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Pooled Analysis of Phosphatidylinositol 3-kinase Pathway Variants and Risk of Prostate Cancer

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Abstract

The phosphatidylinositol 3-kinase (PI3K) pathway regulates various cellular processes, including cellular proliferation and intracellular trafficking and may impact prostate carcinogenesis. Thus, we explored the association between single nucleotide polymorphisms (SNPs) in PI3K genes and prostate cancer. Pooled data from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium were examined for associations between 89 SNPs in PI3K genes (*PIK3C2B, PIK3AP1, PIK3C2A, PIK3CD*, and *PIK3R3*) and prostate cancer risk in 8,309 cases and 9,286 controls. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression. SNP rs7556371 in *PIK3C2B* was significantly associated with prostate cancer risk ($OR_{\text{per allele}}$=1.08 (95% CI: 1.03, 1.14), p-trend = 0.0017) after adjustment for multiple testing (P_{adj}=0.024). Simultaneous adjustment of rs7556371 for nearby SNPs strengthened the association ($OR_{\text{per allele}}$=1.21 (95% CI: 1.09, 1.34); p-trend =0.0003). The adjusted association was stronger for men who were diagnosed before 65 years ($OR_{\text{per allele}}$= 1.47 (95% CI: 1.20, 1.79), p-trend = 0.0001) or had a family history

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($OR_{\text{per allele}}$= 1.57 (95% CI: 1.11, 2.23), p-trend = 0.0114), and was strongest in those with both characteristics ($OR_{\text{per allele}}$= 2.31 (95% CI: 1.07, 5.07), p-interaction = 0.005). Increased risks were observed among men in the top tertile of circulating insulin like growth factor-1 (IGF-1) levels ($OR_{\text{per allele}}$= 1.46 (95% CI: 1.04, 2.06), p-trend=0.075). No differences were observed with disease aggressiveness (≥8/stage T3/T4/fatal). In conclusion, we observed a significant association between *PIK3C2B* and prostate cancer risk, especially for familial, early onset disease, which may be attributable to IGF-dependent PI3K signaling.

Keywords

Prostate cancer; Genetics; Consortium; Phosphatidylinositol 3-kinase; Insulin

Introduction

The phosphatidylinositol 3-kinase (PI3K) pathway regulates various cellular processes such as cell growth, proliferation, apoptosis, motility, differentiation, survival and intracellular trafficking.(1) PI3Ks are heterodimeric lipid kinases that are composed of regulatory and catalytic subunits that catalyze the production of several phosphoinositides critical for the signal transduction in these multiple cellular processes.(2) In addition, numerous growth factors signal through the PI3K pathway(3,4), including insulin-like growth factors (IGF) which have also been linked with prostate carcinogenesis.(5-8) Mutation, amplification, and rearrangement in the PI3K pathway and its downstream targets have been observed in several cancer sites, including prostate cancer.(9-11) Because of the role of PI3Ks in cell proliferation, much of the research on this pathway concerns its potential as a target for anticancer therapies.

Despite the well known role of this pathway in cancer progression, genetic variants in PI3K genes have not been well studied. One study found an association between a *PIK3CA* SNP (rs2865084) and endometrioid ovarian cancer but not overall ovarian cancer risk.(12) Two studies have evaluated the functional variant *Met*326*Ile* (rs3730089) in *PIK3R1* and risk of colon (13) and prostate cancer (14) and found no association with either cancer site. While some studies have looked at polymorphisms in genes of downstream targets of PI3K and prostate cancer risk (15-17), there is minimal information regarding the impact of variants in the PI3K gene family on prostate cancer risk.

Given the limited evaluation of PI3K gene variants, we sought to further explore the association between germline polymorphisms, which may alter the function of these genes and the proteins they encode, and risk of prostate cancer. Pooled data from seven prospective studies in the National Cancer Institute (NCI) Breast and Prostate Cancer Cohort Consortium (BPC3) (18) were examined for associations between 89 single nucleotide polymorphisms (SNPs) in five PI3K pathway genes, *PIK3C2B* (chromosome 1), *PIK3AP1* (chromosome 10), *PIK3C2A* (chromosome 11), *PIK3CD* (chromosome 1), and *PIK3R3* (chromosome 1) and prostate cancer risk in 8,751 cases and 9,742 controls. We also evaluated the effects of these PI3K pathway SNPs and circulating IGF-1 and IGF binding protein 3 (IGFBP-3) on prostate cancer risk in a subgroup of 6,076 men using pre-diagnostic sera.

Methods

Study Population

The BPC3 has been described elsewhere.(18) Briefly, the consortium includes large well-established cohorts assembled in the United States and Europe that have DNA for genotyping and extensive questionnaire data from cohort members. The prostate cancer study includes seven case-control studies nested within these cohorts: the American Cancer Society (ACS)

Cancer Prevention Study II, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC), the European Prospective Investigation into Cancer and Nutrition (EPIC), the Health Professionals Follow-up Study (HPFS), the Physicians Health Study (PHS), the Hawaii-Los Angeles Multiethnic Cohort (MEC), and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). With the exception of MEC and PLCO, most subjects in these cohorts were Caucasian. Cases were identified in each cohort by self report, with subsequent confirmation of the diagnosis from medical records, and/or linkage with population-based tumor registries. Controls were free of prostate cancer at selection and were matched to cases within each cohort by age, ethnicity, and other select factors, such as country of residence for EPIC. Written informed consent was obtained from all subjects, and each cohort study was approved by the appropriate institutional review boards.

IGF-1 and IGFBP-3 Blood Levels

Data on pre-diagnostic blood levels of IGF-1 and IGFBP-3 were available in six of the seven BPC3 cohorts (ATBC, EPIC, HPFS, MEC, PHS and PLCO; IGF-1: N=6,076; IGFBP-3: N=6,059).(5-8,19-21) Samples from three studies (ATBC, HPFS and PHS) were measured in the laboratory of the Cancer Prevention Research Unit, Departments of Medicine and Oncology, Lady Davis Research Institute of the Jewish General Hospital and McGill University, and the remaining three studies (EPIC, MEC and PLCO) were measured in the laboratory of the Hormones and Cancer Team at IARC; all used enzyme-linked immunosorbent assays (ELISA) from Diagnostic System Laboratories (Webster, TX). Bloods levels were categorized into tertiles based on the distribution among the controls. Although the blood level assays were performed at different times, batch effects did not appear to confound the results, and the analyses were pooled across cohorts controlling for study.

Genotyping and SNP selection

Tag SNPs for the five PI3K candidate genes potentially related to the IGF pathway were selected using the CEU HapMap data assuming a minor allele frequency ≥0.02, an Illumina design score >0.4, and an $r^2 \geq 0.8$ for binning.(22) Genotyping in the prostate cancer cases and controls was performed in four laboratories (University of Southern California, Los Angeles, CA, USA; Harvard School of Public Health, Boston, MA, USA; Core Genotyping Facility, National Cancer Institute, Bethesda, MD, USA; and Imperial College, London, UK) using Illumina GoldenGate technology (San Diego, CA, USA), as part of a larger array of 1,536 SNPs in total. Each genotype center genotyped 30 CEU HapMap trios to evaluate inter-lab reproducibility and for the 1,536 SNPs that made up the Illumina platform the inter-lab concordance was 99.5% (before excluding failed SNPs or samples). Within each study, blinded duplicate samples (~5%) were also included and concordance of these samples ranged from 97.2-99.9% across studies.

Data Filtering and Imputation

Any sample where greater than 25% of the SNPs attempted on a given platform failed was removed from the data set. Data were filtered by study to remove poorly performing SNPs: all SNPs that failed on 25% or more samples were excluded from the data set, as were all SNPs that showed statistically significant ($p<10^{-5}$) deviations from HWE genotype frequencies among European-ancestry controls, and all SNPs with MAF<1%. Any SNP that was missing or excluded in more than three studies or exhibited large differences in European-ancestry allele frequencies across cohorts ($F_{st}>0.02$) was excluded from further analysis. The current analysis included 8,751 prostate cases and 9,742 controls. Among these, 8,309 cases and 9,286 controls had genotype information.

The MACH software program (http://www.sph.umich.edu/csg/abecasis/MaCH/index.html) was used to impute SNPs that were polymorphic in any of the HapMap reference panels using observed genotypes from the BPC3 subjects and phased haplotypes from HapMap samples (release #21).(23) Genotypes for European-ancestry subjects were imputed using the CEPH European (CEU) reference panel; those for Japanese Americans were imputed using the combined Han Chinese and Japanese panels (CHB+JPT); those for remaining subjects (African Americans, Latinos, Native Hawaiians) were imputed using a “cosmopolitan” panel of all HapMap samples (CEU+CHB+JPT+YRI).(24) Imputation was performed stratified by study and ethnicity. Poorly imputed SNPs with an estimated correlation between the imputed and true genotypes less than 30% were excluded from analysis.(23) SNPs that were dropped in more than three European-ancestry cohorts were excluded from analysis.

Statistical Analysis

Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age (5-year intervals), cohort (plus country for EPIC), and ethnicity for each SNP with minor allele frequency above 1% were estimated using unconditional logistic regression. Genotypes were coded either as counts of the variant allele (trend test) or as two indicator variables, one for heterozygotes and one for variant homozygotes (two d.f. test). Analyses were performed in all subjects, separately for each ethnicity, and separately for each ethnicity within study. Aggressive disease was defined as Gleason grade ≥ 8 or Stage T3/T4 or fatal prostate cancer. Heterogeneity between studies and interactions between the SNPs and other covariates were assessed by including the cross-product terms as well as the main effect terms in regression models, and the statistical significance of the interaction was evaluated by comparing nested models with and without the cross-product terms using a likelihood ratio test. All analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, North Carolina).

Pairwise linkage disequilibrium (LD) measures (D' and r^2) were estimated and haplotypes were constructed using the expectation-maximization (EM) algorithm (25) in HaploStats. (26,27) Risks for individual haplotypes were calculated among whites only in *PIK3C2B* assuming a log-additive model for each haplotype adjusting for age and cohort. Haplotypes with a frequency of less than 1% were collapsed into a single category, and the most common haplotype was used as the referent.

In order to take into account the large number of tests performed, the number of effective independent variables, M_{eff}, was calculated for each gene by use of the SNP Spectral Decomposition approach (28), and p-values adjusted for multiple testing were calculated using the gene-wide M_{eff} values.

Results

Demographic and prostate cancer related characteristics among BPC3 prostate cancer cases and controls are presented in Table 1. The majority of subjects were white (76.5%) and diagnosed over the age of sixty-five years (70.8%). Aggressive cases of prostate cancer comprised about a quarter of cases (25.3%), and 7.8% of subjects reported a family history of disease in first degree relatives. Family history was not available for the EPIC and PHS cohorts.

Eighty-nine genotyped and 251 imputed SNPs in *PIK3CD*, *PIK3C2A*, *PIK3R3*, *PIK3AP1*, and *PIK3C2B* were evaluated for their association with prostate cancer (Table 2). Among the five PI3K pathway genes, only *PIK3C2B* showed a cluster of SNPs related to prostate cancer risk, where at least one genotyped SNP remained associated with risk after adjustment for the number of effective tests ($p = 0.024$). Specifically, of the 15 genotyped and 89 imputed SNPs, eleven SNPs (1 genotyped, 10 imputed) in *PIK3C2B* showed an association for trend with prostate cancer at $p< 0.01$. Main effect p-values for trend for all SNPs (genotyped and imputed) in PI3K genes and risk of prostate cancer are presented in Supplementary Table 1.

SNPs related to prostate cancer were clustered upstream and in the first two introns of *PIK3C2B* (Table 3). The strongest genotyped association was for rs7556371 with $OR_{per\ allele} = 1.08$ (95% CI: 1.03, 1.14), p-trend = 0.0017; $OR_{het} = 1.05$ (95% CI: 0.94, 1.18) and $OR_{homwt} = 1.15$ (95% CI: 1.03, 1.29) (Table 3). Several surrounding imputed SNPs with $p<0.001$ were highly correlated (r^2=0.89-0.99) with rs7556371 and showed similar magnitudes of association (Table 3). Overall, the strongest signals were for two highly correlated (pairwise r^2=1), imputed SNPs, rs10494852, which is an intronic SNP, and rs11240751, which is located in the promoter region of *PIK3C2B*.

Simultaneous adjustment of rs7556371 for the two other genotyped SNPs in the region, rs6594014 (r^2=0.37) and rs11240748 (r^2=0.10), located between 201.18 and 201.20 Mb, strengthened the prostate cancer risk association observed for rs7556371: $OR_{per\ allele} = 1.21$ (95% CI: 1.09, 1.34), p-trend=0.0003. The ORs and 95% CIs for rs6594014 and rs11240748 were also strengthened with all three SNPs in the same model: $OR_{per\ allele} = 1.11$ (95% CI: 1.01, 1.22), p-trend=0.0284 and $OR_{per\ allele} = 1.14$ (95% CI: 1.03, 1.25), p-trend= 0.0085, respectively. Haplotype analyses indicated that several haplotypes in *PIK3C2B* comprised of rs6594014, rs11240748, and rs7556371 were related to prostate cancer (Supplemental Table 2) but to a lesser degree than the SNPs alone (p-value range 0.02-0.03), suggesting that no single genotyped variant explains all the observed risk and that additional unknown variants in the region may be related to risk.

Stratified analyses of rs7556371 and risk of prostate cancer are presented in Table 4. Because additional adjustment for the other two genotyped SNPs in the region strengthened the main effect of rs7556371, stratified results are presented for both the simple and additionally adjusted models. No statistically significant heterogeneity between cohorts and ethnicity was observed (p-het= 0.14 and 0.37 respectively); however, some heterogeneity in magnitude of the ORs was evident among the different ethnic groups. As expected, the OR for rs7556371 among whites was similar to the overall association, $OR_{per\ allele} = 1.24$ (95% CI: 1.09, 1.41), p-trend=0.0008. The association was stronger for men with prostate cancer diagnosed before age 65 years, $OR_{per\ allele} = 1.47$ (95% CI: 1.20, 1.79), p-trend = 0.0001, p-interaction=0.06 and for men with a family history of prostate cancer, $OR_{per\ allele} = 1.57$ (95% CI: 1.11, 2.23), p-trend = 0.0114, p-interaction=0.02. Men diagnosed before age 65 years who also had a family history were found to have a two-fold increased risk of prostate cancer ($OR_{per\ allele}$ =2.31; 95% CI: 1.07, 5.07), p-trend 0.034, p-interaction=0.005. Increased risks were also observed among obese men (BMI ≥30 kg/m^2), $OR_{per\ allele}$ =1.30 (95% CI: 0.98, 1.71), p-trend =0.0003 and among men in the top tertile of circulating IGF-1 levels, $OR_{per\ allele}$ =1.46 (95% CI: 1.04, 2.06), p-trend =0.075, although interactions with body mass index and IGF-1 levels were not statistically significant. There was no association among tertiles of IGFBP-3 (data not shown). No differences were observed with disease aggressiveness.

Discussion

In this large pooled analysis of prostate cancer cases and controls, we explored the associations between common SNPs in five PI3K pathway genes (*PIK3C2B*, *PIK3AP1*, *PIK3C2A*, *PIK3CD*, and *PIK3R3*) and prostate cancer risk. Among the five genes, we observed significant associations between a cluster of variants located upstream and in the promoter region of *PIK3C2B* and risk of prostate cancer. The association was strongest for the genotyped SNP rs7556371, which remained statistically significant at the $p<0.05$ level after correction for multiple testing. We observed no meaningful significant associations in the other four PI3K pathway genes (*PIK3AP1*, *PIK3C2A*, *PIK3CD*, and *PIK3R3*) and prostate cancer risk.

The phosphoinositol-3-kinase family is divided into three different classes, class I, class II, and class III, based on primary structure, regulation, and *in vitro* lipid substrate specificity.(2) The

PIK3C2B gene codes for the class II PI3K enzyme PIK3C2β, about which, until recently, there was little known. Recently, increased expression of PIK3C2β was found to enhance membrane ruffling and migration speed of cells in cancer cell lines.(29) In addition, PIK3C2β-overexpressing cells have been found to be protected from anoikis and display enhanced proliferation.(29,30) Thus, SNPs in *PIK3C2B* may alter PIK3C2β expression, influencing the migration and survival of tumor cells and promoting prostate carcinogenesis.

An additional mechanism of action potentially linking *PIK3C2B* variants with prostate cancer is through insulin signaling. PI3Ks play a pivotal role in signal transduction pathways linking insulin with many of its cellular responses.(31) Furthermore, class II PI3Ks, including PIK3C2β, have been shown to be activated by insulin.(32) The insulin-like growth factor axis has been related to prostate cancer, with elevated blood levels of IGF-I associated with increased risks.(33,34) Obesity, a chronic hyperinsulinemic state resulting in altered IGF levels, has also been associated with prostate cancer risk.(35-38) In this analysis, we observe increased risks associated with *PIK3C2B* variants among obese men and among men in the top tertile of circulating IGF-1 levels. Although these interactions were not statistically significant, men <65 years who also had a family history of prostate cancer had the highest mean levels of serum IGF-1 and had a higher BMI than other age-family history subgroups. The association between *PIK3C2B* variants and prostate cancer among men <65 years who also had a family history of prostate cancer was not appreciably attenuated after adjustment for IGF-1 levels or BMI suggesting an alternative mechanism; however, a mechanism related to IGF-dependent PI3K signaling cannot be ruled out.

Alternatively, the observed association may be due to effects on another nearby gene. The p53 regulator, *MDM4*, is located within 30 kb of the observed *PIK3C2B* variants and SNPs located within this gene may be in linkage disequilibrium with the genotyped region. MDM4 plays a critical role in p53-dependent apoptosis and thus tumor suppression. (39) Altered expression of MDM4 and in turn p53 could lead to a disruption in apoptotic activity that may promote prostate carcinogenesis. The most significantly associated genotyped SNP in our study, rs7556371, is in strong linkage disequilibrium with rs4245735, which has been associated with MDM4 mRNA expression in lymphocytes.(40) Thus, the observed association with prostate cancer risk in this study may be due to altered mRNA expression of MDM4. While this apoptosis regulatory region may explain the observed effect, the well described interplay of the IGF axis with PI3K signaling and the observed stratified associations with BMI and IGF-1 levels suggest that insulin signaling remains a possible mechanistic pathway.

We observed that the association between *PIK2C2B* variants was modified by age and family history of prostate cancer but not by aggressive disease. Risk appeared to be equally related to aggressive and non-aggressive disease, despite the tendency for familial early-onset disease to present more aggressively in other studies.(41-43) In our study, the percentage of men with aggressive disease among the early-onset, familial cases was comparable to that of the pooled population, and among those with a family history of disease, the association for rs7556371 persisted for non-aggressive prostate cancer. This suggests that the association we observed with *PIK2C2B* variants among men with a family history was not due to the fact that they had aggressive disease.

Despite numerous linkage studies, few genes have been identified as being associated with familial prostate cancer risk. The exploration of prostate cancer susceptibility loci identified from population-based genome-wide scans and family history of prostate cancer has not conclusively identified any loci that explain a substantial portion of inherited risk.(44) In our study, younger men with a family history of disease who carried the variant allele at rs7556371 were 2.3 times more likely to develop prostate cancer. Genetic linkage analyses have observed significant linkage to chromosome 1q23-25 and 1q42-43; however, 1q32, where *PIK3C2B* is located, has not been identified as a region of higher predisposition.(45) Given that familial prostate cancer tends to be diagnosed at a younger age than sporadic prostate cancer and that the risk associated with rs7556371 was greatest among early onset familial cases in our study, this region may be of interest for future exploration in familial studies. It is also possible that this observed subgroup association could be a false-positive finding given the small numbers of subjects diagnosed before age 65 with a positive family history.

Strengths of our study include a large sample of cases and controls drawn from well defined cohorts and a comprehensive SNP tagging approach. Further, mathematical imputation of all variants known to HapMap that were not directly genotyped provided a more comprehensive characterization of the genetic variation of the candidate genes in the PI3K pathway. Despite this, the precise genetic variant driving the association in *PIK3C2B* may not have been completely captured in our genotyping effort or by our imputation analysis. In addition, PSA values were not available for use in adjusting the analysis but likely would not have affected odds ratios significantly. SNPs in *PIK3C2B* have not been associated with risk in individual genome-wide association studies to date; however, this is possibly due to the small effect size, which most genome-wide association studies are underpowered to detect. Although it is possible that our findings are false-positive results, the large sample size, the clustering of the significant variants, and sustained significance after adjusting for multiple testing make this possibility less likely. Future genotyping for the statistically significant imputed SNPs in this analysis, including the *PIK3C2B* promoter region SNP rs11240751, is warranted and may yield additional insight.

In conclusion, this large pooled study has identified a cluster of variants in the class II PI3K gene, *PIK3C2B*, and risk of prostate cancer, especially among men with familial, early-onset disease. The precise genetic variant driving this association, however, is not clear and further studies are needed both to replicate and refine this region of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Reference List

1. Foster FM, Traer CJ, Abraham SM, Fry MJ. The phosphoinositide (PI) 3-kinase family. J Cell Sci 2003;116:3037–40. [PubMed: 12829733]
2. Fruman DA, Meyers RE, Cantley LC. Phosphoinositide kinases. Annu Rev Biochem 1998;67:481–507. [PubMed: 9759495]
3. Russell PJ, Bennett S, Stricker P. Growth factor involvement in progression of prostate cancer. Clin Chem 1998;44:705–23. [PubMed: 9554481]
4. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov 2005;4:988–1004. [PubMed: 16341064]
5. Allen NE, Key TJ, Appleby PN, et al. Serum insulin-like growth factor (IGF)-I and IGF-binding protein-3 concentrations and prostate cancer risk: results from the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev 2007;16:1121–7. [PubMed: 17548673]
6. Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 1998;279:563–6. [PubMed: 9438850]

7. Cheng I, DeLellis HK, Haiman CA, et al. Genetic determinants of circulating insulin-like growth factor (IGF)-I, IGF binding protein (BP)-1, and IGFBP-3 levels in a multiethnic population. J Clin Endocrinol Metab 2007;92:3660–6. [PubMed: 17566087]
8. Platz EA, Pollak MN, Leitzmann MF, Stampfer MJ, Willett WC, Giovannucci E. Plasma insulin-like growth factor-1 and binding protein-3 and subsequent risk of prostate cancer in the PSA era. Cancer Causes Control 2005;16:255–62. [PubMed: 15947877]
9. Boormans JL, Hermans KG, van Leenders GJ, Trapman J, Verhagen PC. An activating mutation in AKT1 in human prostate cancer. Int J Cancer 2008;123:2725–6. [PubMed: 18798258]
10. Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943–7. [PubMed: 9072974]
11. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2002;2:489–501. [PubMed: 12094235]
12. Quaye L, Song H, Ramus SJ, et al. Tagging single-nucleotide polymorphisms in candidate oncogenes and susceptibility to ovarian cancer. Br J Cancer 2009;100:993–1001. [PubMed: 19240718]
13. Li L, Plummer SJ, Thompson CL, Tucker TC, Casey G. Association between phosphatidylinositol 3-kinase regulatory subunit p85alpha Met326Ile genetic polymorphism and colon cancer risk. Clin Cancer Res 2008;14:633–7. [PubMed: 18245521]
14. Paradis AE, Kantoff PW, Giovannucci E, Stampfer MJ, Ma J. Association between the Met326Ile polymorphism of the p85alpha regulatory subunit of phosphatidylinositol 3-kinase and prostate cancer risk: a prospective study. Cancer Epidemiol Biomarkers Prev 2003;12:172–3. [PubMed: 12582030]
15. Haiman CA, Stram DO, Cheng I, et al. Common genetic variation at PTEN and risk of sporadic breast and prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:1021–5. [PubMed: 16702386]
16. Assinder SJ, Dong Q, Kovacevic Z, Richardson DR. The TGF-beta, PI3K/Akt and PTEN pathways: established and proposed biochemical integration in prostate cancer. Biochem J 2009;417:411–21. [PubMed: 19099539]
17. Kang D, Lee KM, Park SK, et al. Lack of association of transforming growth factor-beta1 polymorphisms and haplotypes with prostate cancer risk in the prostate, lung, colorectal, and ovarian trial. Cancer Epidemiol Biomarkers Prev 2007;16:1303–5. [PubMed: 17548703]
18. Hunter DJ, Riboli E, Haiman CA, et al. A candidate gene approach to searching for low-penetrance breast and prostate cancer genes. Nat Rev Cancer 2005;5:977–85. [PubMed: 16341085]
19. Chan JM, Stampfer MJ, Ma J, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. J Natl Cancer Inst 2002;94:1099–106. [PubMed: 12122101]
20. Weiss JM, Huang WY, Rinaldi S, et al. IGF-1 and IGFBP-3: Risk of prostate cancer among men in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Int J Cancer 2007;121:2267–73. [PubMed: 17597108]
21. Woodson K, Tangrea JA, Pollak M, et al. Serum insulin-like growth factor I: tumor marker or etiologic factor? A prospective study of prostate cancer among Finnish men. Cancer Res 2003;63:3991–4. [PubMed: 12873996]
22. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106–20. [PubMed: 14681826]
23. Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. Annu Rev Genomics Hum Genet 2009;10:387–406. [PubMed: 19715440]
24. de Bakker PI, Burtt NP, Graham RR, et al. Transferability of tag SNPs in genetic association studies in multiple populations. Nat Genet 2006;38:1298–303. [PubMed: 17057720]
25. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995;12:921–7. [PubMed: 7476138]
26. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002;70:425–34. [PubMed: 11791212]
27. Lake SL, Lyon H, Tantisira K, et al. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum Hered 2003;55:56–65. [PubMed: 12890927]

28. Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genet Epidemiol 2008;32:361–9. [PubMed: 18271029]
29. Katso RM, Pardo OE, Palamidessi A, et al. Phosphoinositide 3-Kinase C2beta regulates cytoskeletal organization and cell migration via Rac-dependent mechanisms. Mol Biol Cell 2006;17:3729–44. [PubMed: 16775008]
30. Maffucci T, Cooke FT, Foster FM, Traer CJ, Fry MJ, Falasca M. Class II phosphoinositide 3-kinase defines a novel signaling pathway in cell migration. J Cell Biol 2005;169:789–99. [PubMed: 15928202]
31. Shepherd PR, Withers DJ, Siddle K. Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. Biochem J 1998;333(Pt 3):471–90. [PubMed: 9677303]
32. Brown RA, Shepherd PR. Growth factor regulation of the novel class II phosphoinositide 3-kinases. Biochem Soc Trans 2001;29:535–7. [PubMed: 11498023]
33. Gennigens C, Menetrier-Caux C, Droz JP. Insulin-Like Growth Factor (IGF) family and prostate cancer. Crit Rev Oncol Hematol 2006;58:124–45. [PubMed: 16387509]
34. Rowlands MA, Gunnell D, Harris R, Vatten LJ, Holly JM, Martin RM. Circulating insulin-like growth factor peptides and prostate cancer risk: a systematic review and meta-analysis. Int J Cancer 2009;124:2416–29. [PubMed: 19142965]
35. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–38. [PubMed: 12711737]
36. Wright ME, Chang SC, Schatzkin A, et al. Prospective study of adiposity and weight change in relation to prostate cancer incidence and mortality. Cancer 2007;109:675–84. [PubMed: 17211863]
37. Rodriguez C, Freedland SJ, Deka A, et al. Body mass index, weight change, and risk of prostate cancer in the Cancer Prevention Study II Nutrition Cohort. Cancer Epidemiol Biomarkers Prev 2007;16:63–9. [PubMed: 17179486]
38. MacInnis RJ, English DR. Body size and composition and prostate cancer risk: systematic review and meta-regression analysis. Cancer Causes Control 2006;17:989–1003. [PubMed: 16933050]
39. Jeyaraj S, O'Brien DM, Chandler DS. MDM2 and MDM4 splicing: an integral part of the cancer spliceome. Front Biosci 2009;14:2647–56. [PubMed: 19273224]
40. Dixon AL, Liang L, Moffatt MF, et al. A genome-wide association study of global gene expression. Nat Genet 2007;39:1202–7. [PubMed: 17873877]
41. Gronberg H, Isaacs SD, Smith JR, et al. Characteristics of prostate cancer in families potentially linked to the hereditary prostate cancer 1 (HPC1) locus. JAMA 1997;278:1251–5. [PubMed: 9333266]
42. Klein EA, Kupelian PA, Witte JS. Does a family history of prostate cancer result in more aggressive disease? Prostate Cancer Prostatic Dis 1998;1:297–300. [PubMed: 12496870]
43. Rodriguez C, Calle EE, Miracle-McMahill HL, et al. Family history and risk of fatal prostate cancer. Epidemiology 1997;8:653–7. [PubMed: 9345665]
44. Fitzgerald LM, Kwon EM, Koopmeiners JS, Salinas CA, Stanford JL, Ostrander EA. Analysis of recently identified prostate cancer susceptibility loci in a population-based study: associations with family history and clinical features. Clin Cancer Res 2009;15:3231–7. [PubMed: 19366831]
45. Schaid DJ, McDonnell SK, Zarfas KE, et al. Pooled genome linkage scan of aggressive prostate cancer: results from the International Consortium for Prostate Cancer Genetics. Hum Genet 2006;120:471–85. [PubMed: 16932970]

Table 1

Characteristics of study population by cohort.

	Cohort							
Characteristic	**Pooled**	**ACS**	**ATBC**	**EPIC**	**HPFS**	**MEC**	**PHS**	**PLCO**
Prostate Cancer								
Case	8,751	1,296	1,058	953	700	2,320	1,101	1,323
Control	9,742	1,293	1,058	1,320	700	2,290	1,430	1,651
Aggressive Disease[*]								
No	5,572 (63.7)	1,052 (81.2)	335 (31.7)	373 (39.1)	523 (74.7)	1,516 (65.3)	719 (65.3)	1,054 (79.7)
Yes	2,215 (25.3)	204 (15.7)	324 (30.6)	141 (14.8)	155 (22.1)	779 (33.6)	343 (31.2)	269 (20.3)
Missing	964 (11.0)	40 (3.1)	399 (37.7)	439 (46.1)	22 (3.1)	25 (1.1)	39 (3.5)	0 (0)
Age at case diagnosis								
<65	5,397 (29.2)	436 (16.8)	371 (17.5)	1,098 (48.3)	356 (25.4)	1,439 (31.2)	667 (26.4)	1,030 (34.6)
>=65	13,096 (70.8)	2,153 (83.2)	1,745 (82.5)	1,175 (51.7)	1,044 (74.6)	3,171 (68.8)	1,864 (73.7)	1,944 (65.4)
Ethnicity								
White	14,138 (76.5)	2,566 (99.1)	2,116 (100)	2,273 (100)	1315 (93.9)	909 (19.7)	2,415 (95.4)	2,544 (85.5)
Black	1,762 (9.5)	-	-	-	-	1,333 (28.9)	-	429 (14.4)
Hispanic	1,296 (7.0)	-	-	-	-	1,296 (28.1)	-	-
Japanese	933 (5.1)	-	-	-	-	933 (20.2)	-	-
Native Hawaiian	139 (0.7)	-	-	-	-	139 (3.1)	-	-
Other/Unknown	225 (1.2)	23 (0.9)	-	-	85 (6.7)	-	116 (4.6)	1 (0.03)
Body Mass Index kg/m^2								
<25	6,919 (37.4)	985 (38.1)	803 (38.0)	714 (31.4)	493 (35.2)	1,624 (35.2)	1,500 (59.3)	800 (26.9)
25-<30	8,599 (46.5)	1,273 (49.2)	1,014 (48.0)	1,189 (52.3)	465 (33.2)	2,213 (48.0)	948 (37.5)	1,497 (50.3)
≥30	2,501 (13.5)	299 (11.6)	297 (14.0)	364 (16.0)	84 (6.0)	731 (15.9)	83 (3.3)	643 (21.6)
Missing	474 (2.6)	32 (1.2)	2 (0.1)	6 (0.3)	358 (25.6)	42 (0.9)	--	34 (1.1)
Family History[†]								
No	11,582 (62.6)	2,163 (83.6)	1,762	-	1,155 (82.5)	3,783 (82.1)	-	2,719 (91.4)
Yes	1,439 (7.8)	426 (16.5)	89 (4.2)	-	245 (17.5)	424 (9.2)	-	255 (8.6)

	Cohort							
Characteristic	**Pooled**	**ACS**	**ATBC**	**EPIC**	**HPFS**	**MEC**	**PHS**	**PLCO**
Missing	5,472 (29.6)	-	265 (12.5)	2,273 (100)	-	403 (8.7)	2,531 (100)	-

[*] Aggressive disease: Gleason ≥ 8 or Stage 3/4 or fatal.

[†] Prostate cancer in first degree relative.

Table 2

Overview of PI3K genes and risk of prostate cancer.

Gene abbreviation	Genotyped SNPs	Imputed SNPs	Total No. of SNPs	No. of SNPs with P < 0.01	Minimum p-trend for Imputed SNPs	Minimum p-trend for Genotyped SNPs	Adjusted Minimum p-trend for Genotyped SNPs
PIK3CD	15	5	20	0	0.42	0.129	-
PIK3C2A	7	37	44	0	0.099	0.28	-
PIK3R3	10	45	55	0	0.068	0.069	-
PIK3AP1	42	75	117	1[*]	0.0033	0.012	0.396 [‡]
PIK3C2B	15	89	104	11[†]	0.0004	0.0017	0.024 [§]

[*] Imputed SNP

[†] 1 Genotyped, 10 Imputed

[‡] M_{eff} (number of effective tests) = 33 tests

[§] M_{eff} (number of effective tests) = 14 tests

Table 3

Risk of prostate cancer for genotyped or imputed SNPs located between 120.18 and 120.20 Mb in *PIK3C2B*.

SNP chromosomal order	Position	MAF*	r^2*	Gene Neighborhood	Status	Imputation Quality MACH r^2 range (mean)	OR† (95% CI)	p-trend
rs4951389	-36904 G>A	0.301	0.89	PIK3C2B,MDM4	Imputed	0.72-0.90 (0.84)	1.09 (1.03, 1.15)	0.0005
rs10900594	-31199 C>G	0.301	0.89	PIK3C2B,MDM4	Imputed	0.67-0.92 (0.84)	1.09 (1.03, 1.15)	0.0006
rs11240751	-23119 G>A	0.301	0.89	PIK3C2B	Imputed	0.78-0.95 (0.91)	1.09 (1.03, 1.15)	0.0004
rs1398148	IVS2+616 G>C	0.279	0.99	PIK3C2B	Imputed	0.95-0.98 (0.97)	1.08 (1.03, 1.14)	0.0018
rs10494852	IVS2+1158 T>C	0.301	0.89	PIK3C2B	Imputed	0.80-0.99 (0.95)	1.09 (1.03, 1.15)	0.0004
rs7556371	**IVS2+1608 A>G**	**0.279**	-	**PIK3C2B**	**Genotyped**	--	**1.08 (1.03, 1.14)**	**0.0017**
rs11240748	IVS2+5624 C>T	0.237	0.10	PIK3C2B	Genotyped	--	1.01 (0.96, 1.06)	0.8087
rs12402641	IVS2+6165 G>A	0.493	0.38	PIK3C2B	Imputed	0.95-0.99 (0.98)	1.07 (1.02, 1.11)	0.0049
rs4951384	IVS2+6430 T>A	0.279	0.99	PIK3C2B	Imputed	0.93-0.99 (0.98)	1.09 (1.03, 1.15)	0.0015
rs4951382	IVS2+7449 C>T	0.279	0.99	PIK3C2B	Imputed	0.88-0.99 (0.96)	1.09 (1.02, 1.15)	0.0048
rs7519417	IVS2+8992 C>T	0.279	0.99	PIK3C2B	Imputed	0.97-0.99 (0.99)	1.09 (1.03, 1.15)	0.0014
rs6594014	IVS2+9510 A>G	0.478	0.37	PIK3C2B	Genotyped	--	0.96 (0.92, 1.003)	0.0703
rs6692377	IVS2+9787 G>A	0.487	0.41	PIK3C2B	Imputed	0.78-0.98 (0.93)	0.94 (0.90, 0.98)	0.0083

[*] Minor allele frequency (MAF) and r^2 among white controls.

[†] OR per wild-type allele assuming a log-additive model. Adjusted by age, cohort (including country for EPIC), and ethnicity.

Table 4

Stratified analyses of rs7556371 and risk of prostate cancer.

Characteristic			Simple Adjusted*	p-trend	Additionally Adjusted**	p-trend	p-int
	Case	Control	OR (95% CI)		OR (95% CI)		
Overall Risk	7,900	8,476	1.08 (1.03, 1.14)	0.0017	1.21 (1.09, 1.34)	0.0003	
Cohort							
ACS	1,184	1,182	0.98 (0.86, 1.11)	0.7240	1.31 (0.98, 1.75)	0.0698	
ATBC	959	828	1.18 (1.00, 1.38)	0.0456	1.18 (0.80, 1.74)	0.2740	
EPIC	667	1,049	0.99 (0.84, 1.16)	0.8680	1.15 (0.80, 1.66)	0.4537	
HPFS	650	650	1.11 (0.93, 1.34)	0.2325	1.31 (0.92, 1.89)	0.1393	
MEC	2,296	2,254	1.10 (1.01, 1.21)	0.0346	1.08 (0.90, 1.30)	0.3962	
PHS	993	1,098	1.21 (1.06, 1.38)	0.0058	1.38 (1.05, 1.82)	0.0206	
PLCO	1,151	1,415	1.05 (0.93, 1.19)	0.4193	1.33 (1.01, 1.75)	0.0398	0.14
Ethnicity †							
White	5,974	6,383	1.08 (1.02, 1.15)	0.0059	1.24 (1.09, 1.41)	0.0008	
Hispanic	641	646	1.19 (1.01, 1.40)	0.0369	0.91 (0.61, 1.36)	0.6508	
Black	754	909	0.99 (0.86, 1.15)	0.8914	1.13 (0.87, 1.47)	0.3512	
Japanese	461	470	1.11 (0.88, 1.38)	0.3798	0.77 (0.34, 1.73)	0.5272	
Native Hawaiian	70	68	1.46 (0.85, 2.51)	0.1698	0.45 (0.07, 2.62)	0.3648	0.37
Age at case diagnosis (yrs)							
<65	2,087	2,625	1.12 (1.02, 1.23)	0.0158	1.47 (1.20, 1.79)	0.0001	
≥65	5,813	5,851	1.07 (1.01, 1.13)	0.0220	1.13 (0.997, 1.27)	0.0561	0.06
Aggressive Disease							
No	5,155	8,476	1.07 (1.01, 1.13)	0.0163	1.20 (1.07, 1.35)	0.0018	
Yes	2,031	8,476	1.10 (1.01, 1.19)	0.0168	1.16 (0.99, 1.37)	0.0760	
Family History of PCA							
No	5,097	5,515	1.04 (0.98, 1.11)	0.1932	1.12 (0.99, 1.28)	0.0755	
Yes	817	525	1.19 (1.01, 1.41)	0.0341	1.57 (1.11, 2.23)	0.0114	0.02
<65	212	142	1.34 (0.94, 1.92)	0.108	2.31 (1.07, 5.02)	0.034	
≥65	605	383	1.14 (0.93, 1.40)	0.207	1.39 (0.93, 2.07)	0.106	0.005

Characteristic	Case	Control	Simple Adjusted[*] OR (95% CI)	p-trend	Additionally Adjusted[**] OR (95% CI)	p-trend	p-int
Body Mass Index (kg/m^2)							
<25	3,303	3,616	1.11 (1.02, 1.20)	0.0128	1.17 (0.99, 1.38)	0.0624	
25-<30	4,159	4,440	1.07 (0.995, 1.15)	0.0677	1.22 (1.05, 1.43)	0.0121	
≥30	1,447	1,054	1.03 (0.90, 1.18)	0.6358	1.30 (0.98, 1.71)	0.0690	0.46
p-trend			0.003		0.0003		
IGF-1 (ng/ml) [‡]							
T1	758	974	1.04 (0.89, 1.21)	0.6694	0.99 (0.73, 1.35)	0.9567	
T2	878	969	1.08 (0.92, 1.26)	0.3508	1.19 (0.85, 1.67)	0.3116	
T3	911	970	1.11 (0.96, 1.29)	0.1658	1.46 (1.04, 2.06)	0.0287	0.80
p-trend			0.101		0.075		

[*] OR per wild-type allele assuming a log-additive model. Adjusted by age, cohort (including country for EPIC), and ethnicity where appropriate.

[**] Additionally adjusted by rs6594014 and rs11240748.

[†] MAF Hispanic = 0.36; Black = 0.41; Japanese = 0.22; Native Hawaiian = 0.32.

[‡] Tertile 1: < 147.3 ng/ml; Tertile 2: 147.3-204.0 ng/ml; Tertile 3: ≥ 204.1 ng/ml.