Published in final edited form as:
Hum Genet. 2012 July ; 131(7): 1095–1103. doi:10.1007/s00439-011-1136-0.

Validation of prostate cancer risk-related loci identified from genome-wide association studies using family-based association analysis: evidence from the International Consortium for Prostate Cancer Genetics (ICPCG)

Guangfu Jin,
Data Coordinating Center for the ICPCG and Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem NC 27157, USA

Lingyi Lu,
Data Coordinating Center for the ICPCG and Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem NC 27157, USA

Kathleen A. Cooney,
Departments of Internal Medicine and Urology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA. University of Michigan ICPCG Group, Ann Arbor, USA

Anna M. Ray,
Departments of Internal Medicine and Urology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA. University of Michigan ICPCG Group, Ann Arbor, USA

Kimberly A. Zuhlke,
Departments of Internal Medicine and Urology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA. University of Michigan ICPCG Group, Ann Arbor, USA

Ethan M. Lange,
Departments of Genetics and Biostatistics, University of North Carolina, Chapel Hill, NC 27599, USA

Lisa A. Cannon-Albright,
University of Utah ICPCG Group, Division of Genetic Epidemiology, Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT 84108, USA. George E. Wahlen Department of Veterans Affairs Medical Center, Salt Lake City, UT 84148, USA. University of Michigan ICPCG Group, Ann Arbor, USA

Correspondence to: Sarah D. Isaacs, wisaacs@jhmi.edu; Elaine A. Ostrander, eostrand@mail.nih.gov; Jianfeng Xu, jxu@wakehealth.edu.

G. Jin and L. Lu contributed equally to this work.
K.A. Cooney, A.M. Ray, K.A. Zuhlke and E.M. Lange are the members of the University of Michigan ICPCG Group.
L.M. FitzGerald and J.L. Stanford are the members of the FHCRC/NHGRI ICPCG Group.
W.D. Foulkes, G.G. Giles, J.L. Hopper, G. Severi, R. Eeles, D. Easton, Z. Kote-Jarai, and M. Guy are the members of the ACTANE Consortium ICPCG Group.
A. Rinckleb, C. Maier and W. Vogel are the members of the University of Ulm ICPCG Group.
S.N. Thibodeau, S.K. McDonnell and D.J. Schaid are the members of the Mayo Clinic ICPCG Group.
F. Wiklund, H. Grönberg and M. Emanuelsson are the members of the University of Umeå ICPCG Group.
A.S. Whittemore, I. Oakley-Girvan and C.-L. Hsieh are the members of the BC/CA/HI ICPCG Group.
T. Wahlfors, T. Tammela and J. Schleutker are the members of the University of Tampere ICPCG Group.

Nicola J. Camp,
University of Utah ICPCG Group, Division of Genetic Epidemiology, Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT 84108, USA

Craig C. Teerlink,
University of Utah ICPCG Group, Division of Genetic Epidemiology, Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT 84108, USA

Liesel M. FitzGerald,
Division of Public Health Sciences, Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA 98195, USA

Janet L. Stanford,
Division of Public Health Sciences, Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA 98195, USA

Kathleen E. Wiley,
Johns Hopkins University ICPCG Group, Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD 21287, USA

Sarah D. Isaacs,
Johns Hopkins University ICPCG Group, Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD 21287, USA

Patrick C. Walsh,
Johns Hopkins University ICPCG Group, Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD 21287, USA

William D. Foulkes,
Program in Cancer Genetics, McGill University, Montreal, QC H3T 1E2, Canada

Graham G. Giles,
Cancer Epidemiology Centre, Cancer Council Victoria, Carlton, VIC 3053, Australia. Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, Melbourne, VIC 3010, Australia

John L. Hopper,
Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, Melbourne, VIC 3010, Australia

Gianluca Severi,
Cancer Epidemiology Centre, Cancer Council Victoria, Carlton, VIC 3053, Australia. Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, Melbourne, VIC 3010, Australia

Ros Eeles,
The Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK

Doug Easton,
Departments of Public Health and Primary Care and Oncology, University of Cambridge, Cambridge CB1 8RN, UK

Zsofia Kote-Jarai,
The Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK

Michelle Guy,
The Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK

Antje Rinckleb,

Department of Urology, University of Ulm, Ulm, Germany. Institute for Human Genetics, University of Ulm, Ulm, Germany

Christiane Maier,
Department of Urology, University of Ulm, Ulm, Germany. Institute for Human Genetics, University of Ulm, Ulm, Germany

Walther Vogel,
Institute for Human Genetics, University of Ulm, Ulm, Germany

Geraldine Cancel-Tassin,
CeRePP ICPCG Group, Hopital Tenon, Assistance Publique-Hopitaux de Paris, 75020 Paris, France

Christophe Egrot,
CeRePP ICPCG Group, Hopital Tenon, Assistance Publique-Hopitaux de Paris, 75020 Paris, France

Olivier Cussenot,
CeRePP ICPCG Group, Hopital Tenon, Assistance Publique-Hopitaux de Paris, 75020 Paris, France

Stephen N. Thibodeau,
Department of Lab Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA

Shannon K. McDonnell,
Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55905, USA

Daniel J. Schaid,
Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55905, USA

Fredrik Wiklund,
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Henrik Grönberg,
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Monica Emanuelsson,
Oncologic Centre, Umeå University, 90187 Umeå, Sweden

Alice S. Whittemore,
Department of Health Research and Policy, Stanford School of Medicine, Stanford, CA 94305, USA

Ingrid Oakley-Girvan,
Cancer Prevention Institute of California, 2201 Walnut Ave Suite 300, Fremont, CA 94538, USA. Department of Health Research and Policy, Stanford Cancer Institute, Stanford School of Medicine, Stanford, CA 94305, USA

Chih-Lin Hsieh,
Department of Urology, University of Southern California, Los Angeles, CA 90089, USA. Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA 90089, USA

Tiina Wahlfors,
Institute of Biomedical Technology, University of Tampere, BioMediTech, Tampere, Finland. Centre for Laboratory Medicine, Tampere University Hospital, 33520 Tampere, Finland

Teuvo Tammela,
Department of Urology, University of Tampere and Tampere University Hospital, 33520 Tampere, Finland

Johanna Schleutker,
Department of Medical Biochemistry and Genetics, University of Turku, 20014 Turku, Finland

William J. Catalona,
Northwestern University ICPCG Group, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

S. Lilly Zheng,
Data Coordinating Center for the ICPCG and Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem NC 27157, USA

Elaine A. Ostrander,
Cancer Genetics Branch, National Human Genome Research Institute (NHGRI), National Institutes of Health (NIH), Bethesda, MD 20892, USA

William B. Isaacs,
Johns Hopkins University ICPCG Group, Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD 21287, USA

Jianfeng Xu, and
Data Coordinating Center for the ICPCG and Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem NC 27157, USA

International Consortium for Prostate Cancer Genetics

Sarah D. Isaacs: wisaacs@jhmi.edu; Elaine A. Ostrander: eostrand@mail.nih.gov; Jianfeng Xu: jxu@wakehealth.edu

Abstract

Multiple prostate cancer (PCa) risk-related loci have been discovered by genome-wide association studies (GWAS) based on case–control designs. However, GWAS findings may be confounded by population stratification if cases and controls are inadvertently drawn from different genetic backgrounds. In addition, since these loci were identified in cases with predominantly sporadic disease, little is known about their relationships with hereditary prostate cancer (HPC). The association between seventeen reported PCa susceptibility loci was evaluated with a family-based association test using 1,979 hereditary PCa families of European descent collected by members of the International Consortium for Prostate Cancer Genetics, with a total of 5,730 affected men. The risk alleles for 8 of the 17 loci were significantly over-transmitted from parents to affected offspring, including SNPs residing in 8q24 (regions 1, 2 and 3), 10q11, 11q13, 17q12 (region 1), 17q24 and Xp11. In subgroup analyses, three loci, at 8q24 (regions 1 and 2) plus 17q12, were significantly over-transmitted in hereditary PCa families with five or more affected members, while loci at 3p12, 8q24 (region 2), 11q13, 17q12 (region 1), 17q24 and Xp11 were significantly over-transmitted in HPC families with an average age of diagnosis at 65 years or less. Our results indicate that at least a subset of PCa risk-related loci identified by case–control GWAS are also associated with disease risk in HPC families.

Introduction

Genome-wide association studies (GWAS) have succeeded in identifying low penetrance genetic risk factors that account for differing proportions of the hereditary variance associated with complex diseases (Manolio 2010). Using a GWAS approach, several hundred thousand to more than a million single nucleotide polymorphisms (SNPs) are assayed in thousands of individuals to compare the allele frequencies between the cases and the controls for each SNP (Hardy and Singleton 2009). Cases are generally a set of individuals who have been diagnosed with disease by a certain point in time, while controls are unaffected as of a particular reference date. One or more additional case–control studies are generally needed to confirm the GWAS findings, as the risk of false positives is

appreciable. Although reproducibility in multiple independent study populations is a strong argument for the existence of disease-associated SNPs, population stratification between cases and controls due to population admixture or natural selective pressure resulting in false-positive associations remains a concern (Price et al. 2010). In addition, it is critical to know if variants identified by studies of individuals from the general population are associated with disease risk among high-risk families.

Prostate cancer (PCa) has been extensively studied using GWAS, and the results have identified and replicated multiple risk-related SNPs (Amundadottir et al. 2006; Duggan et al. 2007; Eeles et al. 2008; Gudmundsson et al. 2007a, b; Thomas et al. 2008; Yeager et al. 2007). However, most of these variants were identified in case–control datasets in which there may have been the possibility of population stratification. Thus, it is of interest to further assess these findings in family-based studies. In addition, subjects participating in the reported case–control studies were generally recruited from the general population, and thus primarily represent sporadic cancer cases. Although familial cases have been included in some GWAS studies (Eeles et al. 2008), less is known about the relative importance of most of the risk alleles in high-risk or hereditary prostate cancer (HPC) families. In the present study, we sought to assess whether the genetic markers identified by GWAS are also relevant for HPC families. To address this question, we conducted a large family-based association study that included 1,979 Caucasian HPC families collected by members of the International Consortium for Prostate Cancer Genetics (ICPCG) to evaluate 17 loci that demonstrated a previous association with PCa in GWAS.

Subjects and methods

Study population

The ICPCG study population has been described in detail previously (Schaid and Chang 2005; Xu et al. 2005). Twelve groups participated in the present study, including ACTANE (Anglo/Canadian/Texan/Australian/Norwegian/European Union Biomed), BC/CA/HI (British Columbia, California, Hawaii), CeRePP (Centre de Recherche pour les Pathologies Prostatiques), Fred Hutchinson Cancer Research Center/National Human Genome Research Institute (FHCRC/NHGRI), Johns Hopkins University (JHU), Mayo Clinic, Northwestern University, University of Michigan, University of Tampere in Finland, University of Ulm in Germany, University of Umeå in Sweden, and University of Utah.

Each group within the ICPCG recruited its population via different methods of pedigree ascertainment and confirmation of PCa diagnosis (Schaid and Chang 2005). Nevertheless, there was a general consensus that affected individuals were all defined as men affected with PCa that had been confirmed by either medical records or death certificates. The status of self- or relative-reported men without either medical records or death certificate confirmation was considered as “unknown”. In addition, the affection status of all men without a diagnosis of PCa was coded as “unknown”, regardless of whether they had undergone screening for PCa. Hence, all analyses were based on the sharing of marker genotypes among affected men, with no consideration of phenotype for the remaining subjects. In total, 2,068 PCa families were collected by the ICPCG. After 89 families with African, Asian or other non-Caucasian ancestry (56, 15 and 18 families, respectively) were excluded from these analyses, the remaining 1,979 families with Caucasian ancestry were included in this study (Table 1). These families included a total of 5,730 affected members. Research protocols and study documentation were approved by each group’s Institutional Review Board.

SNPs selection

One SNP was selected from each of the 17 loci that had been shown to be significantly associated with PCa risk in previous GWAS ($P < 10^{-8}$) (Amundadottir et al. 2006; Duggan et al. 2007; Eeles et al. 2008; Gudmundsson et al. 2007a, b; Thomas et al. 2008; Yeager et al. 2007) and subsequently in a follow-up fine-mapping study (Sun et al. 2008b). As shown in Table 2, the loci included three independent positions at 8q24, two at 17q12, and one SNP each at chromosome 2p15, 3p12, 6q25, 7p15, 7p21, 9p13, 10q11, 10q26, 11q13, 17q24, 19q13, and Xp11. Moreover, seven additional SNPs at three independent regions of 8q24 (Witte 2007) were also included: two from region 1, three from region 2 and two from region 3. In addition, we included one SNP (rs979200) that was centromeric to the three at 8q24 that was found to be associated with PCa risk in fine-mapping studies (Salinas et al. 2008; Sun et al. 2008a).

Genotyping

All samples were coordinated and genotyped using the MassARRAY iPLEX (Sequenom, Inc., San Diego, CA, USA) at the Center for Cancer Genomics, Wake Forest University. Briefly, PCR assays were performed in a total volume of 5 μL that contained 10 ng of genomic DNA, 3.5 mM of $MgCl_2$, 0.5 U of HotStarTaq polymerase (QIA-GEN Inc., Valencia, CA, USA), 0.5 mM of each dNTP (Invitrogen, Carlsbad, CA, USA), and 0.06 μM of each primer. The PCR cycling conditions were 94°C for 15 min; followed by 45 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 1 min; followed by a final extension at 72°C for 3 min. Shrimp alkaline phosphatase (SAP) treatments were performed in a total volume of 7 μL that contained the entire PCR mixture and 0.3 U of SAP, with incubation at 37°C for 40 min. The iPLEX extension reactions were performed in a total volume of 9 μL that included the entire SAP reaction and 1× iPLEX termination mix, 1× iPLEX enzyme, and 5.625 μM of each extension primer. The samples were desalted with 6 mg of clean resin and then dispensed to a SpectroCHIP. The chips were scanned using the MALDI-TOF MS system, and the genotypes were analyzed by the MassARRAY Typer 3.4 (Sequenom Inc.). Duplicates and negative controls were included in each 96-well plate to ensure quality control (QC). Genotyping was performed by technicians blinded to sample status. The average concordance rate was >98% for the 25 SNPs.

Statistical methods

To test the linkage or linkage disequilibrium (association) of genotypes with HPC, data from nuclear families and (or) sibships were used to perform a family-based association test (FBAT). FBAT evaluates whether particular alleles are transmitted from parents to affected offspring in a proportion that is different from that expected under the null hypothesis of no association between marker and disease. The empirical variance estimator in FBAT was used to perform a valid test of association, accounting for the correlation of transmitted alleles among multiple affected individuals in the same family due to coinheritance. FBAT analysis was carried out using the appropriate software (Laird et al. 2000).

Results

The characteristics of the 1,979 ICPCG Caucasian HPC families are summarized in Table 1. More than 15% of families (305/1,979) had five or more members affected with PCa. Among 1,690 families with available information on age at prostate cancer diagnosis, 796 families (47.1%) had an average age at diagnosis ≤65 years.

As shown in Table 2, 7 of the 17 loci that were originally associated with PCa risk in previous GWAS showed a statistically significant association based on the family-based association tests. These included 8q24 region 2 (rs16901979, $P = 0.031$), 8q24 region 3

(rs6983267, $P = 0.017$), 10q11 (rs10993994, $P = 6.70 \times 10^{-5}$), 11q13 (rs10896449, $P = 2.31 \times 10^{-3}$), 17q12 region 1 (rs4430796, $P = 0.010$), 17q24 (rs1859962, $P = 1.03 \times 10^{-4}$) and Xp11 (rs5945619, $P = 6.92 \times 10^{-4}$). Among eight additional SNPs selected at 8q24, three regions demonstrated one significant SNP each (region 1: rs4242382, $P = 0.011$; region 2: rs1016343, $P = 0.020$; and region 3: rs7837328, $P = 0.019$). For all of the significant SNPs, the direction of association was consistent with the original reports, i.e., the over-transmitted alleles from parents to affected men (S-E(S) >0) were the same as the risk alleles reported in the original studies (Amundadottir et al. 2006; Duggan et al. 2007; Eeles et al. 2008; Gudmundsson et al. 2007a, b; Thomas et al. 2008; Yeager et al. 2007).

To evaluate the role of these selected SNPs in subsets of families characterized by number of affected individuals or early mean age at diagnosis, we performed an FBAT analysis, specifically, in families with five or more affected men, or those with an average age at diagnosis of 65 years or less. As shown in Table 3, among 305 families with five or more affected individuals, previously reported risk alleles for five SNPs in three loci were significantly over-transmitted from parents to affected men, including four SNPs found to be significant in all families: 8q24 region 1 (rs4242382: $P = 0.010$), 8q24 region 2 (rs16901979: $P = 0.034$; and rs1016343: $P = 0.010$), and 17q24 (rs1859962: $P = 1.62 \times 10^{-3}$), and one additional SNP at 8q24 region 1 (rs10091054: $P = 0.045$). For 796 families with average age of diagnosis ≤65 years, the risk alleles for six SNPs were found to be significantly over-transmitted, including five SNPs at 8q24 region 2 (rs16901979: $P = 0.023$), 11q13 (rs10896449: $P = 0.010$), 17q12 region 1 (rs4430796: $P = 0.012$), 17q24 (rs1859962: $P = 8.01 \times 10^{-4}$) and Xp11 (rs5945619: $P = 4.70 \times 10^{-3}$) that were also observed to be significant in all families, and one additional SNP at 3p12 (rs2660753: $P = 0.037$).

Discussion

In this study, we examined PCa risk-related loci that were identified in previous GWAS using a family-based association approach based on 1,979 HPC families of Caucasian descent collected by the ICPCG. Family-based association methods evaluate whether particular alleles are transmitted from parents to affected offspring in a proportion that is different from that expected under the null hypothesis of no association between marker and disease (Lunetta et al. 2000). Among 17 loci analyzed in this study, risk alleles of eight loci were observed to be significantly over-transmitted from parents to affected offspring. Because family-based studies utilize non-transmitted alleles from the same parents as the control, these methods are not susceptible to population stratification. Therefore, the results from our study provide more compelling evidence that these eight loci are truly associated with risk of PCa. This study also demonstrates that typically low penetrance variants identified from GWAS studies are also important for PCa in HPC families.

Family-based studies may be ideal for validating previously identified genetic risk factors from case–control studies by the absence of susceptibility to false-positive results due to population stratification. GWAS studies generally identify common variants of comparatively lower penetrance, while linkage-based family studies identify rare highly penetrant variants. The nine loci associations that were not replicated in this FBAT analysis may simply represent variants of low penetrance. Our study also does not distinguish which of several possible mutations may be causative. Therefore, there may exist mutations in a gene that are weakly penetrant, and that identify a given locus in a GWAS, while a few rare and highly penetrant mutations, such as loss-of-function mutations, segregate in a subset of families and lead to validation in the FBAT analysis. These approaches are also efficient when utilizing the large family-based samples that have been collected in the past for genetic linkage studies. In the present study, 1,979 families with a total of 5,730 affected

members collected from 12 groups in the ICPCG were analyzed. However, the diminished statistical power resulting from the matching of transmitted with non-transmitted alleles from the same family is a limitation of this approach (Lange et al. 2008). We cannot formally exclude the possibility that the remaining nine loci, which were originally associated with PCa risk in case–control studies (Amundadottir et al. 2006; Duggan et al. 2007; Eeles et al. 2008; Gudmundsson et al. 2007a, b; Thomas et al. 2008; Yeager et al. 2007), and for which significant differences between observed and expected allele transmissions were not observed in the current FBAT analyses, were not associated with disease risk in HPC families.

In addition to the validation of findings reported in previous studies, this study provides another level of confirmatory evidence supporting the importance of genetic risk factors in HPC. A GWAS performed by Eeles et al. (2008) using familial or early onset cases reported that genetic variants at 3p12, 6q25, 7q21, 8q24 region 1, region 2 and region 3, 10q11, 11q13, 17q12, 17q24, 19q13 and Xp11 were observed to have genome-wide significant associations with PCa risk, suggesting that these loci may account for some portion of the heritability in families with excess PCa. Our study confirms a role of loci at 8q24 region 1, region 2 and region 3, 10q11, 11q13, 17q12, 17q24, and Xp11, but not at 3p12, 6q25, 7q21 and 19q13.

We further focused on families with larger numbers of affected men as well as those with early onset PCa cases and found three and six loci with significant associations, respectively. Generally, families with such characteristics may be more likely to reflect an important role for genetic factors in disease etiology. However in this study, we noted that some significant loci were not involved in the larger or early onset families. This is not surprising, as GWAS are not aimed at identifying rare highly penetrant loci that would be expected to characterize such families. Thus, these loci may not contribute to the heritability of multiple or early onset PCa. Alternatively, our results may reflect limitations in statistical power associated with the data set. Therefore, we propose that the results based on significantly over-transmitted risk alleles in multiple or early onset families, but not all families, should be interpreted with caution.

Amundadottir et al. (2006) originally identified genetic variants at 8q24 to be associated with PCa risk using a genome-wide linkage scan in 323 extended PCa families followed by two case–control groups of European ancestry and one African American group. Subsequent studies have consistently confirmed these findings and suggested at least three distinct regions within 8q24 that are independently associated with PCa risk (i.e., regions 1, 2 and 3, Table 2) (Gudmundsson et al. 2007a; Haiman et al. 2007; Yeager et al. 2007). In the current study, we genotyped three to four SNPs at each region, and found a significant association for at least one SNP in each locus. Interestingly, the SNP rs16901979 at 8q24 region 2 was also significantly associated with PCa risk in families with five or more affected men, or those with an average age at diagnosis of 65 years or less. These results suggest that genetic variants at three independent regions of 8q24 are all implicated in the inherited risk of PCa in HPC families.

Our study has strengths and limitations. The family-based association design is a strength, offering the ability to reduce the risk of false-positive findings due to population stratification. Another strength is the large number of HPC families included in the analyses that yielded a measure of statistical power that reduces the inefficiency of family-based association studies when considering the matching of transmitted with non-transmitted alleles in the same family. A limitation is the potential for heterogeneous genetic and environmental influences in PCa families collected from multiple locations across the US and Europe.

In summary, we confirmed multiple PCa risk-associated loci identified in GWAS or follow-up studies using a family-based association design. Our results suggest that these regions associated with sporadic PCa risk are not due to population stratification or admixture, and may account for a proportion of the inherited risk associated with HPC families. Finally, this study demonstrates the value of utilizing existing familial samples, originally collected for the purpose of linkage, to identify findings from a GWAS that are relevant for a disease in high-risk families.

Acknowledgments

We would like to express our gratitude to the many families who participated in the studies involved in the International Consortium for Prostate Cancer Genetics (ICPCG). The ICPCG, including the consortium's Data Coordinating Center (DCC), is made possible by a grant from the National Institutes of Health U01 CA89600 (to William B. Isaacs). Additional support to participating groups, or members within groups, is as follows: University of Utah Group: The authors appreciate the support of the University of Utah Huntsman Cancer Institute (to Lisa A. Cannon-Albright). FHCRC/NHGRI Group: Partial support was provided by the Fred Hutchinson Cancer Research Center (to Janet L. Stanford) and National Human Genome Research Institute (to Elaine A. Ostrander). ACTANE Group: We appreciate the support of the CR-UK grant A8385 and the NIHR to the Biomedical Research Centre at The Institute of Cancer Research and Royal Marsden NHS Foundation Trust (to Ros Eeles), and Cancer Research UK (to Doug Easton). University of Umeå Group: Partial support was provided by the Swedish Cancer Society and a Spear grant from the Umeå University Hospital, Umeå, Sweden (to Henrik Grönberg). University of Tampere Group: We appreciate the support of the Competitive Research Funding of the Pirkanmaa Hospital District (9L091), Reino Lahtikari Foundation, Finnish Cancer Organisations, Sigrid Juselius Foundation and Academy of Finland (116437 and 126714) (to Johanna Schleutker). Northwestern University Group: Partial support was provided from Robert H. Lurie Comprehensive Cancer Center and the Urological Research Foundation (to William J. Catalona). University of Michigan Group: Partial support was provided by NIH P50 CA69568, NIH R01 CA79596 (to Kathleen Cooney), and the University of Michigan Comprehensive Cancer Center. Data Coordinating Center: Partial support was provided by NCI CA119069 and CA129684 (to Jianfeng Xu). We also thank other investigators who contributed to this work: ACTANE Group: Daniel Leongamornlert, Ed Saunders, Malgorzata Tymrakiewicz, Lynne O'Brien, Emma Sawyer, Rosemary Wilkinson, and Stephen Edwards from The Institute of Cancer Research, Sutton, Surrey. University of Ulm Group: Manuel Luedeke and Mark Schrader from Department of Urology, University of Ulm, Germany; Josef Hoegel and Christian Kubisch from Institute of Human Genetics, University of Ulm, Germany; and Kathleen Herkommer from Department of Urology, Technical University of Munich, Germany.

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Table 1

Characteristics of 1,979 Caucasian families from 12 groups of the ICPCG

ICPCG member	Families	Number of affected members				Average age of onset[a]		Total number of affected members
		2	3	4	≥5	≤65 years	>65 years	
ACTANE	191	100	73	13	5	110	81	400
BC/CA/HI	83	48	26	9	0	39	44	210
CeRePP	156	92	47	13	4	75	81	364
FHCRC/NHGRI	255	36	91	65	63	135	120	711
JHU	202	26	58	63	55	103	92	631
Mayo Clinic	168	42	75	30	21	67	101	455
Northwestern University	27	23	3	1	0	24	3	59
University of Michigan	281	92	122	44	23	NA	NA	761
University of Tampere	75	39	24	10	1	36	38	193
University of Ulm	189	101	68	17	3	111	78	420
University of Umeå	91	32	35	17	7	29	62	177
University of Utah	261	51	48	39	123	67	194	1,349
Total	1,979	682	670	321	305	796	894	5,730

[a]The average age of onset for affected members was not available (NA) in the University of Michigan group

Table 2

Pooled results from family-based association tests in 12 groups of the ICPCG

Chr.	SNPs	Region	Position	Gene	Allele	RA	RAF	Families[a]	S-E (S)	Var (S)	Z	*P* value
PCa risk SNPs identified in populations of European descent												
2	rs721048	2p15	62,985,235	*EHBP1*	G/A	A	0.178	331	20.9	139.4	1.77	0.077
3	rs2660753	3p12	87,193,364		C/T	T	0.110	229	12.5	103.9	1.23	0.221
6	rs9364554	6q25	106,280,983	*SLC22A3*	C/T	T	0.309	409	10.5	201.0	0.74	0.459
7	rs10486567	7p15	27,943,088	*JAZF1*	C/T	C	0.778	381	20.4	180.9	1.52	0.130
7	rs6465657	7q21	97,654,263	*LMTK2*	T/C	C	0.491	460	17.9	251.6	1.13	0.258
8	rs16901979	8q24 (region 2)	128,194,098		C/A	A	0.062	102	13.9	41.6	2.15	0.031
8	rs6983267	8q24 (region 3)	128,482,487		G/T	G	0.561	449	36.2	230.3	2.39	0.017
8	rs1447295	8q24 (region 1)	128,554,220		C/A	A	0.163	289	17.3	123.9	1.56	0.120
9	rs1571801	9p13	123,467,194	*DAB2IP*	G/T	T	0.278	388	13.7	190.0	1.00	0.319
10	rs10993994	10q11	51,219,502	*MSMB*	C/T	T	0.427	455	61.4	237.6	3.99	6.70×10^{-5}
10	rs4962416	10q26	126,686,862	*CTBP2*	A/G	G	0.267	424	22.0	192.5	1.58	0.113
11	rs10896449	11q13	68,751,243		G/A	G	0.549	446	50.0	269.5	3.05	2.31×10^{-3}
17	rs11649743	17q12 (region 2)	33,149,092	*HNF1B*	C/T	C	0.838	258	9.3	116.7	0.87	0.387
17	rs4430796	17q12 (region 1)	33,172,153	*HNF1B*	T/C	T	0.590	477	42.9	277.4	2.58	0.010
17	rs1859962	17q24	66,620,348		G/T	G	0.526	462	58.1	223.8	3.88	1.03×10^{-4}
19	rs2735839	19q13	56,056,435	*KLK2*/*KLK3*	G/A	G	0.869	260	2.4	91.8	0.25	0.804
X	rs5945619	Xp11	51,074,708	*NUDT10*/*NUDT11*	A/G	G	0.428	365	46.6	189.1	3.39	6.92×10^{-4}
Additional SNPs genotyped at 8q24												
8	rs979200	8q24	127,992,902		C/T	C	0.660	437	8.2	208.0	0.57	0.570
8	rs1016343	8q24 (region 2)	128,162,479		C/T	T	0.245	388	32.2	192.5	2.32	0.020
8	rs13254738	8q24 (region 2)	128,173,525		A/C	C	0.341	420	3.8	231.0	0.25	0.803
8	rs6983561	8q24 (region 2)	128,176,062		A/C	C	0.060	126	8.4	48.5	1.21	0.226
8	rs7837328	8q24 (region 3)	128,492,309		G/A	A	0.452	462	37.7	259.5	2.34	0.019
8	rs7000448	8q24 (region 3)	128,510,352		C/T	T	0.396	449	8.2	217.5	0.56	0.577
8	rs4242382	8q24 (region 1)	128,586,755		G/A	A	0.156	281	26.5	107.6	2.56	0.011
8	rs10090154	8q24 (region 1)	128,601,319		C/T	T	0.155	268	19.8	103.7	1.94	0.052

RA risk allele reported in original studies, *RAF* risk allele frequency

[a] Informative family number

Table 3

Results from family-based association tests in subsets of families with five or more affected members or average age of onset less than 65 years

Chr.	SNPs	Region	RA	Affected members ≥5				Average age of onset ≤65 years			
				RAF	Families[a]	S-E (S)	*P* value	RAF	Families[a]	S-E (S)	*P* value
PCa risk SNPs identified in population of European descent											
2	rs721048	2p15	A	0.170	61	7.6	0.180	0.169	135	6.0	0.454
3	rs2660753	3p12	T	0.127	50	7.8	0.108	0.113	87	15.1	0.037
6	rs9364554	6q25	T	0.318	79	6.2	0.380	0.315	176	8.6	0.379
7	rs10486567	7p15	C	0.789	73	−4.9	0.441	0.770	149	6.9	0.377
7	rs6465657	7q21	C	0.479	87	13.9	0.086	0.500	192	13.2	0.237
8	rs16901979	8q24 (region 2)	A	0.054	18	7.3	0.034	0.076	43	11.3	0.023
8	rs6983267	8q24 (region 3)	G	0.551	95	8.5	0.262	0.548	185	8.3	0.431
8	rs1447295	8q24 (region 1)	A	0.142	57	5.4	0.379	0.158	111	−0.4	0.951
9	rs1571801	9p13	T	0.288	72	11.2	0.094	0.270	164	7.8	0.399
10	rs10993994	10q11	T	0.440	94	10.3	0.195	0.424	183	15.5	0.110
10	rs4962416	10q26	G	0.303	78	10.7	0.092	0.265	176	5.4	0.567
11	rs10896449	11q13	G	0.554	88	8.5	0.326	0.553	180	29.2	0.010
17	rs11649743	17q12 (region 2)	C	0.835	40	−3.9	0.338	0.841	98	7.0	0.295
17	rs4430796	17q12 (region 1)	T	0.577	84	13.6	0.055	0.590	193	27.8	0.012
17	rs1859962	17q24	G	0.552	83	23.6	1.62×10^{-3}	0.509	180	31.2	8.01×10^{-4}
19	rs2735839	19q13	G	0.865	47	2.3	0.606	0.873	111	0.8	0.898
23	rs5945619	Xp11	G	0.416	69	9.1	0.138	0.438	152	25.6	4.70×10^{-3}
Additional SNPs genotyped at 8q24											
8	rs979200	8q24	C	0.677	84	−1.6	0.815	0.665	171	4.8	0.630
8	rs1016343	8q24 (region 2)	T	0.241	71	16.0	0.010	0.242	157	16.5	0.073
8	rs13254738	8q24 (region 2)	C	0.336	84	8.5	0.283	0.325	166	−0.5	0.965
8	rs6983561	8q24 (region 2)	C	0.054	26	7.0	0.059	0.071	54	9.7	0.065
8	rs7837328	8q24 (region 3)	A	0.434	92	3.4	0.696	0.451	180	9.5	0.367
8	rs7000448	8q24 (region 3)	T	0.388	80	1.4	0.850	0.387	176	−0.6	0.951
8	rs4242382	8q24 (region 1)	A	0.133	56	12.2	0.010	0.149	103	1.4	0.840
8	rs10090154	8q24 (region 1)	T	0.133	52	9.3	0.045	0.150	96	1.3	0.841

RA risk allele reported in original studies, *RAF* risk allele frequency

[a] Informative family number