Insulin-like Growth Factor Pathway Genetic Polymorphisms, Circulating IGF1 and IGFBP3, and Prostate Cancer Survival

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Background The insulin-like growth factor (IGF) signaling pathway has been implicated in prostate cancer (PCa) initiation, but its role in progression remains unknown.

Methods Among 5887 PCa patients (704 PCa deaths) of European ancestry from seven cohorts in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium, we conducted Cox kernel machine pathway analysis to evaluate whether 530 tagging single nucleotide polymorphisms (SNPs) in 26 IGF pathway-related genes were collectively associated with PCa mortality. We also conducted SNP-specific analysis using stratified Cox models adjusting for multiple testing. In 2424 patients (313 PCa deaths), we evaluated the association of prediagnostic circulating IGF1 and IGFBP3 levels and PCa mortality. All statistical tests were two-sided.

Results The IGF signaling pathway was associated with PCa mortality ($P = .03$), and *IGF2-AS* and *SSTR2* were the main contributors (both $P = .04$). In SNP-specific analysis, 36 SNPs were associated with PCa mortality with P_{trend} less than .05, but only three SNPs in the *IGF2-AS* remained statistically significant after gene-based corrections. Two were in linkage disequilibrium ($r^2 = 1$ for rs1004446 and rs3741211), whereas the third, rs4366464, was independent ($r^2 = 0.03$). The hazard ratios (HRs) per each additional risk allele were 1.19 (95% confidence interval [CI] = 1.06 to 1.34; $P_{trend} = .003$) for rs3741211 and 1.44 (95% CI = 1.20 to 1.73; $P_{trend} < .001$) for rs4366464. rs4366464 remained statistically significant after correction for all SNPs ($P_{trend.corr} = .04$). Prediagnostic IGF1 ($HR_{highest\ vs\ lowest\ quartile} = 0.71$; 95% CI = 0.48 to 1.04) and IGFBP3 (HR = 0.93; 95% CI = 0.65 to 1.34) levels were not associated with PCa mortality.

Conclusions The IGF signaling pathway, primarily *IGF2-AS* and *SSTR2* genes, may be important in PCa survival.

Abundant experimental evidence indicates that the insulin-like growth factor (IGF) signaling pathway is important for cell survival and tumorigenesis (1,2). Epidemiological research, focused primarily on IGF1 and IGF binding protein 3 (IGFBP3) and risk of incident prostate cancer, suggests that higher circulating IGF1 were associated with increased risk of prostate cancer (3), with mixed findings for IGFBP3 levels (4). However, little is known about the role of prediagnostic circulating levels of IGF1 and/or IGFBP3 in prostate cancer survival.

Data on genetic variations in IGF-related genes and prostate cancer survival are sparse, limited by relatively small number of fatal outcomes and assessment of only a handful of single nucleotide polymorphisms (SNPs) related to risk of prostate cancer, as identified by tagging SNPs or from genome-wide association studies (5,6). To the best of our knowledge, a systematic evaluation of genetic variants of IGF pathway–related genes and progression to fatal prostate cancer is lacking.

The National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3), pooled data from multiple large cohort studies, was designed to examine associations of variations in genes that mediate the steroid hormone and the IGF signaling pathway with breast and prostate cancer risk (7). With an average 8.9 years of follow-up among 5887 prostate cancer patients of European ancestry in BPC3, we aimed to 1) use a novel kernel machine pathway analysis and SNP-specific analysis to evaluate whether common variations among 26 genes involved in the synthesis, metabolism, and regulation of IGFs were associated with prostate cancer mortality; and 2) investigate the associations of prediagnostic circulating IGF1 and IGFBP3 levels with prostate cancer mortality in a subset of 2424 patients.

Methods

Study Population

The BPC3 consists of seven nested case–control studies of prostate cancer from prospective cohort studies in the United States and Europe: Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC), American Cancer Society Cancer Prevention Study II (CPS-II), European Prospective Investigation into Cancer and Nutrition (EPIC), Health Professionals Follow-up Study (HPFS), Multiethnic Cohort Study (MEC), Physicians' Health Study (PHS), and Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial (7). Prostate cancer case patients were ascertained through population-based registries, self-report, or death certificates and verified by medical records. Height, body weight, and family history of prostate cancer were obtained by self-report. Data on disease stage (Jewett–Whitmore classification) and grade (Gleason score) were collected from each cohort. Written informed consent was obtained from all subjects, and each study was approved by the institutional review boards at their respective institutions. Details of vital status follow-up and determination of cause of death are described in the Supplementary Methods (available online).

SNP Selection and Genotyping

A total of 590 SNPs in 26 genes involved in the synthesis, metabolism, and regulation of insulin-like growth factors were genotyped (Figure 1). After restricting to self-reported European ancestry, a total of 5887 prostate cancer patients were included in this analysis. Two approaches were taken to evaluate linkage disequilibrium (LD) patterns and select the SNPs for this analysis as described elsewhere (7,8). Genotyping was performed in six laboratories: National Cancer Institute Core Genotyping Facility (Gaithersburg, MD), University of Southern California (Los Angeles, CA), University of Hawaii (Honolulu, HI), Harvard School of Public Health (Boston, MA), Imperial College (London, UK), and Cambridge University (Cambridge, UK). A total of 40 SNPs from *GNRH1*, *GNRHR*, *IGF1*, *IGFBP1*, and *IGFBP3* were genotyped using TaqMan (Applied Biosystems, Foster City, CA). The remaining SNPs were genotyped by Illumina Golden Gate platform (San Diego, CA). Interlaboratory concordance was evaluated by genotyping 94 samples from the SNP 500 cancer panel (9) for the TaqMan SNPs and 30 HapMap CEU (Utah residents with ancestry from northern and western Europe) trios for the Illumina panel, with concordance rates greater than 99% between laboratories.

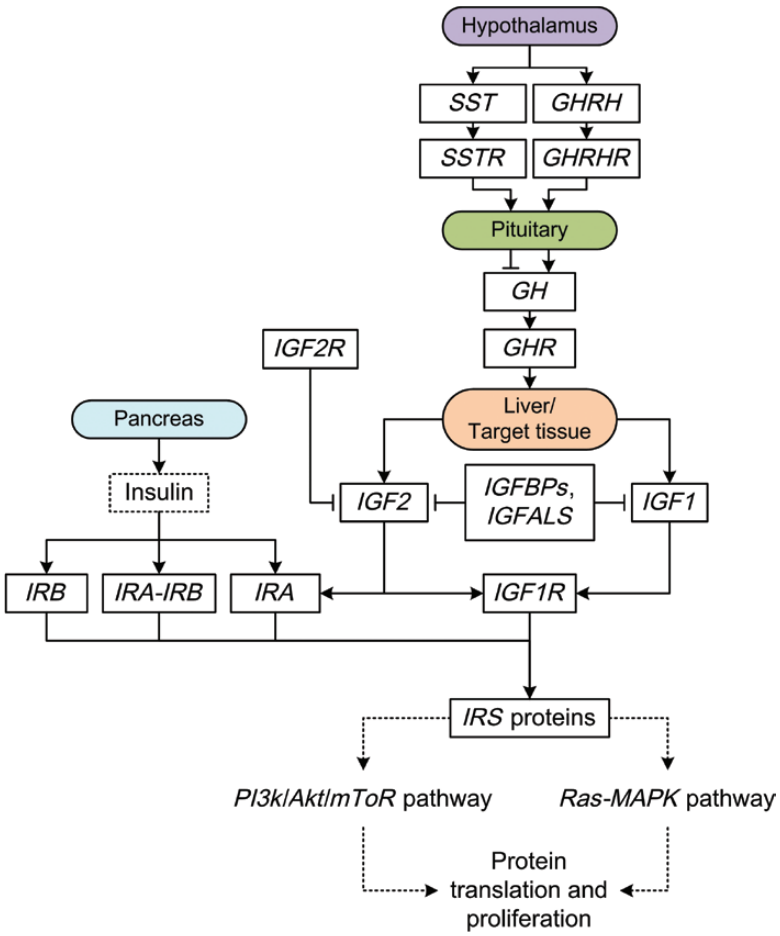

Figure 1. IGF signaling pathway. Genes included in this analysis were *SST, SSTR1-5, GHRH, GHRHR, GHR, IGF1, IGF1R, IGFBP1-6, IGF2-AS, IGF2R, IGFALS, INSR, IRS1, IRS2* (shown in Figure 1) and *POU1F1, GNRH1*, and *GNRHR* (not shown); the insulin receptor is encoded by a single gene, *INSR*, from which alternate splicing during transcription results in either *IRA* or *IRB* isoforms; the insulin gene (INS) was not genotyped, and genes in PI3k/Akt/mTOR and Ras-MAPK pathway were not included in this analysis.

Genotype data from the Taqman and Illumina platforms were filtered separately. Any sample in which more than 25% of the SNPs attempted on a given platform failed was removed from the dataset. Within each study, any SNP that failed in 25% or more of the samples, exhibited a statistically significant ($P < 10^{-5}$) deviation from Hardy–Weinberg proportions among European-ancestry controls, or had a minor allele frequency less than 1% was removed from the dataset. SNPs that were missing in more than 25% of the prostate cancer patients or showed large differences in allele frequency among subjects with European ancestry across studies (fixation index $F_{st} > 0.02$) were also excluded from analysis. For each gene region, SNPs that were polymorphic in any of the HapMap reference panels were imputed using MACH (10). Genotypes were imputed by cohort using the CEPH (Utah residents with ancestry from northern and western Europe) European (CEU) reference panel for subjects of European ancestry (release No. 21). Imputed data was filtered by study, and poorly imputed SNPs (r^2 <0.3) were removed from analysis.

Circulating IGF1 and IGFBP3 levels

Prediagnostic measurements of IGF1 and IGFBP3 were available for five of the seven cohorts (ATBC, EPIC, HPFS, PHS, and PLCO; n = 2445) (11–15). Details of sample collection and storage were described previously. Samples from ATBC, HPFS, and PHS were measured in the Pollak laboratory (McGill University, Montreal, QC, Canada), and the remaining studies were measured in the laboratory of the Hormones and Cancer Team at International Agency for Research on Cancer (IARC) with enzyme-linked immunosorbent assays (Diagnostic System Laboratories, Webster, TX). We excluded cohort and assay batch-specific statistical outliers (n = 21) based on the generalized extreme studentized deviate many-outlier detection approach, setting alpha to 0.05 for both IGF1 and IGFBP3 blood levels (16).

Statistical Analysis

IGF Gene Pathway. The kernel machine Cox regression framework (17,18), a novel and comprehensive approach for pathway analysis of censored survival outcomes, was used to assess associations with deaths from prostate cancer and other causes for SNP sets defined by all 26 genes in the IGF pathway and each gene individually after adjusting for continuous age and study cohort. Because genotyped SNPs may be imperfect surrogates for the true casual SNP, their individual relative risks are likely to be modest, and a multimarker global test will more effectively capture the true effect. The kernel machine accounts for LD in an SNP set, leading to a powerful test with reduced degrees of freedom. More attractively, it can also capture potential nonlinear SNP effects, SNP–SNP interactions (epistasis), and the joint effects of multiple causal variants without requiring a priori knowledge of directionality. The kernel machine tests whether an SNP set is associated with event time of interest after adjusting for covariables, and the test statistic under the null follows a mixture of χ^2 distributions, which can be approximated by resampling methods. Logistic kernel machines have been applied in a variety of traits and diseases (19,20).

SNP-specific analyses were conducted by stratified Cox proportional hazards models under a log-additive hazards assumption and stratified by study cohort, allowing different baseline hazards for each study. Follow-up was defined from the date of prostate cancer diagnosis to the date of any death or last follow-up. The assumption of proportionality was verified by testing each SNP and time since diagnosis, and no violation was identified. All analyses were adjusted for age at diagnosis and further adjusted for stage and Gleason score at diagnosis. To correct for multiple testing with possible presence of LD, the number of effective SNPs, M_{eff}, was calculated for each gene using a spectral decomposition approach (21). For gene-based *P* value correction, nominal *P* values for each SNP were multiplied by the M_{eff} for the gene. For the pathway-based correction, the M_{eff} values for all 26 genes were summed to correct the *P* values.

Cumulative incidence of prostate cancer death by years since diagnosis were plotted for statistically significant SNPs after gene-level–based correction using competing-risks regression by the method of Fine and Gray (22).

Stratified analysis of statistically significant SNPs and prostate cancer mortality association by age at diagnosis (<65 or ≥65 years) and BMI (<25, 25–30, or ≥30 kg/m^2), Gleason score (2–6, 7, or 8–10) and stage (A/B or C/D) were conducted under a dominant model as a result of limited sample size. To assess effect modification, we added a product term of statistically significant SNPs with the variables above and computed *P* values from log likelihood ratio test.

Circulating IGF1 and IGFBP3 Levels. We created batch-specific (n = 10) quartiles for IGF1 and IGFBP3 and assessed their associations with prostate cancer mortality simultaneously by stratified Cox proportional hazards models adjusting for age at diagnosis. Models were also additionally adjusted for BMI assessed at the baseline of each study to assess possible confounding or stage and Gleason score at diagnosis to evaluate possible mediation. Tests for trend were done by treating the median concentration for each quartile as a continuous variable. Stratified analysis by stage and Gleason score at diagnosis were also performed. To account for the possibility of reverse causation in which an undiagnosed tumor could affect biomarker levels, sensitivity analyses were conducted by excluding cases diagnosed within 2 years of blood draw.

Analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC), R (The R Foundation for Statistical Computing; http://www.r-project.org/foundation/), and Stata 12 (StataCorp, College Station, TX). All statistical tests were two-sided. A *P* value of less than .05 was considered statistically significant.

Results

During an average follow-up of 8.9 years among the 5887 case patients, 1,999 patients died, 704 of whom had prostate cancer as the underlying cause of death. Among the 2424 men in the subgroup of biomarker analysis, 313 of the 810 deaths were due to prostate cancer. Compared with those who were either alive at last follow-up or had died from other causes, patients who died from prostate cancer had higher Gleason score and clinical stage at diagnosis but similar BMI (Table 1; Supplementary Table 1, available online).

IGF Gene Pathway and Prostate Cancer Mortality

Pathway Analysis. A total of 530 SNPs were included in the genetic analysis. Kernel machine pathway analysis suggests that this set of SNPs covering all 26 genes in the IGF signaling pathway

Table 1. Characteristics of prostate cancer patients in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium*

Characteristic	PCa death (n = 704)	Censored (n = 5183)	Total (n = 5887)
Age at diagnosis, y, mean (SD)	69.1 (7.1)	68.3 (6.4)	68.4 (6.5)
Diagnosis to prostate cancer death/ censoring, y, mean (SD)	5.3 (3.8)	9.4 (3.9)	8.9 (4.1)
Body mass index, kg/m^2			
18–24.9	265 (38)	2030 (39)	2295 (39)
25–29.9	342 (49)	2393 (46)	2735 (46)
≥30	78 (11)	572 (11)	650 (11)
Missing	19 (2)	188 (4)	207 (4)
Family history			
Yes	39 (6)	576 (11)	615 (10)
No	358 (51)	3038 (59)	3396 (58)
Missing	307 (44)	1569 (30)	1876 (32)
Gleason score			
2–6	115 (16)	2567 (50)	2682 (46)
7	225 (32)	1465 (28)	1690 (29)
8–10	217 (31)	485 (9)	702 (12)
Missing	147 (21)	666 (13)	813 (14)
Stage			
A or B	259 (37)	3801 (73)	4060 (69)
C or D	343 (49)	702 (14)	1045 (18)
Missing	102 (14)	680 (13)	782 (13)
Biomarker subcohort			
No. of patients	313	2111	2424
Age at blood draw, y, mean (SD)	64.0 (7.8)	63.0 (6.9)	63.1 (7.1)
Circulating IGF1, ng/mL, median (IQR)	161 (124–212)	182 (142–228)	179 (139–227)
Circulating IGFBP3, ng/mL, median (IQR)	3110 (2544–3753)	3613 (2597–4333)	3544 (2899–4290)

* Data are No. (%) unless otherwise specified.

was associated with prostate cancer mortality (P = .03) (Table 2). When testing the SNP set of each gene, *IGF2-AS* (9 SNPs; P = .04) and *SSTR2* (14 SNPs; P = .04) showed statistically significant associations with prostate cancer mortality. The overall pathway P values were .05 without either *IGF2-AS* or *SSTR2* and .08 without both *IGF2-AS* and *SSTR2*, suggesting both *IGF2-AS* and *SSTR2* may contribute to the progression to fatal prostate cancer. Neither the overall pathway nor *IGF2-AS* or *SSTR2* were associated with risk of dying from causes other than prostate cancer.

SNP-Specific Analysis. A total of 36 SNPs were associated with prostate cancer mortality with P_{trend} < .05 (Supplementary Table 2, available online). After correcting for multiple testing at gene level, three SNPs, all in IGF2 antisense gene (*IGF2-AS*, 11p15.5), were statistically significantly associated with prostate cancer–specific mortality. Two of these SNPs, rs1004446 (intron) and rs3741211(3'-UTR), were in LD with each other (r^2 = 1 in 1000 Genome CEU population) but independent with the third SNP rs4366464 (intron) (r^2 = 0.03). For rs3741211, each additional A allele was associated with a 19% (hazard ratio [HR] = 1.19; 95% confidence interval [CI] = 1.06 to 1.34; P_{trend} = .003) increased risk of prostate cancer–specific mortality. For rs4366464, each additional minor allele G was associated with a 44% (HR = 1.44; 95% CI = 1.20 to 1.73) increased risk of prostate cancer mortality (P_{trend} = <.001) (Table 3; Supplementary Figure 1, available online). The association for rs4366464 remained statistically significant after further correcting for multiple testing of all SNPs ($P_{trend.corr}$ = .04; M_{eff} = 424). When mutually adjusted for each other, the hazard ratios remained similar for rs3741211 (HR = 1.15; 95% CI = 1.03 to 1.30) and rs4366464 (HR = 1.37; 95% CI = 1.13 to 1.67), suggesting independent additive effects of the two SNPs on prostate cancer progression. Cohort-specific associations (Figure 2) also indicated the robustness of these associations, and minimal heterogeneities were observed (rs3741211: I^2 < 0.05%, $P_{heterogenity}$ = .44; rs4366464: I^2 < 0.05%, $P_{heterogenity}$ = .55).

SNP rs4366464 or rs3741211 was not statistically significantly associated with either Gleason score or stage (data not shown). After additionally adjusting for these clinical parameters, the association between rs3741211 and prostate cancer death remained unchanged, whereas the hazard ratio for rs4366464 was slightly attenuated. Neither rs3741211 nor rs4366464 was associated with risk of dying from other causes (Table 3). These data suggest that the association between the two SNPs in *IGF2-AS* and prostate cancer mortality were independent of tumor characteristics and specific to death from prostate cancer.

Joint effect analysis suggests that for rs3741211, the association with prostate cancer mortality tended to be stronger among men with cancer diagnosed at younger age or patients with BMI less than 25 kg/m^2 (Supplementary Figure 2, available online). For rs4366464, the association was stronger among men diagnosed at younger age. For both SNPs, the associations were somewhat stronger among patients with higher stage (C or D) or higher Gleason score (≥7). However, only interaction between rs3741211 and stage was statistically significant (P = .02).

Circulating IGF1 and IGFBP3 and Prostate Cancer Mortality

IGF1 levels were statistically significantly correlated with IGFBP3 (r = 0.52; P < .001). Prediagnositic circulating levels of IGF1 ($HR_{highest\ vs\ lowest\ quartile}$ = 0.71; 95% CI = 0.48 to 1.04) and IGFBP3 (HR = 0.93; 95% CI = 0.65 to 1.34) were not associated with prostate cancer

Table 2. IGF pathway analyses for prostate cancer–specific mortality and mortality of other causes by kernel machine*

Gene abbreviation	Gene name	Chromosomal region	No. of SNPs included	*P* for PCa death†	*P* for other death†
Pathway					
Total pathway	—	—	530	.03	.14
Pathway w/o *IGF2-AS*	—	—	521	.05	.14
Pathway w/o *SSTR2*	—	—	516	.05	.13
Pathway w/o *IGF2-AS* and *SSTR2*	—	—	507	.08	.13
Gene					
GHR	Growth hormone receptor	5p13-p12	34	.16	.61
GHRH	Growth hormone releasing hormone	20q11.2	9	.14	.75
GHRHR	Growth hormone releasing hormone receptor	7p14	26	.38	.85
GNRH1	Gonadotropin-releasing hormone 1	8p21-p11.2	3	.86	.55
GNRHR	Gonadotropin-releasing hormone receptor	4q21.2	6	.15	.07
IGF1	Insulin-like growth factor 1	12q23.2	14	.35	.12
IGF1R	Insulin-like growth factor 1 receptor	15q26.3	112	.36	.20
IGF2-AS	IGF2 antisense RNA	11p15.5	9	.04	.40
IGF2R	Insulin-like growth factor 2 receptor	6q26	68	.09	.16
IGFALS	Insulin-like growth factor binding protein, acid labile subunit	16p13.3	7	.34	.54
IGFBP1	Insulin-like growth factor binding protein 1	7p13-p12	7	.29	.22
IGFBP2,5	Insulin-like growth factor binding protein 2 and 5	2q33-q36	36	.06	.34
IGFBP3	Insulin-like growth factor binding protein 3	7p13-p12	8	.77	.22
IGFBP4	Insulin-like growth factor binding protein 4	17q12-q21.1	7	.69	.67
IGFBP6	Insulin-like growth factor binding protein 6	12q13	7	.76	.54
INSR	Insulin receptor	19p13.3-p13.2	53	.07	.19
IRS1	Insulin receptor substrate 1	2q36	8	.53	.85
IRS2	Insulin receptor substrate 2	13q34	13	.38	.82
POU1F1	POU class 1 homeobox 1	3p11	6	.55	.42
SST	Somatostatin	3q28	16	.66	.03
SSTR1	Somatostatin receptor 1	14q13	19	.31	.06
SSTR2	Somatostatin receptor 2	17q24	14	.04	.50
SSTR3	Somatostatin receptor 3	22q13.1	18	.96	.96
SSTR4	Somatostatin receptor 4	20p11.2	26	.24	.41
SSTR5	Somatostatin receptor 5	16p13.3	4	.78	.91

* PCa = prostate cancer; SNP = single nucleotide polymorphism.

† *P* values were calculated using kernel machine Cox regression framework and were two-sided.

mortality in the model mutually adjusted for each other and age at diagnosis (Table 4). The hazard ratios were similar after additionally adjusting for stage and Gleason score at diagnosis in the model, or BMI at baseline, or excluding IGF1 and IGFBP3 measurements within 2 years of prostate cancer diagnosis (data not shown). In subgroup analysis, higher IGF1 levels were statistically significantly associated with lower prostate cancer mortality (P_{trend} = .02) among men diagnosed with more advanced tumors (stage C or D).

Discussion

To the best of our knowledge, this analysis of IGF pathway genes in relation to prostate cancer mortality among prostate cancer patients is the largest study to date. Using the kernel machine pathway analysis, a powerful test allowing assessment of the joint associations of variants in a predefined pathway, we demonstrated that the IGF pathway was statistically significantly associated with prostate cancer mortality and two genes, *IGF2-AS* and *SSTR2*, may play important roles in prostate cancer progression. Using SNP-specific association analysis, we further identified two SNPs, rs3741211 and rs4366464 in *IGF2-AS*, that were statistically significantly associated with prostate cancer mortality.

Additionally, among a subset of 2424 patients, we found no overall associations between prediagnostic circulating levels of IGF1 and IGFBP3 and prostate cancer mortality. The null associations between *IGF1* and *IGFBP3* genes and prostate cancer mortality suggest that their roles in the progression of prostate cancer were limited. In previous analyses of BPC3 patients, genetic variations in *IGF1* and *SSTR5* were associated with circulating levels of IGF1, and *IGFBP3* and *IGFALS* genes were associated with IGFBP3 levels (8,23). However, none of the SNPs in these genes were associated with prostate cancer mortality in our analysis, which is in line with the null findings between circulating levels of IGF1 and IGFBP3 and prostate cancer mortality. Although these findings should be interpreted with caution given the heterogeneities in blood collection, sample storage, and assay variation across the cohorts, the findings are not surprising because recent prospective studies did not support stronger associations of IGF1 levels with risk of advanced prostate cancer, favoring the hypothesis that common germline variations or circulating levels of IGF1 may contribute to early growth of prostate carcinogenesis (4), but not during progression.

The role of *IGF2-AS* and *IGF2* in prostate cancer initiation and progression is largely underexplored. A previous genome-wide

Table 3. Single nucleotpide polymorphisms in *IGF2-AS* associated with prostate cancer–specific mortality after gene-based *P* value correction*

SNP	Risk allele	RAF	Chromosomal region	Position	Genotype	Person-years	PCa death			Other death		
							No.	HR (95% CI)†	HR (95% CI)‡	No.	HR (95% CI)†	HR (95% CI)‡
rs3741211	A	0.626	11p15.5	2169110	GG	6945	64	1.00 (referent)	1.00 (referent)	182	1.00 (referent)	1.00 (referent)
					GA	23425	312	1.40 (1.07 to 1.83)	1.43 (1.09 to 1.87)	558	0.89 (0.75 to 1.05)	0.89 (0.75 to 1.05)
					AA	19562	295	1.55 (1.18 to 2.03)	1.50 (1.15 to 1.97)	500	0.93 (0.79 to 1.11)	0.93 (0.78 to 1.10)
					AA/GA	42987	607	1.47 (1.13 to 1.90)	1.46 (1.13 to 1.90)	1058	0.91 (0.78 to 1.07)	0.90 (0.77 to 1.06)
					per allele	—	—	1.19 (1.06 to 1.34)	1.16 (1.04 to 1.30)	—	0.99 (0.91 to 1.07)	0.98 (0.90 to 1.07)
					P_{trend}§	—	—	.003	.01	—	.75	.66
					$P_{trend.corr}$§	—	—	.02	.08	—	1.00	1.00
rs4366464	G	0.066	11p15.5	2164799	CC	44133	566	1.00 (referent)	1.00 (referent)	1099	1.00 (referent)	1.00 (referent)
					GC	6596	117	1.39 (1.14 to 1.70)	1.32 (1.08 to 1.62)	157	0.96 (0.82 to 1.14)	0.96 (0.81 to 1.14)
					GG	169	6	2.87 (1.28 to 6.44)	2.34 (1.04 to 5.25)	7	1.93 (0.92 to 4.08)	1.88 (0.89 to 3.97)
					GG/GC	6764	123	1.43 (1.18 to 1.74)	1.35 (1.11 to 1.65)	164	0.99 (0.84 to 1.16)	0.98 (0.83 to 1.16)
					per allele	—	—	1.44 (1.20 to 1.73)	1.36 (1.13 to 1.63)	—	1.01 (0.86 to 1.18)	1.00 (0.86 to 1.18)
					P_{trend}§	—	—	.0001	.001	—	.92	.96
					$P_{trend.corr}$§	—	—	.0008	.01	—	1.00	1.00

* CI = confidence interval; HR = hazard ratio; PCa = prostate cancer; SNP = single nucleotide polymorphism; RAF = risk allele frequency in patients who did not die from prostate cancer.

† The Cox model was stratified by study cohort and adjusted for age at diagnosis.

‡ The model was additionally adjusted for Gleason score and stage at diagnosis. Because adding body mass index to the multivariable model did not alter the hazard ratios, we decided not to present results adjusted for body mass index.

§ P_{trend} were calculated using stratified Cox proportional hazards models under a log-additive hazards assumption and were two-sided. $P_{trend.corr}$ were P_{trend} after gene-based correction for multiple testing (M_{eff} = 8).

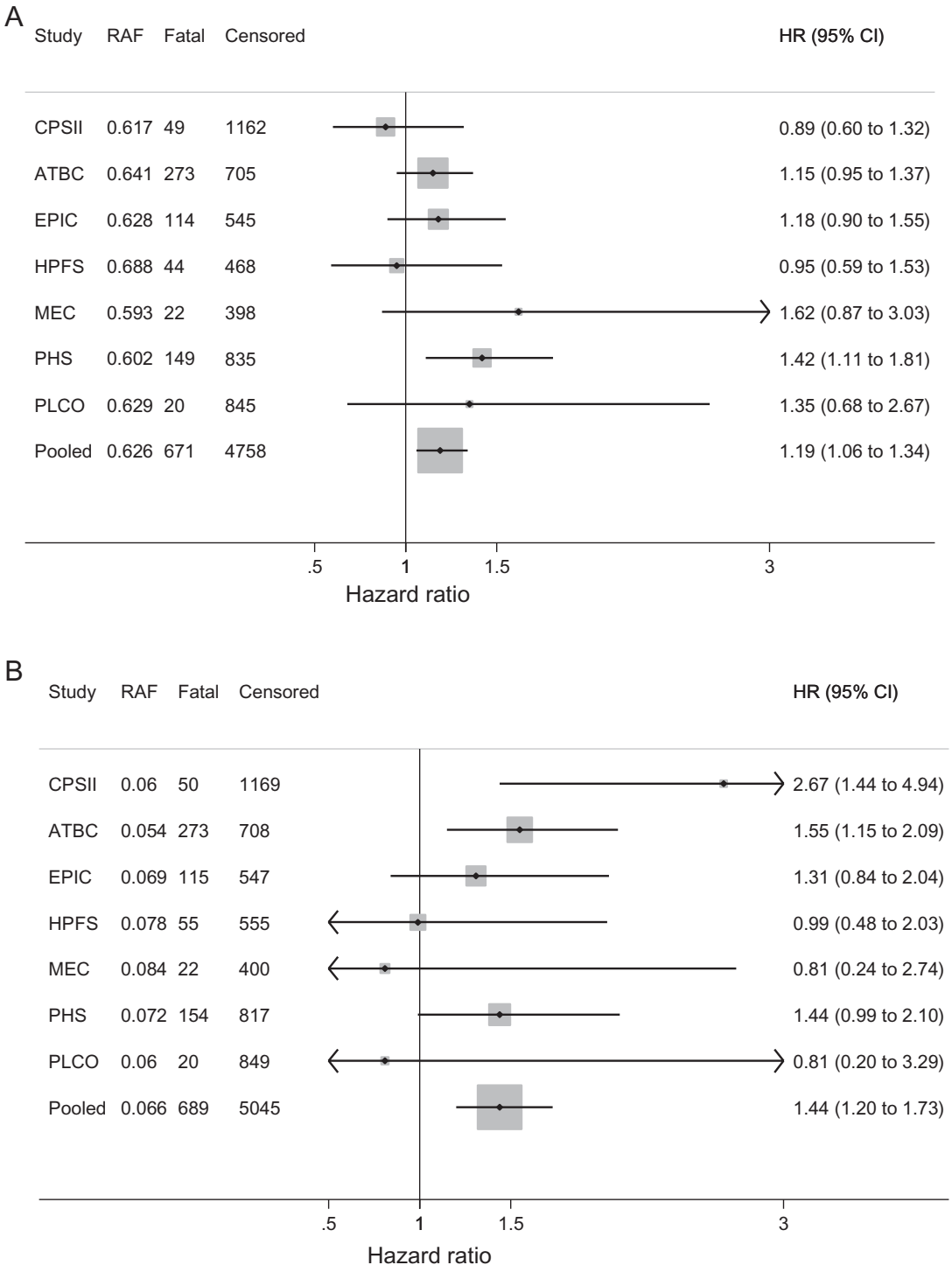

Figure 2. Association of *IGF2-AS* single nucleotide polymorphism rs3741211 and rs4366464 with prostate cancer–specific mortality by study cohort. Hazard ratios (HRs; **diamonds**) and 95% confidence intervals (CIs; **error bars**) calculated for the association for the individual studies and the pooled analysis for rs3741211 (**A**) and rs4366464 (**B**) are shown. **Size of gray square** represents percentage weight of each study. RAF = risk allele frequency. ATBC = Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CI = confidence interval; CPS-II = American Cancer Society Cancer Prevention Study II; EPIC = European Prospective Investigation into Cancer and Nutrition; HPFS = Health Professionals Follow-up Study; HR = hazard ratio; MEC = Multiethnic Cohort Study; PHS = Physicians' Health Study; PLCO = Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.

association study identified SNP rs7127900 in *IGF2-AS* as associated with risk of incident prostate cancer (24) but not with prostate cancer mortality (5). This SNP was not in LD with the two SNPs we identified (r^2 = 0.01 for rs3741211 and r^2 = 0.003 for rs4366464 in 1000 Genome CEU population).

IGF2 is a peptide growth factor that is homologous to both IGF1 and insulin; interaction of IGF2 with insulin receptor subtype A (IRA) may play a role both in fetal growth and cancer biology (25). *IGF2-AS* expresses a paternally imprinted antisense transcript of the *IGF2* gene. It is transcribed in the opposite

Table 4. Circulating levels of IGF1 and IGFBP3 and prostate cancer–specific mortality in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium

	Quartile*								
	Q1		Q2		Q3		Q4		
Outcome/ biomarker	Fatal/ censored	HR (95% CI)	Fatal/ censored	HR (95% CI)	Fatal/ censored	HR (95% CI)	Fatal/ censored	HR (95% CI)	P_{trend}§
All cases									
Model 1†									
IGF1	101/501	1.00 (referent)	80/529	0.86 (0.63 to 1.17)	68/541	0.74 (0.53 to 1.05)	64/540	0.71 (0.48 to 1.04)	.08
IGFBP3	102/499	1.00 (referent)	77/534	0.83 (0.61 to 1.13)	59/549	0.67 (0.47 to 0.95)	75/529	0.93 (0.65 to 1.34)	.35
Model 2‡									
IGF1	101/501	1.00 (referent)	80/529	0.84 (0.62 to 1.14)	68/541	0.77 (0.55 to 1.09)	64/540	0.77 (0.52 to 1.14)	.18
IGFBP3	102/499	1.00 (referent)	77/534	0.77 (0.57 to 1.06)	59/549	0.59 (0.41 to 0.84)	75/529	0.93 (0.65 to 1.35)	.28
Stage A or B†									
IGF1	35/357	1.00 (referent)	23/375	0.81 (0.46 to 1.41)	27/363	1.05 (0.59 to 1.89)	19/374	0.75 (0.37 to 1.53)	.53
IGFBP3	39/347	1.00 (referent)	25/376	0.65 (0.37 to 1.12)	13/381	0.37 (0.19 to 0.74)	27/365	0.77 (0.41 to 1.46)	.23
Stage C or D†									
IGF1	48/56	1.00 (referent)	38/80	0.73 (0.46 to 1.16)	32/91	0.58 (0.35 to 0.94)	30/89	0.52 (0.30 to 0.90)	.02
IGFBP3	40/64	1.00 (referent)	40/82	0.99 (0.63 to 1.55)	34/83	0.91 (0.55 to 1.52)	34/87	1.26 (0.73 to 2.19)	.38
Gleason<7†									
IGF1	19/246	1.00 (referent)	13/267	0.72 (0.34 to 1.53)	14/272	0.78 (0.36 to 1.70)	12/297	0.68 (0.28 to 1.68)	.49
IGFBP3	19/247	1.00 (referent)	15/274	0.86 (0.42 to 1.79)	11/283	0.68 (0.30 to 1.55)	13/278	0.85 (0.36 to 2.02)	.64
Gleason ≥7†									
IGF1	56/174	1.00 (referent)	48/180	0.93 (0.62 to 1.41)	30/188	0.64 (0.39 to 1.06)	35/172	0.81 (0.47 to 1.40)	.33
IGFBP3	57/169	1.00 (referent)	34/182	0.72 (0.46 to 1.12)	40/179	0.85 (0.54 to 1.34)	38/184	0.83 (0.49 to 1.41)	.63

* Batch-specific (n = 10) quartiles were used. All models were stratified by study cohort and simultaneously adjusted for IGF1 and IGFBP3. CI = confidence interval; HR = hazard ratio.

† Adjusted for age at diagnosis

‡ Adjusted for age, Gleason score, and stage at diagnosis

§ P_{trend} values were calculated by treating the median concentration for each quartile as a continuous variable and were two-sided.

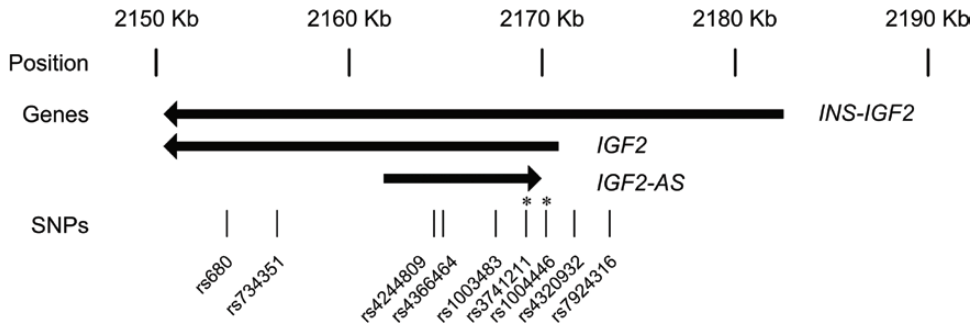

Figure 3. Gene map of *IGF2-AS/IGF2/INS* region and single nucleotide polymorphisms (SNPs) genotyped in *IGF2-AS* (n = 9). Only SNPs rs1004446 and rs3741211 have an r^2 greater than 0.8, indicated by an asterisk (*).

direction to the *IGF2* transcripts, with some genomic regions shared with *IGF2* (Figure 3) (26). *IGF2-AS* and *IGF2* were overexpressed in Wilms' tumor through loss of imprinting (26,27). Loss of imprinting of *IGF2* is generally manifested by the activation of the normally silenced maternal allele with the subsequent expression of both gene copies. Evidence from Wilms' tumor, colorectal cancer, and ovarian cancer suggests that the biallelic *IGF2* expression also correlates with aberrant *IGF2/H19* methylation (28,29). IGF2 levels were increased in prostate tumor–associated tissues, and a widespread *IGF2* loss of imprinting throughout the peripheral prostate in men with prostate cancer was observed but not in samples of benign prostatic hyperplasia or other adult tissues, suggesting that epigenetic modification may play an important role in prostate cancer carcinogenesis (30). Overexpression of *IGF2* and/or *IRA* has been proposed as a potential mechanism of resistance to IGF1R-directed therapies (31).

SSTR2 has been documented in experimental and clinical prostate cancer research but not in population studies. Somatostatin exerts inhibitory effects on cancer cells, including prostate, through five specific G-protein-coupled membrane receptors, SSTR1–5, with SSTR2 being predominant in human cancers (32,33). Its analogs, octreotide and lanreotide, which have high affinity for SSTR2, have been used to treat hormone-refractory prostate cancers (34,35) but are still under development.

The major strength of this study is the use of a large cohort consortium to study genetic predispositions, which are less likely to be affected by screening and treatment. Another strength is our

comprehensive evaluations of genetic variants in the IGF pathway using pathway, SNP-specific, and study cohort–specific analysis. However, additional genotyping to narrow down the region harboring the causal allele, followed by functional work on the identified variants and validations in other independent studies and/or races/ethnicities are necessary. Lack of patient treatment information was another limitation. However, associations of IGF genetic polymorphisms or biomarkers with prostate cancer mortality were unlikely to be affected by treatment because the two SNPs we identified, rs3741211 and rs4366464, were not associated with tumor characteristics (stage and Gleason score), the major determinants of treatment.

In summary, in this large consortium analysis of prostate cancer, both pathway and SNP-specific analyses showed that germline variations in *IGF2-AS* gene were associated with prostate cancer mortality, independent of stage and Gleason score and specific to prostate cancer. In contrast, neither genetic polymorphisms nor prediagnostic circulating levels of IGF1 and IGFBP3 were associated with prostate cancer mortality. Pathway analysis suggests that *SSTR2* may also play a role in prostate cancer progression, but SNP-specific analysis failed to show any statistically significant SNP in this gene after gene-level correction. Further research on the role of *IGF2/IGF2-AS* and *SSTR2* is needed.

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