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PTGS2 and *IL6* genetic variation and risk of breast and prostate cancer: results from the Breast and Prostate Cancer Cohort Consortium (BPC3)

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Genes involved in the inflammation pathway have been associated with cancer risk. Genetic variants in the *interleukin-6* (*IL6*) and *prostaglandin-endoperoxide synthase-2* (*PTGS2,* encoding for the COX-2 enzyme) genes, in particular, have been related to several cancer types, including breast and prostate cancers. We conducted a study within the Breast and Prostate Cancer Cohort Consortium to examine the association between *IL6* and *PTGS2* polymorphisms and breast and prostate cancer risk. Twenty-seven polymorphisms, selected by pairwise tagging, were genotyped on 6292 breast cancer cases and 8135 matched controls and 8008 prostate cancer cases and 8604 matched controls. The large sample sizes and comprehensive single nucleotide polymorphism tagging in this study gave us excellent power to detect modest effects for common variants. After adjustment for multiple testing, none of the associations examined remained statistically significant at $P = 0.01$. In analyses not adjusted for multiple testing, one *IL6* polymorphism (rs6949149) was marginally associated with breast cancer risk (TT versus GG, odds ratios (OR): 1.32; 99% confidence intervals (CI): 1.00–1.74, $P_{trend} = 0.003$) and two were marginally associated with prostate cancer risk (rs6969502-AA versus rs6969502-GG, OR: 0.87, 99% CI: 0.75–1.02; $P_{trend} = 0.002$ and rs7805828-AA versus rs7805828-GG, OR: 1.11, 99% CI: 0.99–1.26; $P_{trend} = 0.007$). An increase in breast cancer risk was observed for the *PTGS2* polymorphism rs7550380 (TT versus GG, OR: 1.38, 99% CI: 1.04–1.83). No association was observed between *PTGS2* polymorphisms and prostate cancer risk. In conclusion, common genetic variation in these two genes might play at best a limited role in breast and prostate cancers.

Introduction

Chronic inflammation has been proposed as an important mechanism involved in the initiation and progression of epithelial tumors by inducing cell division and proliferation, inhibiting apoptosis and promoting angiogenesis (1). Cumulating epidemiological and experimental evidence indicate an implication of chronic inflammation in the development of breast and prostate cancers (2–5). Proliferative inflammatory atrophy, a possible precursor of high-grade prostatic intraepithelial neoplasia, and chronic prostatitis have been associated with local elevated proinflammatory cytokine levels within the prostate as well as with the development of prostate cancer (6). Similarly, an increased risk of prostate cancer has been observed among men with a history of sexually transmitted infections, most of these being associated with chronic prostatic inflammation (7). Regarding breast cancer, it has been hypothesized that a peritumoral inflammatory infiltrate might be associated with the tumor development. *In vivo* studies have shown that, in case of chronic inflammation, breast tumor-associated leukocytes release proinflammatory cytokines and pro-growth factors that activate immune response and enhance tumor promotion (4).

The interleukin-6 (IL-6) plays a major role in the process of inflammation, particularly in the transition from acute to chronic inflammation. During acute inflammation, IL-6 is one of the most potent proinflammatory cytokines, inducing and regulating the production of acute phase proteins. It can also control the extent of the inflammatory response by stimulating the production of anti-inflammatory cytokines. When IL-6 is continuously expressed (i.e. in chronic states of inflammation), it is then exclusively proinflammatory and enhances monocyte recruitment at the site of inflammation (8). IL-6 has been implicated in the proliferation and growth of prostate cancer cells (9). In breast cancer, IL-6 has been shown to inhibit the growth of cancer cells but promote the development of metastases (10).

Abbreviations: BMI, body-mass-index; BPC3, Breast and Prostate Cancer Cohort Consortium; CI, confidence intervals; CPS-II, Cancer Prevention Study II; IL-6, interleukin-6; EPIC, European Prospective Investigation into Cancer and Nutrition; OR, odds ratios; PTGS2, prostaglandin-endoperoxide synthase 2; SNP, single nucleotide polymorphism.

The COX-2 enzyme is implicated in the conversion of arachidonic acid into prostaglandins that stimulate cell proliferation and angiogenesis. In epidemiological studies, the use of non-steroidal anti-inflammatory drugs, which are known to inhibit COX-2, has been associated with a risk reduction of many cancers (11–13), including breast (14) and prostate cancers (15). COX-2 overexpression has been observed in many tumor types, including breast (16) and prostate (17) cancers.

Polymorphisms of genes involved in the inflammatory pathway have been previously associated with cancer. Polymorphisms in the *prostaglandin-endoperoxide synthase 2* (*PTGS2*) gene, which encodes for the COX-2 enzyme, have been, in particular, related to cancers of the breast (18–20) and prostate (21–24). The gene coding for the inflammatory cytokine IL-6 has also been related to breast cancer in some studies (25,26), but not in others (27,28).

We conducted a study within the Breast and Prostate Cancer Cohort Consortium (BPC3) to examine the association between *PTGS2* and *IL6* gene variants and breast and prostate cancer risk. BPC3 is a consortium of case–control studies nested within large prospective cohorts.

Materials and methods

Study population

The BPC3 has been described in detail elsewhere (29). Briefly, the consortium includes large well-established cohorts assembled in the USA and Europe that have DNA for genotyping and extensive questionnaire data from cohort members. Written informed consent was obtained from all subjects and each cohort has been approved by the appropriate institutional review board. The breast cancer study includes six case–control studies nested within the following cohorts: the American Cancer Society Cancer Prevention Study II (CPS-II) (30), the European Prospective Investigation into Cancer and Nutrition (EPIC) (31), the Harvard Nurses' Health Study (NHS) (32) and Women's Health Study (WHS) (33), the Hawaii-Los Angeles Multiethnic Cohort (MEC) (34) and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) (35). The prostate cancer study includes seven case–control studies nested within these cohorts: CPS-II, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) (36), EPIC, the Health Professionals Follow-up Study (37), MEC, the Physicians Health Study (PHS) (38) and PLCO.

With the exception of MEC and PLCO, most members of these cohorts are Caucasian. The MEC includes US Caucasians (24% in women and 19% in men), African-Americans (22% in women and 29% in men), Latinos (20% in women and 28% in men), Japanese (24% in women and 21% in men) and native Hawaiians (11% in women and 3% in men). The PLCO includes US Caucasians (90% in women and 86% in men), African-Americans (4% in women and 14% in men) and Asians (4% in women).

Cases were confirmed by medical records, pathology reports and/or linkage with population-based tumor registries. High-stage (stage $\geq T_{3b}$, N_1 or M_1) and high-grade (Gleason score ≥ 8) prostate cancer cases were classified as aggressive tumors. Advanced breast cancer cases were defined as breast tumors with regional metastases to lymph nodes or other adjacent tissues ['regional' by Surveillance, Epidemiology and End Results (SEER) Program staging] or metastases to distant organs ('distant' by SEER staging). In addition, for NHS and PLCO, which had used American Joint Committee on Cancer (AJCC) staging guidelines, breast tumors >2 cm in diameter without lymph node involvement or other regional spread (AJCC stage II) were also included. In the various EPIC recruitment centers, due to different coding practices at the cancer registries, codes provided information about either distant metastases only or about local plus distant metastases combined.

Controls were matched to cases by ethnicity and age, and in some cohorts, additional matching criteria were employed (for example, EPIC matched on country of residence). A total of 6292 breast cancer cases and 8135 matched controls and 8008 prostate cancer cases and 8604 matched controls were included in the present analysis. Baseline characteristics of cases and controls included in this study have been described elsewhere (39,40).

SNP selection and genotyping

Single nucleotide polymorphism (SNP) selection was designed to comprehensively capture common genetic variation in the *PTGS2* and *IL6* gene regions. To this end, we followed a tagging approach. Gene regions were defined as 30 kb upstream of the start of translation and 20 kb downstream of the polyA tail. All polymorphisms in each gene region with minor allele frequency $\geq 5\%$ in Caucasians from the International HapMap Project (version 22; http://www.hapmap.org) were included. Tagging SNPs were selected with the use of the Tagger program within Haploview (http://www.broad.mit.edu/mpg/haploview/; http://www.broad.mit.edu/mpg/tagger/) (41), using pairwise tagging with a minimum r^2 of 0.8 (42).

The 27 SNPs included in this study were genotyped together with 1509 SNPs in the sex steroid and insulin/growth factor pathways. Genotyping in the BPC3 case–control samples was conducted using the GoldenGate assay and Illumina BeadArray™ technology in four laboratories (University of Southern California, Los Angeles, CA; National Cancer Institute, Rockville, MD; Imperial College, London, UK and Harvard School of Public Health, Boston, MA). Thirty CEU (Utah residents with ancestry from northern and western Europe) trios, used by HapMap, were genotyped in all labs to evaluate inter-lab reproducibility. For SNPs passing design, manufacturing and quality control metrics, the 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 analysis

Any sample where >25% of the 1536 SNPs attempted on the oligo pool assay platform failed was removed from the dataset (3% of subjects in the breast cancer dataset and 2% of subjects in the prostate cancer dataset). In the breast cancer dataset, one SNP (rs7550380) failed genotyping on $\geq 25\%$ samples in EPIC and one SNP (rs6949149) was monomorphic in CPS-II and NHS. In the prostate cancer dataset, the SNP rs5277 failed genotyping on $\geq 25\%$ samples in CPS-II, ATBC and PLCO and rs7550380 failed genotyping in EPIC, MEC and PHS. One SNP (rs4648261) showed statistically significant ($P < 10^{-5}$) deviation from Hardy–Weinberg equilibrium genotype frequencies among CPS-II European-ancestry controls. Analyses on these SNPs were performed excluding subjects from these particular cohorts. No SNP exhibited large differences in European-ancestry allele frequencies across cohorts (fixation index, $F_{st} < 0.02$). A summary of SNPs included in the present study with differences in allele frequencies between cohorts and F_{st} are presented in Table 1.

We analyzed the association between cancer risk and genotypes using unconditional logistic regression adjusted for age at diagnosis/selection as control (in 5 year intervals), study and ethnicity. Genotypes were coded either as counts of minor alleles (log-additive model, trend test) or as two indicator variables, one for heterozygotes and one for minor allele homozygotes. Odds ratios (OR) and 99% confidence intervals (99% CI) were calculated. We performed these analyses in all subjects, separately for each study within subjects of European ancestry, and in MEC and PLCO, separately for each ethnicity. Analyses were also conducted separately for various cancer subtypes (aggressive versus indolent tumors for prostate cancer cases and advanced versus non-advanced tumors or estrogen and progesterone receptor positive versus negative tumors for breast cancer cases) and by various subgroups of cancer risk factors such as family history of breast or prostate cancer (at least one first-degree relative diagnosed with breast or prostate cancer versus none), body-mass-index (BMI) (<25, 25–30, 30+) and age at diagnosis ($\leq$ or >65 for prostate cancer cases; $\leq$ or >55 for breast cancer cases). We tested for heterogeneity in trend odds ratios using I^2, a measure of the proportion of variance in log odds ratios (43). Further adjustment for multiple testing at the gene level was performed by multiplying the observed *P*-value for trend by the effective number of independent tests (Meff) as defined by Gao *et al.* (44). The number of independent tests was calculated separately for men and women, using white controls only. All possible SNP × SNP interaction models within a gene were analyzed in a log-additive way and likelihood ratio tests of the model with and without interaction terms were performed. All *P*-values presented are a two-tailed and a multiple testing corrected *P*-value of <0.01 was considered statistically significant.

Results

Breast cancer

A total of 6292 breast cancer cases and 8135 matched controls were included in the present study. The mean age at diagnosis of the cases was 63.1 years (SD: 8.5 years). Seventy-two percent of cases with available data were non-advanced cases and 82% were estrogen-positive tumors. Seventy-nine percent of the cases and 75% of the controls were postmenopausal and 78% of all women were of European ancestry.

One polymorphism in the *IL6* gene (rs6949149) showed an increased risk of breast cancer among homozygotes for the minor allele T (OR: 1.32; 99% CI: 1.00–1.74, $P_{trend} = 0.003$) (Table II). When the analyses were stratified by ethnicity, this effect was significant only among African-American women (OR: 5.77; 99% CI: 1.03–32.51, $P_{trend} < 0.0001$) and the proportion of variation due to heterogeneity between ethnicities was 76% (supplementary Table 1 is available at *Carcinogenesis* Online). Among other ethnic groups, the effect for this particular SNP was in the same direction but not statistically significant for women of European ancestry (OR: 2.07; 99% CI: 0.63–6.78; $P_{trend} = 0.06$) and Hispanic (OR: 1.70; 99%

Table I. SNP information

Gene	chromosome	SNP	SNP position (hg 18)	Region	Δaf[a]	F_{st}[b]
IL6	7	rs6949149	22715682	Intergenic	0.05	0.0054
		rs4552807	22717544	Intergenic	0.07	0.0025
		rs6969502	22718951	Intergenic	0.05	0.0010
		rs6952003	22719230	Intergenic	0.03	0.0010
		rs10156056	22720613	Intergenic	0.03	0.0007
		rs7776857	22721293	Intergenic	0.05	0.0018
		rs7801617	22724607	Intergenic	0.03	0.0017
		rs7805828	22725087	Intergenic	0.06	0.0019
		rs2056576	22727727	Intergenic	0.04	0.0012
		rs12700386	22729534	Intergenic	0.04	0.0009
		rs1800795	22733170	5′ flanking region	0.07	0.0030
		rs2069840	22735097	Intronic	0.06	0.0013
		rs2069861	22738179	3′ flanking region	0.02	0.0012
		rs10242595	22740756	Intergenic	0.08	0.0047
		rs11766273	22742188	Intergenic	0.02	0.0007
PTGS2	1	rs10911902	184898940	Intergenic	0.05	0.0031
		rs4648298	184908305	3′-UTR	0.01	0.0009
		rs2206593	184909052	3′-UTR	0.01	0.0003
		rs5275	184909681	3′-UTR	0.02	0.0004
		rs5277	184914820	Exonic (synonymous)	0.02	0.0006
		rs4648261	184915627	Intronic	0.01	0.0006
		rs2745557	184915844	Intronic	0.02	0.0003
		rs20417	184916944	5′ flanking region	0.03	0.0013
		rs689466	184917374	5′ flanking region	0.02	0.0003
		rs12042763	184918499	Intergenic	0.02	0.0005
		rs7550380	184931128	Intergenic	0.04	0.0025
		rs2383529	184935715	Intergenic	0.03	0.0005

[a]Maximum difference in allele frequencies for Caucasians between cohorts.
[b]Fixation index.

CI: 0.76–3.83; $P_{trend} = 0.06$). No association with breast cancer risk was observed for rs6949149 among Asians and Hawaiians.

In the *PTGS2* gene, homozygotes for the T allele of rs7550380 had a statistically significant increased risk of breast cancer (OR: 1.38; 99% CI: 1.04–1.83) (Table III). This effect was slightly stronger in women of European ancestry (OR: 1.55; 99% CI: 0.82–2.95) and 47% of the total variation was due to heterogeneity between ethnicities. The direction of the effect was similar only among Hawaiians (OR: 3.77; 99% CI: 0.31–45.66) (supplementary Table 1 is available at *Carcinogenesis* Online).

No substantial heterogeneity was observed across cohorts or other subgroups (≤55 years versus >55 years at diagnosis; advanced versus non-advanced tumors; estrogen/progesterone receptor positive versus negative tumors; at least one first-degree relative diagnosed with breast cancer versus none; BMI: <25, 25–30, 30+).

Prostate cancer

A total of 8008 prostate cancer cases and 8604 matched controls were included in the study. Seventy-four percent of the subjects were of European origin. The mean age at diagnosis of the cases was 68.4 years (SD: 6.4 years). Aggressive cases represented 35% of the cases with available data on stage of the tumor.

Results for the association between *IL6* polymorphisms and prostate cancer risk are presented in Table IV. Under a co-dominant model, the following SNPs were associated with prostate cancer risk: rs6969502 (GA versus GG, OR: 0.92, 99% CI: 0.84–1.00; AA versus GG, OR: 0.87, 99% CI: 0.75–1.02; $P_{trend} = 0.002$) and rs7805828 (GA versus GG, OR: 1.10, 99% CI: 1.00–1.20; AA versus GG, OR: 1.11, 99% CI: 0.99–1.26; $P_{trend} = 0.007$). For rs6969502, a stronger effect was observed among subjects with a family history of prostate cancer (log-additive model, OR: 0.76, 99% CI: 0.61–0.97, $P_{trend} = 0.003$) compared with subjects with no family history of prostate cancer (log-additive model, OR: 0.94, 99% CI: 0.86–1.02, $P_{trend} = 0.04$) and 87% of the total variation was due to heterogeneity between men with and without a family history of prostate cancer (supplementary Table 2 is available at *Carcinogenesis* Online).

We found no evidence that genetic variation in *PTGS2* was significantly related to prostate cancer risk among all subjects (Table V) or among any of the subgroups we examined. Only one *PTGS2* SNP (rs2383529) was associated with risk among men with BMI < 25 (AG versus AA, OR: 1.16, 99% CI: 1.00–1.35; GG versus AA, OR: 1.24, 99% CI: 0.89–1.63; $P_{trend} = 0.004$) but not among men with BMI between 25 and 30 or ≥30. Eighty-five percent of the total variation for this polymorphism was due to heterogeneity between the different BMI categories (supplementary Table 3 is available at *Carcinogenesis* Online).

No heterogeneity of the associations between *PTGS2* and prostate cancer was observed across cohorts, by age at diagnosis or tumor aggressiveness.

In order to take into account the large number of tests performed in this study, we calculated the number of effective independent SNPs. Thirteen independent tests were obtained for *IL6* in women and 12 in men, giving a gene-adjusted significant threshold for significance of 0.0008. For *PTGS2*, the Meff was 11 among women and 10 among men, resulting in a gene-adjusted significance level of 0.001. Using these corrected *P*-values, no SNP was significantly associated with either breast or prostate cancer risk. Of all 171 tests of statistical models including pairwise interactions tested for each cancer site, none showed a *P*-value $<10^{-3}$. The minimum observed *P*-value for interaction ($P = 0.0014$) was observed between SNPs rs2056576 and rs12700386 of *IL6* in relation to prostate cancer.

Discussion

With >6000 breast cancer cases and 8000 prostate cancer cases, our study is the largest to examine the association between these two cancers and *PTGS2* and *IL6* genes. We had excellent power (>90%) to detect common variants (frequency 10% or greater overall) with relative risks of 1.2 per copy or greater, while controlling for the number of associations considered.

Despite the large size of the study, we found no evidence of an association between *PTGS2* genetic variants and prostate cancer risk.

Table II. Association of *IL6* SNPs with breast cancer in the BPC3

SNP	Cases[a]	Controls[a]	OR (99% CI)[b]	P_{trend}[c]	Gene-adjusted P_{trend}[d]
rs6949149					
GG	3272	4422	1.00	0.003	0.10
GT	992	1185	1.12 (0.98–1.29)		
TT	269	299	1.32 (1.00–1.74)		
rs4552807					
AA	1913	2466	1.00	0.84	>0.80
AT	2835	3646	1.04 (0.93–1.16)		
TT	1486	1949	1.01 (0.89–1.15)		
rs6969502					
GG	3445	4585	1.00	0.14	>0.80
GA	2214	2766	1.06 (0.96–1.17)		
AA	585	728	1.06 (0.89–1.25)		
rs6952003					
TT	3527	4500	1.00	0.31	>0.80
TA	2334	3060	0.97 (0.89–1.07)		
AA	385	523	0.94 (0.78–1.13)		
rs10156056					
GG	4862	6250	1.00	0.34	>0.80
GC	1303	1700	0.99 (0.89–1.10)		
CC	106	163	0.83 (0.60–1.16)		
rs7776857					
TT	3422	4447	1.00	0.51	>0.80
TG	2317	3008	1.01 (0.92–1.12)		
GG	513	643	1.05 (0.88–1.24)		
rs7801617					
GG	4898	6301	1.00	0.32	>0.80
GA	1236	1599	0.99 (0.88–1.11)		
AA	122	187	0.83 (0.61–1.14)		
rs7805828					
GG	2276	2873	1.00	0.38	>0.80
GA	2967	3843	0.98 (0.89–1.08)		
AA	1020	1376	0.96 (0.84–1.09)		
rs2056576					
CC	3011	3821	1.00	0.40	>0.80
CT	2596	3381	0.98 (0.89–1.08)		
TT	640	869	0.96 (0.82–1.11)		
rs12700386					
CC	4187	5386	1.00	0.65	>0.80
CG	1811	2415	0.97 (0.88–1.06)		
GG	224	280	1.04 (0.82–1.32)		
rs1800795					
GG	2847	3707	1.00	0.66	>0.80
GC	2523	3324	1.01 (0.91–1.11)		
CC	820	1035	1.03 (0.89–1.19)		
rs2069840					
CC	2993	3758	1.00	0.84	>0.80
CG	2560	3491	0.94 (0.86–1.03)		
GG	686	845	1.05 (0.90–1.22)		
rs2069861					
CC	5264	6813	1.00	0.88	>0.80
CT	947	1229	1.01 (0.89–1.14)		
TT	39	49	1.02 (0.58–1.78)		
rs10242595					
GG	2487	3197	1.00	0.71	>0.80
GA	2645	3446	1.01 (0.92–1.11)		
AA	1138	1474	0.97 (0.84–1.12)		
rs11766273					
GG	5550	7222	1.00	0.37	>0.80
GA	696	871	1.04 (0.90–1.20)		
AA	31	34	1.20 (0.63–2.29)		

[a]Numbers may not add up to 100% of subjects due to genotyping failure.
[b]Unconditional logistic regression adjusted for age at diagnosis/selection as control (in five year intervals), study and ethnicity.
[c]*P*-value for trend before adjustment for multiple testing.
[d]*P*-value for trend after adjustment for multiple testing at the gene level.

Table III. Association of *PTGS2* SNPs with breast cancer in the BPC3

SNP	Cases[a]	Controls[a]	OR (99% CI)[b]	P_{trend}[c]	Gene-adjusted P_{trend}[d]
rs10911902					
CC	4349	5628	1.00	0.72	>0.80
CT	1726	2215	0.99 (0.90–1.09)		
TT	180	230	0.98 (0.75–1.27)		
rs4648298					
TT	6037	7770	1.00	0.17	>0.80
TC	237	342	0.90 (0.72–1.12)		
CC	1	3	0.38 (0.02–7.64)		
rs2206593					
GG	5629	7344	1.00	0.13	>0.80
GA	625	743	1.12 (0.96–1.29)		
AA	24	36	0.87 (0.44–1.73)		
rs5275					
AA	2697	3512	1.00	0.54	>0.80
AG	2664	3501	0.98 (0.89–1.08)		
GG	772	933	1.06 (0.92–1.23)		
rs5277					
CC	4679	5978	1.00	0.37	>0.80
CG	1443	1939	0.97 (0.87–1.07)		
GG	142	197	0.95 (0.71–1.26)		
rs4648261					
CC	5938	7750	1.00	0.07	0.77
CT	311	348	1.18 (0.96–1.45)		
TT	6	9	0.85 (0.22–3.34)		
rs2745557					
GG	4402	5784	1.00	0.18	>0.80
GA	1671	2091	1.05 (0.95–1.17)		
AA	174	217	1.06 (0.81–1.39)		
rs20417					
CC	4394	5694	1.00	0.24	>0.80
CG	1646	2166	1.00 (0.90–1.10)		
GG	214	232	1.24 (0.96–1.60)		
rs689466					
TT	4020	5143	1.00	0.18	>0.80
TC	1928	2562	0.97 (0.88–1.07)		
CC	299	410	0.90 (0.73–1.12)		
rs12042763					
GG	3460	4425	1.00	0.47	>0.80
GT	2168	2846	0.97 (0.88–1.07)		
TT	375	497	0.98 (0.81–1.18)		
rs7550380					
GG	3355	3923	1.00	0.05	0.55
GT	1320	1540	1.02 (0.91–1.15)		
TT	195	173	1.38 (1.04–1.83)		
rs2383529					
AA	3752	4886	1.00	0.85	>0.80
AG	2101	2698	1.02 (0.92–1.12)		
GG	383	505	0.99 (0.82–1.20)		

[a]Numbers may not add up to 100% of subjects due to genotyping failure.
[b]Unconditional logistic regression adjusted for age at diagnosis/selection as control (in five year intervals), study and ethnicity.
[c]*P*-value for trend before adjustment for multiple testing.
[d]*P*-value for trend after adjustment for multiple testing at the gene level.

For breast cancer, a 38% increased risk was observed for homozygotes of the T allele of the *PTGS2* polymorphism rs7550380. This effect even reached 50% among women of European ancestry. To our knowledge, this polymorphism was never genotyped in previous studies on *PTGS2* genetic variation and breast cancer. Although this SNP is not located within the *PTGS2* coding region, it is in strong linkage disequilibrium with other polymorphisms located in the gene, and one of them could be causally related to differences in breast cancer risk. Another polymorphism (rs5275) has been associated with breast cancer risk in two previous studies, one showing an increase in risk (20), whereas in the other one, a decrease in risk was observed (19). Some of the subjects included in the second study (19) were reanalyzed here using a different genotyping method. We could not confirm any association of this SNP with breast cancer risk in the present study.

Although little is known about the functionality of *PTGS2* variants, one can speculate that they might affect prostaglandin expression and

Table IV. Association of *IL6* SNPs with prostate cancer in the BPC3

SNP	Cases[a]	Controls[a]	OR (99% CI)[b]	P_{trend}[c]	Gene-adjusted P_{trend}[d]
rs6949149					
GG	5703	6125	1.00	0.04	0.48
GT	1317	1460	0.93 (0.83–1.05)		
TT	244	276	0.83 (0.64–1.10)		
rs4552807					
AA	2372	2626	1.00	0.28	>0.80
AT	3530	3770	1.04 (0.95–1.16)		
TT	2029	2133	1.05 (0.93–1.19)		
rs6969502					
GG	4435	4588	1.00	0.002	0.02
GA	2810	3136	0.92 (0.84–1.00)		
AA	697	809	0.87 (0.75–1.02)		
rs6952003					
TT	4669	5098	1.00	0.16	>0.80
TA	2825	2987	1.03 (0.95–1.13)		
AA	483	498	1.08 (0.90–1.28)		
rs10156056					
GG	6074	6382	1.00	0.02	0.24
GC	1749	1996	0.92 (0.84–1.02)		
CC	169	203	0.90 (0.69–1.20)		
rs7776857					
TT	4332	4726	1.00	0.80	>0.80
TG	2965	3066	1.05 (0.96–1.15)		
GG	677	787	0.93 (0.79–1.07)		
rs7801617					
GG	6052	6430	1.00	0.33	>0.80
GA	1719	1873	0.99 (0.90–1.10)		
AA	203	268	0.86 (0.68–1.14)		
rs7805828					
GG	2922	3306	1.00	0.007	0.08
GA	3780	3960	1.10 (1.00–1.20)		
AA	1255	1299	1.11 (0.99–1.26)		
rs2056576					
CC	3694	4077	1.00	0.31	>0.80
CT	3430	3546	1.08 (0.99–1.18)		
TT	843	944	1.00 (0.87–1.15)		
rs12700386					
CC	5169	5720	1.00	0.07	>0.80
CG	2528	2529	1.10 (1.02–1.21)		
GG	277	316	0.95 (0.77–1.19)		
rs1800795					
GG	3594	3832	1.00	0.10	>0.80
GC	3218	3402	0.99 (0.90–1.09)		
CC	1125	1274	0.91 (0.80–1.03)		
rs2069840					
CC	3866	4333	1.00	0.05	0.60
CG	3332	3404	1.10 (1.01–1.21)		
GG	776	842	1.04 (0.90–1.20)		
rs2069861					
CC	6681	7222	1.00	0.55	>0.80
CT	1217	1285	1.03 (0.91–1.15)		
TT	46	49	1.05 (0.61–1.77)		
rs10242595					
GG	3285	3505	1.00	0.74	>0.80
GA	3252	3564	0.99 (0.91–1.09)		
AA	1393	1450	1.03 (0.91–1.18)		
rs11766273					
GG	7042	7532	1.00	0.08	>0.80
GA	913	999	0.95 (0.83–1.06)		
AA	40	59	0.67 (0.39–1.14)		

[a]Numbers may not add up to 100% of subjects due to genotyping failure.
[b]Unconditional logistic regression adjusted for age at diagnosis/selection as control (in five year intervals), study and ethnicity.
[c]*P*-value for trend before adjustment for multiple testing.
[d]*P*-value for trend after adjustment for multiple testing at the gene level.

Table V. Association of *PTGS2* SNPs with prostate cancer in the BPC3

SNP	Cases[a]	Controls[a]	OR (99% CI)[b]	P_{trend}[c]	Gene-adjusted P_{trend}[d]
rs10911902					
CC	5626	6072	1.00	0.57	>0.80
CT	2138	2232	1.02 (0.93–1.12)		
TT	213	261	0.85 (0.67–1.10)		
rs4648298					
TT	7597	8100	1.00	0.25	>0.80
TC	342	420	0.92 (0.75–1.10)		
CC	7	13	0.80 (0.22–2.54)		
rs2206593					
GG	7189	7692	1.00	0.34	>0.80
GA	788	872	0.95 (0.83–1.09)		
AA	24	29	0.90 (0.43–1.82)		
rs5275					
AA	3419	3664	1.00	0.44	>0.80
AG	3465	3709	1.02 (0.93–1.11)		
GG	1006	1092	1.04 (0.91–1.19)		
rs5277					
CC	3674	3962	1.00	0.27	>0.80
CG	927	1088	0.95 (0.83–1.09)		
GG	80	103	0.91 (0.62–1.36)		
rs4648261					
CC	6501	7058	1.00	0.42	>0.80
CT	266	307	0.97 (0.78–1.22)		
TT	3	10	0.32 (0.06–1.84)		
rs2745557					
GG	5614	5954	1.00	0.43	>0.80
GA	2098	2338	0.96 (0.87–1.05)		
AA	229	235	1.03 (0.80–1.31)		
rs20417					
CC	5561	5999	1.00	0.13	>0.80
CG	2155	2299	1.05 (0.95–1.14)		
GG	259	268	1.10 (0.88–1.41)		
rs689466					
TT	5089	5530	1.00	0.62	>0.80
TC	2493	2652	1.00 (0.92–1.09)		
CC	403	398	1.06 (0.88–1.29)		
rs12042763					
GG	4595	4878	1.00	0.54	>0.80
GT	2802	3071	0.96 (0.88–1.05)		
TT	487	498	1.02 (0.86–1.22)		
rs7550380					
GG	2847	2916	1.00	0.16	>0.80
GT	1035	1082	1.06 (0.92–1.21)		
TT	112	129	1.16 (0.81–1.64)		
rs2383529					
AA	4686	5050	1.00	0.13	>0.80
AG	2709	2900	1.04 (0.95–1.13)		
GG	550	589	1.09 (0.92–1.30)		

[a]Numbers may not add up to 100% of subjects due to genotyping failure.
[b]Unconditional logistic regression adjusted for age at diagnosis/selection as control (in five year intervals), study and ethnicity.
[c]*P*-value for trend before adjustment for multiple testing.
[d]*P*-value for trend after adjustment for multiple testing at the gene level.

activity. Prostaglandins have been shown to stimulate angiogenesis and to promote tumor cell proliferation (45). Furthermore, prostaglandin E_2 has been shown to increase aromatase expression within the breast and therefore the conversion of androgens to estrogens, which in turn stimulate breast tumor growth (16). Another possible mechanism of action of COX-2 on tumorigenesis is DNA damages that can be caused by free radicals formed during the peroxidation of lipids by COX-2 (46,47).

Our results at most only weakly support the earlier evidence of an association between genetic variation in the *IL6* gene and breast cancer risk. One polymorphism (rs6949149) was marginally associated with an increased risk of breast cancer for carriers of TT versus GG genotypes. Previous studies on breast cancer risk and *IL6* genetic variation were focusing on one particular polymorphism, rs1800795, which is located in the promoter region of *IL6* and is thought to have

a functional effect on gene transcription (48), showing either a positive (49,50), negative (25) or null (26–28) association with breast cancer. We found no indication of an association between rs1800795 and breast cancer risk overall or in any subgroup analyses.

Before consideration for multiple testing, several *IL6* polymorphisms were associated with prostate cancer risk in our study. None of the previous publications found a significant association between *IL6* polymorphisms and prostate cancer risk but they were all of smaller size both in terms of number of subjects included or number polymorphisms genotyped (51–53).

IL-6 is a pleiotropic cytokine that has been implicated in the inhibition of growth in breast cancer cell lines but also in the promotion of metastases. Besides, it has been shown to upregulate angiogenesis and to stimulate aromatase activity within breast tissues (10,54). IL-6 also promotes prostate cancer cell proliferation *in vivo* and might be involved in the transition from estrogen-dependent to estrogen-independent state of prostate tumor (9,55).

The strengths of the BPC3 include its unprecedented sample size and comprehensive characterization of variation around the *PTGS2* and *IL6* loci. Limitations of our study is the much smaller sample size and the less comprehensive tagging for the groups of non-European ancestry, making difficult the testing and the interpretation of the results in these subgroups. Another limitation is the fact that only two genes related to the inflammation pathway have been analyzed here. As the inflammation pathway is highly complex, it is possible that breast and prostate cancers depend on variation in multiple genes in this pathway, which may also be related to risk directly or in combination with other genes. However, the two genes included here are also the most intensively studied genes in the pathway from the point of view of polymorphisms and disease risk.

In conclusion, we observed some indication of an association between genes involved in the inflammation pathway and breast and prostate cancer risk. However, after consideration for multiple testing at the gene level, no SNP included in this study was significantly associated with cancer risk. Therefore, given the weak significance of the associations observed, these genes represent at best a minor risk factor for breast and prostate cancers.

Supplementary material

Supplementary Tables 1–3 can be found at http://carcin.oxfordjournals.org/

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References

1. Coussens,L.M. *et al*. (2002) Inflammation and cancer. *Nature*, **420**, 860–867.
2. Nelson,W.G. *et al*. (2003) Prostate cancer. *N. Engl. J. Med.*, **349**, 366–381.
3. De Marzo,A.M. *et al*. (2007) Inflammation in prostate carcinogenesis. *Nat. Rev. Cancer*, **7**, 256–269.
4. DeNardo,D.G. *et al*. (2007) Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. *Breast Cancer Res.*, **9**, 212.
5. Lithgow,D. *et al*. (2005) Chronic inflammation and breast pathology: a theoretical model. *Biol. Res. Nurs.*, **7**, 118–129.
6. Vasto,S. *et al*. (2008) Inflammation and prostate cancer. *Future Oncol.*, **4**, 637–645.
7. Dennis,L.K. *et al*. (2002) Meta-analysis of measures of sexual activity and prostate cancer. *Epidemiology*, **13**, 72–79.
8. Gabay,C. (2006) Interleukin-6 and chronic inflammation. *Arthritis Res. Ther.*, **8** (suppl. 2), S3.
9. Culig,Z. *et al*. (2005) Interleukin-6 regulation of prostate cancer cell growth. *J. Cell. Biochem.*, **95**, 497–505.
10. Hong,D.S. *et al*. (2007) Interleukin-6 and its receptor in cancer: implications for translational therapeutics. *Cancer*, **110**, 1911–1928.
11. Dai,Y. *et al*. (2006) Non-steroidal anti-inflammatory drugs in prevention of gastric cancer. *World J. Gastroenterol.*, **12**, 2884–2889.
12. Rayyan,Y. *et al*. (2002) The role of NSAIDs in the prevention of colon cancer. *Cancer Invest.*, **20**, 1002–1011.
13. Schildkraut,J.M. *et al*. (2006) Analgesic drug use and risk of ovarian cancer. *Epidemiology*, **17**, 104–107.
14. Harris,R.E. *et al*. (1996) Nonsteroidal antiinflammatory drugs and breast cancer. *Epidemiology*, **7**, 203–205.
15. Jacobs,E.J. *et al*. (2005) A large cohort study of aspirin and other nonsteroidal anti-inflammatory drugs and prostate cancer incidence. *J. Natl Cancer Inst.*, **97**, 975–980.
16. Howe,L.R. (2007) Inflammation and breast cancer. Cyclooxygenase/prostaglandin signaling and breast cancer. *Breast Cancer Res.*, **9**, 210.
17. Hussain,T. *et al*. (2003) Cyclooxygenase-2 and prostate carcinogenesis. *Cancer Lett.*, **191**, 125–135.
18. Gao,J. *et al*. (2007) Functional polymorphisms in the cyclooxygenase 2 (COX-2) gene and risk of breast cancer in a Chinese population. *J. Toxicol. Environ. Health A*, **70**, 908–915.
19. Cox,D.G. *et al*. (2007) A polymorphism in the 3′ untranslated region of the gene encoding prostaglandin endoperoxide synthase 2 is not associated with an increase in breast cancer risk: a nested case-control study. *Breast Cancer Res.*, **9**, R3.
20. Langsenlehner,U. *et al*. (2006) The cyclooxygenase-2 (PTGS2) 8473T>C polymorphism is associated with breast cancer risk. *Clin. Cancer Res.*, **12**, 1392–1394.
21. Cheng,I. *et al*. (2007) COX2 genetic variation, NSAIDs, and advanced prostate cancer risk. *Br. J. Cancer*, **97**, 557–561.
22. Danforth,K.N. *et al*. (2008) Polymorphic variants in PTGS2 and prostate cancer risk: results from two large nested case-control studies. *Carcinogenesis*, **29**, 568–572.
23. Shahedi,K. *et al*. (2006) Genetic variation in the COX-2 gene and the association with prostate cancer risk. *Int. J. Cancer*, **119**, 668–672.
24. Panguluri,R.C. *et al*. (2004) COX-2 gene promoter haplotypes and prostate cancer risk. *Carcinogenesis*, **25**, 961–966.
25. Slattery,M.L. *et al*. (2007) IL6, aspirin, nonsteroidal anti-inflammatory drugs, and breast cancer risk in women living in the southwestern United States. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 747–755.
26. Smith,K.C. *et al*. (2004) Cytokine gene polymorphisms and breast cancer susceptibility and prognosis. *Eur. J. Immunogenet.*, **31**, 167–173.
27. Gonzalez-Zuloeta Ladd,A.M. *et al*. (2006) Interleukin 6 G-174 C polymorphism and breast cancer risk. *Eur. J. Epidemiol.*, **21**, 373–376.
28. Skerrett,D.L. *et al*. (2005) Cytokine genotype polymorphisms in breast carcinoma: associations of TGF-beta1 with relapse. *Cancer Invest.*, **23**, 208–214.
29. Hunter,D.J. *et al*. (2005) A candidate gene approach to searching for low-penetrance breast and prostate cancer genes. *Nat. Rev. Cancer*, **5**, 977–985.
30. Calle,E.E. *et al*. (2002) The American Cancer Society Cancer Prevention Study II Nutrition Cohort: rationale, study design, and baseline characteristics. *Cancer*, **94**, 2490–2501.
31. Riboli,E. *et al*. (2002) European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr.*, **5**, 1113–1124.
32. Colditz,G.A. *et al*. (2005) The Nurses' Health Study: lifestyle and health among women. *Nat. Rev. Cancer*, **5**, 388–396.
33. Rexrode,K.M. *et al*. (2000) Baseline characteristics of participants in the Women's Health Study. *J. Womens Health Gend. Based Med.*, **9**, 19–27.
34. Kolonel,L.N. *et al*. (2000) A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am. J. Epidemiol.*, **151**, 346–357.
35. Hayes,R.B. *et al*. (2000) Etiologic and early marker studies in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial. *Control Clin. Trials*, **21**, 349S–355S.
36. The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group. (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N. Engl. J. Med.*, **330**, 1029–1035.
37. Giovannucci,E. *et al*. (2003) Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 84–89.
38. Chan,J.M. *et al*. (2002) Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. *J. Natl Cancer Inst.*, **94**, 1099–1106.

39. Feigelson,H.S. *et al*. (2006) Haplotype analysis of the HSD17B1 gene and risk of breast cancer: a comprehensive approach to multicenter analyses of prospective cohort studies. *Cancer Res.*, **66**, 2468–2475.
40. Kraft,P. *et al*. (2005) Genetic variation in the HSD17B1 gene and risk of prostate cancer. *PLoS Genet.*, **1**, e68.
41. de Bakker,P.I. *et al*. (2005) Efficiency and power in genetic association studies. *Nat. Genet.*, **37**, 1217–1223.
42. Carlson,C.S. *et al*. (2004) Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am. J. Hum. Genet.*, **74**, 106–120.
43. Higgins,J.P. *et al*. (2002) Quantifying heterogeneity in a meta-analysis. *Stat. Med.*, **21**, 1539–1558.
44. Gao,X. *et al*. (2008) A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet. Epidemiol.*, **32**, 361–369.
45. Cao,Y. *et al*. (2002) Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. *J. Cell. Physiol.*, **190**, 279–286.
46. Lee,S.H. *et al*. (2005) Cyclooxygenase-2-mediated DNA damage. *J. Biol. Chem.*, **280**, 28337–28346.
47. Blair,I.A. (2001) Lipid hydroperoxide-mediated DNA damage. *Exp. Gerontol.*, **36**, 1473–1481.
48. Fishman,D. *et al*. (1998) The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J. Clin. Invest.*, **102**, 1369–1376.
49. Iacopetta,B. *et al*. (2004) The -174 G/C gene polymorphism in interleukin-6 is associated with an aggressive breast cancer phenotype. *Br. J. Cancer*, **90**, 419–422.
50. Hefler,L.A. *et al*. (2005) Interleukin-1 and interleukin-6 gene polymorphisms and the risk of breast cancer in Caucasian women. *Clin. Cancer Res.*, **11**, 5718–5721.
51. Kesarwani,P. *et al*. (2008) Association between -174 G/C promoter polymorphism of the interleukin-6 gene and progression of prostate cancer in North Indian population. *DNA Cell Biol.*, **27**, 505–510.
52. Michaud,D.S. *et al*. (2006) Genetic polymorphisms of interleukin-1B (IL-1B), IL-6, IL-8, and IL-10 and risk of prostate cancer. *Cancer Res.*, **66**, 4525–4530.
53. Sun,J. *et al*. (2004) Interleukin-6 sequence variants are not associated with prostate cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 1677–1679.
54. Knupfer,H. *et al*. (2007) Significance of interleukin-6 (IL-6) in breast cancer (review). *Breast Cancer Res. Treat.*, **102**, 129–135.
55. Culig,Z. (2005) Interleukin-6 polymorphism: expression and pleiotropic regulation in human prostate cancer. *J. Urol.*, **174**, 417.

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