribution, or 2.1-fold for the top 10%, compared with the population average. These risks correspond to absolute lifetime risks of prostate cancer of ~26% and 18%, respectively, by age 80 y, based on recent reports of incidence rates for England and Wales, compared with the population average of 9% (Cancer Research UK Factsheets).

In total, the 15 reported susceptibility variants explain ~16% of the familial risk of prostate cancer. Additional variants are likely to be identified through further analysis of genome-wide association studies, and these will improve the predictive power of genetic profiling for prostate cancer risk. Large-scale collaborations such as PRACTICAL will be needed to estimate with confidence and precision the combined prostate cancer risks associated with all variants.

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Figure 1.

Forrest plots for the tested SNPs shown individually by study center for each SNP: rs2660753 (3p12), rs9364554 (6q25), rs6465657 (7q21), rs10993994 (10q11), rs7931342 (11q13), rs2735839 (19q13), and rs5945619 (Xp11).

Table 1

The PRACTICAL Group and sample sets

	Total no.		Used no.		Type of study		Age range of Case identification		Male controls identification	
	Controls	Cases	Controls	Cases	Cases	Case-control	cases (y)	cases (y)	Matching and ascertainment	Matching and ascertainment
FHCRC (Seattle, WA)	1,335	1,409	1,252	1,252	1,254	Population based	35-74	35-74	Age, telephone dialing	Age, telephone dialing
HaPCS (Hannover, Germany)	504	499	501	499	499	Hospital based	42-82	42-82	Ethnic origin, blood donors	Ethnic origin, blood donors
Mayo (Minnesota)	492	859	484	859	851	Hospital-based cases	40-86	40-86	Geographically, population via epidemiology study	Geographically, population via epidemiology study
MCCS (Melbourne, Australia)	280	322	278	322	320	Population based	49-81	49-81	Age, electoral roll	Age, electoral roll
Montreal (Canada)	146	147	146	147	147	Hospital based	49-84	49-84	Ethnic origin, population volunteers	Ethnic origin, population volunteers
ProtecT (United Kingdom)	449	465	408	465	448	Population based	46-71	46-71	PSA screen detected disease	Ethnic origin, age
Queensland (Australia; incorporating the ProsCan Study and the Retrospective Queensland Study)	177	527	171	527	516	Population based	43-87	43-87	Identified through clinicians in public hospitals and private clinics	Sex only, blood donors
San Francisco (California)	256	391	247	391	367	Population based	44-79	44-79	Age, race/ethnicity origin, random digit dialing	Age, race/ethnicity origin, random digit dialing
Tampere (Finland)	919	801	913	801	799	Population based	43-77	43-77	Sex only, blood donors	Sex only, blood donors
UKGPCS (United Kingdom; incorporating The Prostate Cancer Research Foundation Study)	375	343	368	343	327	Population and hospital based	36-84	36-84	Ethnic origin and age, GP practices	Ethnic origin and age, GP practices
ULM (Germany)	214	517	212	517	516	Population and hospital based	40-84	40-84	Geographically, age $\pm$ 5 y	Geographically, age $\pm$ 5 y
USC (California)	545	1,173	541	1,173	1,156	Population based	42-94	42-94	Age, geographically, neighborhood block walking	Age, geographically, neighborhood block walking
Valais (Switzerland)	221	170	221	170	170	Population based	49-84	49-84	Age, geographically, blood donors, patients in private clinics	Age, geographically, blood donors, patients in private clinics
Total	5,913	7,623	5,742	7,623	7,370					

Abbreviations: FHCRC, Fred Hutchinson Cancer Research Center; MCCS, Melbourne Collaborative Cohort Study; UKGPCS, U.K. Genetic Prostate Cancer Study; USC, University of Southern California.

Table 2

Combined ORs (95% CI) for tested SNPs

Marker	Chrom	Alleles	MAF (W/B)	Per-allele OR (95% CI)	Het OR (95% CI)	Hom OR (95% CI)	P_{trend}	Homogeneity P
rs2660753	3p12	C/T	0.11/0.50	1.08 (1.00-1.16)	1.11 (1.01-1.21)	1.06 (0.84-1.34)	0.06	0.90
rs9364554	6q25	C/T	0.28/0.06	1.14 (1.08-1.21)	1.20 (1.11-1.29)	1.22 (1.06-1.39)	3×10^{-6}	0.40
rs6465657	7q21	T/C	0.47/0.87	1.12 (1.07-1.18)	1.10 (1.01-1.20)	1.27 (1.14-1.40)	7×10^{-6}	0.23
rs10993994	10q11	C/T	0.39/0.58	1.25 (1.18-1.31)	1.21 (1.11-1.31)	1.57 (1.42-1.74)	10^{-17}	0.57
rs7931342	11q13	G/T	0.50/0.24	0.85 (0.80-0.89)	0.87 (0.80-0.94)	0.71 (0.65-0.79)	3×10^{-11}	0.71
rs2735839	19q13	G/A	0.15/0.30	0.89 (0.83-0.95)	0.86 (0.79-0.94)	0.90 (0.71-1.15)	0.0007	0.91
rs5945619	Xp11	T/C	0.35/0.40	1.29 (1.20-1.39)		1.29 (1.20-1.39)	10^{-11}	0.29

Abbreviations: W, Whites; B, Blacks.

Table 3
Per-allele ORs (95% CI) associated with a family history of prostate cancer among cases compared with men without a family history

Marker	OR (95% CI)
rs2660753 (3p12)	0.92 (0.80-1.07)
rs9364554 (6q25)	1.06 (0.94-1.20)
rs6465657 (7q21)	0.96 (0.86-1.07)
rs10993994 (10q11)	1.08 (0.98-1.21)
rs7931342 (11q13)	0.89 (0.79-0.99)
rs2735839 (19q13)	1.02 (0.88-1.18)
rs5945619 (Xp11)	1.06 (0.89-1.25)

Table 4
ORs (95% CI) by age (at diagnosis for cases and age at interview for controls)

Marker	<55	55-59	60-64	65-69	70+	P _{trend}
rs2660753 (3p12)	0.96 (0.77-1.18)	1.01 (0.83-1.23)	1.06 (0.86-1.28)	1.25 (1.01-1.54)	1.33 (1.06-1.66)	0.07
rs9364554 (6q25)	1.14 (0.97-1.34)	1.06 (0.91-1.22)	1.28 (1.11-1.47)	1.03 (0.88-1.22)	1.19 (1.00-1.42)	0.82
rs6465657 (7q21)	1.20 (1.03-1.38)	1.20 (1.04-1.37)	1.02 (0.90-1.17)	1.16 (1.00-1.34)	1.06 (0.90-1.25)	0.17
rs10993994 (10q11)	1.32 (1.14-1.53)	1.35 (1.18-1.54)	1.13 (1.00-1.29)	1.26 (1.08-1.46)	1.45 (1.24-1.70)	0.64
rs7931342 (11q13)	0.87 (0.75-1.01)	0.81 (0.71-0.93)	0.78 (0.69-0.89)	0.83 (0.72-0.96)	0.90 (0.77-1.05)	0.66
rs2735839 (19q13)	0.82 (0.67-1.00)	0.73 (0.61-0.88)	0.95 (0.71-1.13)	0.85 (0.69-1.04)	1.02 (0.83-1.15)	0.18
rs5945619 (Xp11)	1.34 (1.07-1.68)	1.25 (1.02-1.52)	1.48 (1.22-1.79)	1.21 (0.97-1.50)	1.27 (1.01-1.60)	0.91

Table 5
ORs (95% CI) by tumor grade

Marker	Gleason 1-4	Gleason 5-7	Gleason 8+	P_{trend}
rs2660753 (3p12)	1.04 (0.74-1.44)	1.14 (1.02-1.28)	0.94 (0.76-1.18)	0.25
rs9364554 (6q25)	0.94 (0.75-1.20)	1.12 (1.03-1.22)	1.15 (0.99-1.35)	0.06
rs6465657 (7q21)	0.87 (0.70-1.07)	1.16 (1.07-1.25)	0.98 (0.84-1.13)	0.89
rs10993994 (10q11)	1.06 (0.86-1.32)	1.27 (1.18-1.37)	1.14 (0.99-1.32)	0.73
rs7931342 (11q13)	0.92 (0.75-1.12)	0.86 (0.80-0.93)	0.84 (0.73-0.97)	0.36
rs2735839 (19q13)	0.91 (0.67-1.25)	0.91 (0.81-1.01)	0.95 (0.77-1.17)	0.54
rs5945619 (Xp11)	1.03 (0.76-1.41)	1.34 (1.21-1.50)	1.37 (0.95-1.45)	0.45

Table 6
ORs (95% CI) by approximate quantiles of the risk score

Marker	OR (95% CI)	Predicted OR
<1%	1.0	1.0
1-10%	1.36 (0.78-2.40)	1.22
10-25%	1.86 (1.07-3.22)	1.61
25-75%	2.35 (1.35-4.08)	2.03
75-90%	3.06 (1.76-4.08)	2.42
90-99%	3.50 (2.00-6.12)	2.97
>99%	3.52 (1.87-6.64)	3.81