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Original Contribution

Interactions Between Genome-wide Significant Genetic Variants and Circulating Concentrations of Insulin-like Growth Factor 1, Sex Hormones, and Binding Proteins in Relation to Prostate Cancer Risk in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium

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Genome-wide association studies (GWAS) have identified many single nucleotide polymorphisms (SNPs) associated with prostate cancer risk. There is limited information on the mechanistic basis of these associations, particularly about whether they interact with circulating concentrations of growth factors and sex hormones, which may be important in prostate cancer etiology. Using conditional logistic regression, the authors compared per-allele odds ratios for prostate cancer for 39 GWAS-identified SNPs across thirds (tertile groups) of circulating concentrations of insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3), testosterone, androstenedione, androstanediol glucuronide, estradiol, and sex hormone-binding globulin (SHBG) for 3,043 cases and 3,478 controls in the Breast and Prostate Cancer Cohort Consortium. After allowing for multiple testing, none of the SNPs examined were significantly associated with growth factor or hormone concentrations, and the SNP-prostate cancer associations did not differ by these concentrations, although 4 interactions were marginally significant (*MSMB*-rs10993994 with androstenedione (uncorrected $P = 0.008$); *CTBP2*-rs4962416 with IGFBP-3 (uncorrected $P = 0.003$); 11q13.2-rs12418451 with IGF-1 (uncorrected $P = 0.006$); and 11q13.2-rs10896449 with SHBG (uncorrected $P = 0.005$)). The authors found no strong evidence that associations between GWAS-identified SNPs and prostate cancer are modified by circulating concentrations of IGF-1, sex hormones, or their major binding proteins.

gene-environment interaction; gonadal steroid hormones; insulin-like growth factor binding protein 3; insulin-like growth factor I; molecular epidemiology; prostatic neoplasms

Abbreviations: BPC3, Breast and Prostate Cancer Cohort Consortium; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; GWAS, genome-wide association study(ies); IGF, insulin-like growth factor; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; MCCS, Melbourne Collaborative Cohort Study; OR, odds ratio; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; SD, standard deviation; SHBG, sex hormone-binding globulin; SNP, single nucleotide polymorphism.

Common genetic variants associated with prostate cancer risk were identified recently in genome-wide association studies (GWAS) (1–13). Many of these loci are located in intergenic regions, and their functions remain unclear. Some studies have investigated whether these associations differ according to other established or possible risk factors for

prostate cancer, including age, ethnicity, family history, body mass index, and diabetes, and have found no evidence for interaction (14, 15). However, little is known about whether these genetic associations are modified by circulating concentrations of insulin-like growth factors (IGFs) or steroid sex hormones. The IGF system is related to proliferation, tumor growth, and inhibition of apoptosis, and men with relatively high circulating concentrations of insulin-like growth factor 1 (IGF-1) are at increased risk of prostate cancer, as shown in a pooled reanalysis of the worldwide prospective data (16). Sex steroid hormones have long been hypothesized to be related to prostate cancer development, mainly because of the growth-promoting activities of testosterone and its derivatives (17), although circulating concentrations are not clearly associated with the risk of prostate cancer (18). However, single nucleotide polymorphisms (SNPs) in the 8q24 region have been associated with testosterone and androstenedione concentrations (19), suggesting that these genetic variants may influence prostate cancer risk through hormonal pathways.

To investigate the mechanistic basis for the association between GWAS-identified SNPs and prostate cancer risk, we examined the interactions between 39 SNPs and circulating concentrations of IGF-1, insulin-like growth factor binding protein 3 (IGFBP-3), testosterone, androstenedione, androstanediol glucuronide, estradiol, and sex hormone-binding globulin (SHBG) among 3,043 cases and 3,478 controls in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3).

MATERIALS AND METHODS

Source and study population

The BPC3 was established in 2004 to investigate common genetic variation for breast and prostate cancer and has combined resources from 10 cohort studies in the United States, Europe, and Australia (the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, Cancer Prevention Study II, the European Prospective Investigation into Cancer and Nutrition (EPIC), the Health Professionals Follow-up Study, the Melbourne Collaborative Cohort Study (MCCS), the Multi-Ethnic Cohort Study, the Nurses' Health Study, the Physicians' Health Study, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), and the Women's Health Study). Incident cancer cases were identified through linkage to cancer registries or through self-reports that were confirmed by medical records and/or pathology reports. With the exception of the case-cohort study design of MCCS, the BPC3 consists of a series of case-control studies nested within each cohort, where controls are matched to cases on age, ethnicity, and geographic region, depending on the cohort. Detailed information about this consortium and its component studies can be found elsewhere (20, 21).

The current study on gene-hormone interactions (the term "hormones" will be used in this paper to denote IGF-1, steroid sex hormones, and their binding proteins) and prostate cancer risk excluded 2 female cohorts (the Nurses' Health Study and the Women's Health Study) and cohorts that had no data or limited data on prediagnostic circulating hormone levels (Cancer Prevention Study II and the Multi-Ethnic Cohort Study). Men were excluded if they had prevalent cancer at recruitment or if they were not of white European ancestry, leaving a total of 3,043 prostate cancer cases and 3,478 controls available for analysis. All participants provided informed consent, and approval of the study was obtained from institutional review boards or ethics committees at the participating institutions.

Genotyping

A total of 39 SNPs were genotyped based on published GWAS for prostate cancer. The SNPs were: rs721048, rs1465618, rs12621278, rs2660753, rs4857841, rs17021918, rs12500426, rs7679673, rs9364554, rs10486567, rs6465657, rs1512268, rs2928679, rs4961199, rs1016343, rs7841060, rs16901979, rs620861, rs6983267, rs1447295, rs4242382, rs7837688, rs16902094, rs1571801, rs10993994, rs4962416, rs7127900, rs12418451, rs7931342, rs10896449, rs11649743, rs4430796, rs7501939, rs1859962, rs266849, rs2735839, rs5759167, rs5945572, and rs5945619. For rs12418451 and rs2928679, genotypes from rs12418451 or rs10896438 ($r^2 = 0.964$ in the HapMap CEU population) and from rs2928679 or rs13264338 ($r^2 = 0.966$ in the HapMap CEU population) were used, respectively. The MCCS did not have genotype data on rs4961199, rs16901979, and rs16902094.

Genotyping was performed using the TaqMan assay (Applied Biosystems, Foster City, California) at 7 genotyping laboratories in 4 countries: the Core Genotyping Facility at the National Cancer Institute, Harvard School of Public Health, the University of Southern California, the German Cancer Research Center, the University of Cambridge, Imperial College London, and the Genetic Epidemiology Laboratory of the University of Melbourne. The median genotyping success rate was 98.7% overall (interquartile range, 97.4%–99.6%; range, 82.4%–100%). Blinded duplicate samples (approximately 5%) were included within each study, and the concordance rate was greater than 99%. All autosomal SNPs were in Hardy-Weinberg equilibrium ($P > 0.02$).

Circulating hormone concentrations

Prediagnostic circulating concentrations of IGF-1, IGFBP-3, testosterone, androstenedione, androstanediol glucuronide, estradiol, and SHBG were measured in specialized laboratories. All laboratory personnel were blinded to the case-control status of the samples. Detailed information on assay methods can be found elsewhere (22–33).

Free testosterone and free estradiol concentrations were calculated using the law of mass action from the measured values of testosterone, estradiol, and SHBG, assuming a constant serum albumin concentration of 43 g/L (34). These calculated values correlate highly with the free hormone concentrations measured by equilibrium dialysis (35, 36).

Statistical analysis

Conditional logistic regression models with adjustment for age at blood draw (as a continuous variable in years)

were used to assess the association between each SNP and prostate cancer risk. To increase comparability in the matching process, we created new matched sets using age at blood draw (in 2-year intervals), cohort, and country (within EPIC). Odds ratios and their 95% confidence intervals were calculated per copy of the minor allele carried, which assumes a log-additive increase in risk for each risk allele, as a prior BPC3 study found no evidence of departure from an additive model for these SNPs (14).

The associations between circulating concentrations of the 9 hormones and risk of prostate cancer were examined using conditional logistic regression models comparing cohort-specific thirds (tertile groups) of the hormone concentrations, calculated among controls, after adjustment for age at blood draw (continuous) and body mass index (continuous), which was calculated as weight in kilograms divided by height in meters squared. The cutpoints for the cohort-specific thirds of the hormone concentrations are shown in Web Table 1 (http://aje.oxfordjournals.org/). Likelihood ratio tests were used to evaluate the heterogeneity of associations between cohorts.

Geometric mean values and 95% confidence intervals for the circulating hormone concentrations were calculated by genotype for each SNP (common homozygotes, heterozygotes, rare homozygotes), using linear regression models with a natural logarithmic transformation for the hormones and adjustment for age at blood draw (continuous), case-control status, cohort, and country (within EPIC). Further adjustment for body mass index made little difference in the risk estimates, and results are not presented here. When this analysis was restricted to controls only, the results were very similar, so data on all participants combined are presented. Likelihood ratio tests were used to evaluate the heterogeneity of associations by body mass index (continuous).

To test for gene-hormone interactions in relation to prostate cancer risk, the per-allele odds ratios for prostate cancer for each SNP were compared across cohort-specific thirds of each of the 9 hormone concentrations using conditional logistic regression models adjusted for age at blood draw and body mass index. The *P* values for interaction were calculated using 1-df likelihood ratio tests based on per-allele odds ratios and a continuous hormone variable.

All reported *P* values are 2-sided and uncorrected for multiple hypothesis testing, but they are interpreted in view of the 351 (39 SNPs $\times$ 9 hormones) comparisons made. Using the Bonferroni correction, only an uncorrected *P* value less than 0.00014 would be regarded as statistically significant. All statistical analyses were performed using STATA, version 11 (StataCorp LP, College Station, Texas).

RESULTS

Selected demographic and serologic characteristics of the 3,043 prostate cancer cases and 3,478 controls are shown by cohort in Web Table 2. The cases were on average aged 62.9 years (standard deviation (SD), 6.9) at the time of blood draw and aged 67.8 years (SD, 6.7) at cancer diagnosis, while the controls were on average aged 60.2 years (SD, 8.6) at blood draw. Cases and controls had overall mean body mass indices of 26.3 (SD, 3.4), and 26.6 (SD, 3.6), respectively. The geometric mean values for circulating concentrations of the 9 hormones differed by cohort.

Table 1 shows the per-allele association between the 39 GWAS-identified SNPs and prostate cancer risk. The directions of all associations were consistent with previous GWAS findings (1–10, 13, 37, 38), but 6 SNPs (rs1465618, rs12621278, rs2660753, rs12500426, rs2928679, and rs266849) were not significantly associated with risk in this study. The strongest association was observed for rs4242382, located at 8q24 (odds ratio (OR) = 1.47, 95% confidence interval (CI): 1.31, 1.66; $P = 1.63 \times 10^{-10}$), and the weakest association was observed for rs266849, near *KLK3* (OR = 0.98, 95% CI: 0.89, 1.08; $P = 0.63$).

Table 2 shows odds ratios and 95% confidence intervals for tertiles of circulating hormone concentrations and prostate cancer risk. Circulating concentrations of IGF-1 and IGFBP-3 were significantly associated with risk; compared with men in the lowest third, men in the highest third had 17% (OR = 1.17, 95% CI: 1.02, 1.34; *P*-trend = 0.03) and 23% (OR = 1.23, 95% CI: 1.07, 1.42; *P*-trend = 0.003) higher risks, respectively. SHBG was inversely associated with risk (OR = 0.83, 95% CI: 0.71, 0.96; *P*-trend = 0.01). Sex steroid hormones were not associated with risk. Risk estimates did not vary significantly between the different cohorts (all *P*-heterogeneity values ≥ 0.05).

The distributions of the circulating hormone concentrations by genotype for the SNPs nominally associated with hormone levels ($P < 0.05$) are shown in Table 3 (the distributions across all SNPs are provided in Web Table 3). We conducted 351 tests of association, and 17 results were conventionally significant; 18 were expected to be significant by chance alone. None of the associations remained significant after allowance for multiple tests. On the basis of *P* values, the strongest associations were between rs12621278 (located in *ITGA6*) and testosterone concentrations ($P = 0.005$) and between rs6465657 (located in *LMTK2*) and SHBG concentrations ($P = 0.003$). Homozygous carriers of the G allele of rs12621278 had lower average testosterone concentrations (geometric mean = 11.8 nmol/L, 95% CI: 9.76, 14.2) than homozygous carriers of the A allele (geometric mean = 15.7 nmol/L, 95% CI: 15.5, 15.9) and heterozygotes (geometric mean = 16.1 nmol/L, 95% CI: 15.5, 16.7), but this association was based on only 19 participants with a GG genotype. Homozygous (geometric mean = 41.5 nmol/L, 95% CI: 40.3, 42.7) and heterozygous (geometric mean = 42.6 nmol/L, 95% CI: 41.8, 43.5) carriers of the C allele of rs6465657 had slightly higher concentrations of SHBG than TT carriers (geometric mean = 40.3 nmol/L, 95% CI: 39.3, 41.4). The associations between the genetic variants and circulating hormone concentrations did not vary significantly by body mass index (results not shown).

To investigate whether the genetic associations with prostate cancer were stronger for specific strata of circulating hormone concentrations, we evaluated 351 gene-environment interactions (Figure 1 and Web Tables 4–12). Only 15 findings were significant at the 0.05 level (Figure 1); 18 were expected to be significant by chance alone, and none of these were significant after allowance for multiple comparisons.

Table 1. Per-Allele Associations Between 39 Single Nucleotide Polymorphisms Identified in Genome-wide Association Studies and Risk of Prostate Cancer in the Breast and Prostate Cancer Cohort Consortium

Single Nucleotide Polymorphism	Chromosome	Gene	No. of Cases	No. of Controls	Minor Allele Frequency (in Controls)	Per-Allele Odds Ratio[a]	95% Confidence Interval	*P* Value
rs721048	2	*EHBP1*	2,921	3,374	0.18 (A)	1.12	1.02, 1.24	0.02
rs1465618	2	*THADA*	2,911	3,027	0.21 (T)	1.07	0.98, 1.18	0.13
rs12621278	2	*ITGA6*	2,920	3,033	0.06 (G)	0.91	0.77, 1.07	0.24
rs2660753	3	—[b]	2,928	3,369	0.11 (T)	1.11	0.99, 1.24	0.08
rs4857841	3	*EEFSEC*	2,671	3,013	0.28 (A)	1.15	1.06, 1.25	0.001
rs17021918	4	*PDLIM5*	2,906	3,039	0.35 (T)	0.87	0.80, 0.94	5.67×10^{-04}
rs12500426	4	*PDLIM5*	2,871	3,002	0.46 (A)	1.05	0.98, 1.14	0.18
rs7679673	4	*TET2*	2,915	3,034	0.42 (A)	0.86	0.80, 0.93	2.02×10^{-04}
rs9364554	6	*SLC22A3*	2,927	3,358	0.27 (T)	1.11	1.02, 1.20	0.02
rs10486567	7	*JAZF1*	2,908	3,381	0.24 (A)	0.84	0.77, 0.92	2.15×10^{-04}
rs6465657	7	*LMTK2*	2,918	3,359	0.46 (C)	1.09	1.01, 1.17	0.02
rs1512268	8	*NKX3-1*	2,940	3,056	0.44 (T)	1.11	1.03, 1.20	0.007
rs2928679	8	*SLC25A37*	2,809	2,787	0.46 (A)	1.03	0.96, 1.12	0.42
rs4961199	8	*CPNE3* and *CNGB3*	2,411	2,186	0.15 (A)	1.13	1.01, 1.28	0.04
rs1016343	8	—	2,734	3,046	0.20 (T)	1.20	1.09, 1.32	1.34×10^{-04}
rs7841060	8	—	2,704	3,006	0.20 (G)	1.22	1.11, 1.34	3.68×10^{-05}
rs16901979	8	—	2,407	2,174	0.03 (A)	1.41	1.12, 1.76	0.003
rs620861	8	—	2,586	2,882	0.37 (T)	0.91	0.84, 0.99	0.03
rs6983267	8	—	2,902	3,368	0.50 (T)	0.79	0.74, 0.86	2.17×10^{-09}
rs1447295	8	—	2,738	3,051	0.10 (A)	1.42	1.26, 1.61	2.59×10^{-08}
rs4242382	8	—	2,984	3,435	0.10 (A)	1.47	1.31, 1.66	1.63×10^{-10}
rs7837688	8	—	2,687	3,018	0.10 (T)	1.44	1.27, 1.63	9.65×10^{-09}
rs16902094	8	—	2,278	2,052	0.17 (G)	1.15	1.03, 1.30	0.02
rs1571801	9	—	2,793	2,863	0.24 (A)	1.16	1.06, 1.28	0.001
rs10993994	10	*MSMB*	2,883	3,341	0.40 (T)	1.21	1.12, 1.30	1.27×10^{-06}
rs4962416	10	*CTBP2*	2,799	3,292	0.27 (C)	1.09	1.00, 1.18	0.05
rs7127900	11	—	2,898	3,044	0.20 (A)	1.21	1.10, 1.33	6.96×10^{-05}
rs12418451	11	—	2,294	1,918	0.28 (A)	1.28	1.16, 1.41	5.05×10^{-07}
rs7931342	11	—	2,929	3,332	0.50 (T)	0.82	0.76, 0.89	5.21×10^{-07}
rs10896449	11	—	2,877	3,357	0.50 (A)	0.81	0.75, 0.87	1.61×10^{-08}
rs11649743	17	*TCF2*	2,890	3,375	0.19 (A)	0.90	0.82, 1.00	0.04
rs4430796	17	*TCF2*	2,780	2,786	0.49 (G)	0.79	0.73, 0.85	3.53×10^{-09}
rs7501939	17	*TCF2*	2,665	3,002	0.40 (T)	0.82	0.76, 0.89	1.01×10^{-06}
rs1859962	17	—	2,931	3,392	0.48 (T)	0.85	0.79, 0.92	1.50×10^{-05}
rs266849	19	*KLK3*	2,863	3,322	0.19 (G)	0.98	0.89, 1.08	0.63
rs2735839	19	*KLK3*	2,864	3,318	0.15 (A)	0.90	0.81, 1.00	0.05
rs5759167	22	*BIK*	2,906	3,035	0.50 (T)	0.83	0.77, 0.89	9.39×10^{-07}
rs5945572	X	*NUDT11*	2,705	3,002	0.35 (A)	1.11	1.05, 1.18	3.96×10^{-04}
rs5945619	X	*NUDT11*	2,933	3,353	0.35 (C)	1.11	1.05, 1.17	2.95×10^{-04}

[a] Results were obtained from a conditional logistic regression model matched for age at blood draw, cohort, and country (within the European Prospective Investigation into Cancer and Nutrition) and adjusted for age at blood draw (years; continuous).
[b] Intergenic region.

Two SNPs on chromosome 10 (rs10993994 and rs4962416) and 2 SNPs on chromosome 11 (rs12418451 and rs10896449) showed potential gene-environment interactions with circulating concentrations of androstenedione, IGFBP-3, IGF-1, and SHBG, respectively. The per-allele odds ratio for prostate cancer for rs10993994 (located in *MSMB*) was

Table 2. Associations Between Insulin-like Growth Factor and Steroid Sex Hormone Concentrations and Risk of Prostate Cancer in the Breast and Prostate Cancer Cohort Consortium

Hormone	No. of Cases	No. of Controls	Tertile of Hormone Concentration						*P*-Trend[a]	*P*-Heterogeneity (by Cohort)[b]
			First		Second		Third			
			OR[c]	95% CI	OR[c]	95% CI	OR[c]	95% CI		
Insulin-like growth factor 1, nmol/L	2,771	3,055	1.00	Reference	1.10	0.96, 1.25	1.17	1.02, 1.34	0.03	0.23
Insulin-like growth factor binding protein 3, nmol/L	2,772	3,054	1.00	Reference	1.11	0.97, 1.27	1.23	1.07, 1.42	0.003	0.36
Testosterone, nmol/L	2,282	2,762	1.00	Reference	1.02	0.89, 1.18	1.00	0.86, 1.16	0.99	0.06
Free testosterone, pmol/L	2,257	2,742	1.00	Reference	0.97	0.84, 1.13	1.06	0.92, 1.23	0.44	0.05
Androstenedione, nmol/L	1,034	1,651	1.00	Reference	1.09	0.89, 1.33	1.03	0.84, 1.26	0.77	0.36
Androstanediol glucuronide, nmol/L	2,293	2,704	1.00	Reference	0.98	0.85, 1.13	1.01	0.88, 1.17	0.87	0.25
Estradiol, pmol/L	1,255	1,779	1.00	Reference	0.89	0.73, 1.08	1.04	0.86, 1.26	0.70	0.05
Free estradiol, pmol/L	1,255	1,778	1.00	Reference	0.90	0.74, 1.09	1.04	0.85, 1.26	0.73	0.52
Sex hormone-binding globulin, nmol/L	2,307	2,789	1.00	Reference	0.93	0.80, 1.07	0.83	0.71, 0.96	0.01	0.50

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] The ordinal hormone variable (0, 1, 2) was used in the calculation of *P*-trend.

[b] Likelihood ratio tests were used to evaluate heterogeneity between the cohorts.

[c] Results were obtained from a conditional logistic regression model using thirds of hormone concentration (see Web Table 1), matched for age at blood draw, cohort, and country (within the European Prospective Investigation into Cancer and Nutrition), and adjusted for age at blood draw (years; continuous) and body mass index.

significantly higher for men in the lowest third of androstenedione concentration (Figure 1; OR = 1.36, 95% CI: 1.10, 1.68), intermediate for the second third (OR = 1.19, 95% CI: 0.97, 1.46), and null for the highest third (OR = 0.98, 95% CI: 0.79, 1.22; *P*-interaction = 0.008). The per-allele association of rs4962416 (located in *CTBP2*) and prostate cancer risk was significant only for men in the top third of IGFBP-3 concentration (Figure 1; OR = 1.30, 95% CI: 1.11, 1.52), while it was null for the middle and lower thirds (middle third: OR = 0.95, 95% CI: 0.82, 1.12; lowest third: OR = 1.02, 95% CI: 0.87, 1.19; *P*-interaction = 0.003). Similar suggestive interactions were observed for 2 SNPs in 11q13.2; the association between rs12418451 and risk was stronger for the lowest third of IGF-1 concentration (Figure 1; OR = 1.48, 95% CI: 1.22, 1.79; *P*-interaction = 0.006), while the association between rs10896449 and risk was stronger for the top third of SHBG concentration (Figure 1; OR = 0.71, 95% CI: 0.61, 0.82; *P*-interaction = 0.005). However, none of these 4 SNPs (rs10993994, rs4962416, rs12418451, and rs10896449) were associated with circulating levels of the hormone of interest: androstenedione (*P* = 0.52), IGFBP-3 (*P* = 0.48), IGF-1 (*P* = 0.81), and SHBG (*P* = 0.91), respectively (Web Table 3). Notably, the integrin α-6 (*ITGA6*) SNP rs12621278, which was associated with testosterone concentrations (Table 3; *P* = 0.005), also showed some evidence for a gene-testosterone interaction (Figure 1; *P*-interaction = 0.04), but this might have been a chance finding given the large number of tests performed.

DISCUSSION

To our knowledge, this was the first study to investigate potential interactions between polymorphisms for prostate cancer identified from GWAS and circulating IGF and sex steroid hormone concentrations. The BPC3 is in a unique position to explore gene-environment interactions because it consists of 10 well-established cohort studies (of which 6 contributed to this analysis) with prospectively collected blood specimens, high-quality biomarker assays, and genotyping data for thousands of participants. With 3,043 prostate cancer cases and 3,478 controls, this study had more than 80% power to detect a multiplicative interaction effect of 1.7, assuming an allele frequency of 30% and an SNP or hormone main effect on prostate cancer of 1.1, but for some SNP-hormone combinations the power was reduced because of missing values. Of the 351 tests for gene-hormone interaction conducted, only 15 were conventionally significant (18 would be expected by chance alone), and therefore, in view of the multiple testing, these findings are likely to be due to chance.

This study found that the circulating hormone concentrations did not strongly differ by genotype, suggesting that the low-penetrance susceptibility loci investigated here are unlikely to affect prostate cancer risk through mechanisms involving the IGF system or endogenous sex hormones. We found several weak associations, which were possibly due to chance, given the large number of tests performed. The concentration of testosterone among men with the GG genotype

Table 3. Distribution of Insulin-like Growth Factor Proteins and Steroid Sex Hormones According to Single Nucleotide Polymorphisms Identified in Genome-wide Association Studies for 17 Nominally Significant Findings in the Breast and Prostate Cancer Cohort Consortium

Hormone and Single Nucleotide Polymorphism	Zygosity and Hormone Concentration[a]						*P* Value[b]
	Common Homozygote		Heterozygote		Rare Homozygote		
	GM	95% CI	GM	95% CI	GM	95% CI	
Insulin-like growth factor 1, nmol/L							
rs266849	22.6	22.3, 22.9	23.0	22.6, 23.3	24.0	22.8, 25.2	0.03
Insulin-like growth factor binding protein 3, nmol/L							
rs2660753	117	116, 117	117	116, 119	124	118, 131	0.04
rs7679673	116	115, 118	117	116, 118	119	117, 121	0.03
Testosterone, nmol/L							
rs12621278	15.7	15.5, 15.9	16.1	15.5, 16.7	11.8[c]	9.76, 14.2	0.005
rs6465657	15.6	15.3, 16.0	15.9	15.7, 16.2	15.3	15.0, 15.7	0.03
Androstenedione, nmol/L							
rs7679673	3.81	3.71, 3.91	3.81	3.73, 3.90	3.60	3.47, 3.74	0.03
rs16901979	4.38	4.24, 4.52	4.89	4.51, 5.30	2.78[c]	1.39, 5.54	0.01
Androstanediol glucuronide, nmol/L							
rs1859962	12.4	12.0, 12.8	13.0	12.7, 13.3	13.2	12.8, 13.6	0.02
Estradiol, pmol/L							
rs2660753	109	108, 111	108	105, 110	97.3[c]	89.1, 106	0.03
rs10486567	108	107, 110	110	108, 112	104	99.1, 109	0.04
Free estradiol, pmol/L							
rs17021918	1.61	1.57, 1.65	1.56	1.52, 1.60	1.51	1.44, 1.59	0.04
rs4242382	1.58	1.56, 1.61	1.51	1.46, 1.56	1.58[c]	1.36, 1.83	0.03
rs16902094	1.37	1.28, 1.46	1.30	1.21, 1.39	1.19	1.04, 1.37	0.04
Sex hormone-binding globulin, nmol/L							
rs12621278	41.3	40.6, 41.9	43.5	41.6, 45.6	33.9[c]	26.8, 42.9	0.02
rs6465657	40.3	39.3, 41.4	42.6	41.8, 43.5	41.5	40.3, 42.7	0.003
rs4242382	41.1	40.5, 41.7	43.2	41.9, 44.6	40.9	36.2, 46.3	0.02
rs16902094	43.7	41.4, 46.2	46.1	43.3, 49.0	48.7	42.9, 55.3	0.02

Abbreviations: CI, confidence interval; GM, geometric mean.

[a] Results were obtained from a linear regression analysis of the association between natural log-transformed hormone concentrations and single nucleotide polymorphisms with adjustment for age at blood draw (years; continuous), case-control status, cohort, and country (within the European Prospective Investigation into Cancer and Nutrition).

[b] Test of the difference between the 3 geometric means using the *F* distribution. Conventional *P* values are shown; all *P* values were nonsignificant after allowance for multiple testing.

[c] The total sample size in this genotype subgroup was 50 observations or fewer.

of the *ITGA6*-rs12621278 SNP, which encodes a cell-surface protein, was slightly lower than that in carriers of the A allele, while the G allele has been reported to be associated with a lower risk of prostate cancer in GWAS (1). Another weak association was observed between the lemur tyrosine kinase 2 (*LMTK2*)-rs6465657 SNP, which encodes for a serine/threonine kinase, and SHBG concentration. Homozygous or heterozygous carriers of the C allele, which was associated with an increased risk of prostate cancer in this study, had slightly higher concentrations of SHBG than TT carriers. To our knowledge, no other study has examined the potential effects of these genes on hormone levels, and confirmatory data are needed. However, we had no prior expectation for gene-hormone associations because none of the genetic regions identified through GWAS are known to be closely involved in hormone metabolism. In a separate study that was performed in 563 controls in the PLCO cohort, Chu et al. (19) reported a possible association between SNPs in

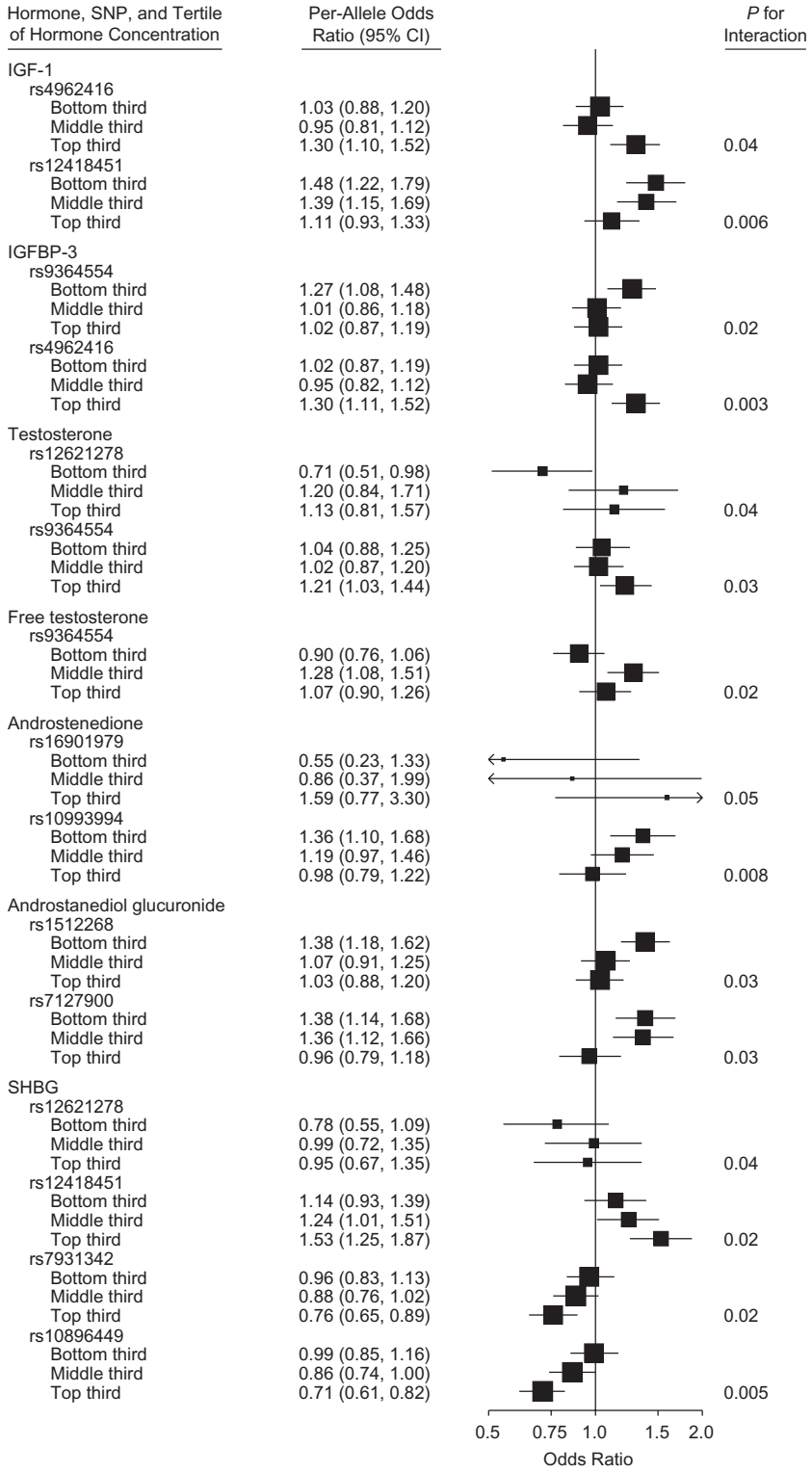

Figure 1. Per-allele associations between single nucleotide polymorphisms (SNPs) identified in genome-wide association studies and risk of prostate cancer, according to circulating concentrations of insulin-like growth factor and steroid sex hormones, for the 15 nominally significant interactions in the Breast and Prostate Cancer Cohort Consortium. Results were obtained from a conditional logistic regression model using cohort-specific thirds of the hormone concentrations (see Web Table 1), matched for age at blood draw, cohort, and country (within the European Prospective Investigation into Cancer and Nutrition), and adjusted for age at blood draw (years; continuous) and body mass index. The *P* values for interaction were calculated using 1-df likelihood ratio tests based on per-allele odds ratios and a continuous hormone variable. Conventional *P* values are shown; all *P* values were nonsignificant after allowance for multiple testing. Bars, 95% confidence interval (CI). (IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SHBG, sex hormone-binding globulin).

the 8q24 region and testosterone and androstenedione concentrations. The current study included data from 6 cohorts, including the PLCO cohort, and genotyped 9 SNPs in 8q24 that were in linkage disequilibrium with the SNPs identified in the previous study, none of which were associated with androgen concentrations.

The directions of the relative risks for the main effects of the SNPs on prostate cancer risk in our study are consistent with previous GWAS findings (1–10, 13, 37, 38). Six SNPs were not associated with risk in our study, most likely because of smaller sample sizes, as all of these 6 SNPs were significantly associated with risk in a larger sample of the BPC3 study (14). Similarly for hormones, the directions and magnitudes of the main effects were similar to those previously published in larger pooled analyses (16, 18).

Although in this study we found no strong evidence for gene-hormone interactions in relation to prostate cancer risk, there were 4 marginally significant interactions (after allowance for multiple tests) for 2 SNPs in chromosome 10 (rs10993994 and rs4962416) and 2 SNPs in chromosome 11 (rs12418451 and rs10896449) with circulating concentrations of androstenedione, IGFBP-3, IGF-1, and SHBG, respectively. Although no mechanisms that could predict such interactions are known, possible indirect links have been suggested for the chromosome 10 variants and hormone metabolism. The rs10993994 SNP resides in the proximal promoter of the microseminoprotein β gene (*MSMB*) (3) and has been shown to affect multiple binding sites for transcription and splicing factors, while this SNP lies less than 50 base pairs downstream of androgen and estrogen receptor binding sites (2). The rs4962416 SNP resides in the fifth intron of the C-terminal binding protein 2 gene (*CTBP2*) (3), and *CTBP2* expression has been associated with activation of the phosphatidylinositol 3-kinase pathway (39), which is largely mediated by upstream IGF signals (40). However, in view of the multiple comparisons in our study, these interactions may also have arisen from chance and should be reexamined in larger studies.

Several factors should be considered in interpreting our findings. This study investigated possible interactions between 39 GWAS-identified prostate cancer-associated SNPs and circulating concentrations of 9 hormones in a large, Caucasian, multicountry prospective setting, but none of these interactions were significant after allowing for multiple comparisons. However, we cannot exclude the possibility that there may be modest gene-hormone interactions that this study had insufficient statistical power to detect. For this reason, we did not report results of interaction analyses by stage and grade of prostate cancer. In general, lack of statistical interaction does not imply lack of biologic (causal) interaction. Moreover, absence of interaction on the multiplicative scale does not imply absence of a ''public health interaction,'' where the absolute scale (risk difference) is used (41). In addition, recently published GWAS have added several new prostate cancer SNPs to the 39 SNPs studied here (37, 42). Therefore, more studies, with a larger number of participants, are needed to reexamine our findings, to study untested GWAS-identified SNPs, and to evaluate the gene-hormone interactions for prostate cancer in people of other ethnicities.

In conclusion, our study found no strong evidence that the SNP-prostate cancer association differed by circulating concentrations of IGF-1, steroid sex hormones, or their major binding proteins or that the polymorphisms studied were related to blood levels of those hormones.

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