Common Polymorphisms in the Prostaglandin Pathway Genes and Their Association with Breast Cancer Susceptibility and Survival

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Abstract

Purpose: There is evidence that genetic variation in the prostaglandin pathway affects cancer susceptibility and progression. Conflicting data from several studies exist for the association of *PTGS2* (cyclooxygenase 2) polymorphisms with breast cancer risk. We investigated associations between common germ-line variations in seven genes in the prostaglandin pathway and breast cancer susceptibility and survival among women with invasive breast cancer in the SEARCH study.

Experimental Design: DNA samples from 9,030 cases and controls were genotyped for 64 single nucleotide polymorphisms tagging known common variants (minor allele frequency > 0.05) in *PTGS1, PTGS2, TBXAS1, PTGIS, PTGES, PTGDS*, and *PGDS* with a two-stage case-control study design.

Results: Four tagging single nucleotide polymorphisms showed modest association with breast cancer susceptibility. All four fit a recessive genetic model. Minor allele homozygotes for *PTGISrs5602* [odds ratio (OR), 1.15; 95% confidence interval (95% CI), 1.04-1.27; $P = 0.005$], *PTGISrs8183919* (OR, 1.22; 95% CI, 1.06-1.41; $P = 0.006$), and *TBXASrs41727* (OR, 1.83; 95% CI, 1.22-2.73; $P = 0.003$) are associated with an increased risk compared with common allele carriers. For *PTGISrs44627* minor allele homozygotes (OR, 0.66; 95% CI, 0.5-0.86; $P = 0.002$), a protective effect was observed.

Conclusion: Specific *PTGIS* and *TBXAS1* variants may affect breast cancer susceptibility, but common variants in *PTGS1, PTGS2, PTGES, PTGDS*, and *PGDS* have no major role in breast cancer susceptibility. None of the variants in the seven genes studied appear to affect survival. Further larger studies correlating clinical and genotypic data are required to establish if the clinical utility of prostaglandin-targeted therapies, as chemoprevention agents, is influenced by an individual's profile of genetic variants in key prostaglandin pathway genes.

Breast cancer is the most common cancer among women in developed countries and accounts for 18% of all cancers (1). Although a few, rare, strongly predisposing alleles are well established (e.g., deleterious alleles of *BRCA1* and *BRCA2*), these account for only 2% to 5% of all breast cancer cases (2) and <25% of the excess familial risk of breast cancer can be explained by variants in the known susceptibility genes (3). It is likely that the unexplained excess is due to multiple low to moderate risk alleles in numerous genes, that is, a polygenic model of cancer susceptibility (4).

There has been increasing interest in the role of the prostaglandin pathway in both cancer susceptibility and tumor progression, with a particular focus on the enzyme cyclooxygenase (COX) 1/2. COX is also known as prostaglandin H_2 synthase 1/2. The gene encoding this protein is *PTGS1/PTGS2*. Prostaglandins are ubiquitous substances found in many tissues and organs throughout the body. During their normal function, they act on a variety of cell types mediating processes such as inflammation, muscle constriction, calcium movement, cell growth control, vascular constriction, and platelet aggregation. The prostaglandin pathway has long been pharmacologically manipulated using enzyme antagonists such as COX2 inhibitors.

Proteins in this pathway have been identified as playing a role in breast cancer metastasis (5), angiogenesis (6), and prognosis (7) and a potential role in breast cancer risk reduction (8). It is postulated that inflammation, activated secondary signaling pathways, and their associated consequences may also be important in the etiology of cancer (6, 9, 10) Prostaglandins are derived from the arachidonic acid (an essential fatty acid) metabolic pathway. Figure 1 and Table 1

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Translational Relevance

There is increasing evidence that genetic variation in the prostaglandin pathway affects cancer susceptibility and progression. This study investigates the associations between common germ-line variations in seven genes in the prostaglandin pathway and breast cancer susceptibility and survival among women with invasive breast cancer using DNA from 9,030 cases and controls. Our results suggest that specific *PTGIS* and *TBXAS1* variants may affect breast cancer susceptibility, but *PTGS1, PTGS2, PTGES, PTGDS*, and *PGDS* common variants have no major role in breast cancer susceptibility. None of the variants in the seven genes studied appear to affect survival. In colorectal cancer, prostaglandin-targeted treatments have been used in chemoprevention. Our study opens up the possibility that only individuals with particular genotypes may benefit from these therapies, thus affecting their clinical utility in breast cancer patients. This study has a clear translational effect and invites further investigation in the wider clinical trial setting.

illustrate the key enzymes, their main functions, and the genes encoding the proteins in this pathway.

The rate-limiting factor for production of prostaglandins is COX. COX1 and COX2 are isoenzymes. The substitution of isoleucine at position 523 in COX1 with valine in COX2 is the difference that allows for selective inhibition. Both catalyze the oxidation of essential fatty acids giving rise to protein products further down the pathway.

Although this study focuses on germ-line variations in genes in the prostaglandin pathway, there is also considerable somatic data available concerning this pathway. The prostaglandin pathway gene most thoroughly investigated with regards its association with cancer is *PTGS2*. Regular intake of aspirin, a nonselective COX inhibitor, is found to be associated with a reduction in breast cancer risk in some studies (11, 12), although other studies have been less conclusive (13, 14). Continuous use of nonsteroidal anti-inflammatory drugs, via local *PTGS2* blockade, causes a reduction in prostaglandin E_2 and aromatase synthesis, which may have wider implications in the use of such agents as an adjunct to aromatase inhibitor therapy (11). *PTGS2* overexpression has been noted in invasive breast cancer and ductal carcinoma *in situ* tumors, with protein expression levels varying greatly between studies (15–26). The role of genetic variation in *PTGS2* in the etiology of breast cancer is unclear. One common variant, *PTGS2* 8473 $t > c$ (rs5275), has been evaluated in several case-control studies, but the results have been inconclusive (11, 27–30).

Further down the prostaglandin pathway, *PTGES* is responsible for the formation of prostaglandin E_2, which acts via the G-protein-coupled receptors, EP1 to EP4. In murine models of breast cancer, *in vitro* inhibition of the EP1, EP2, and EP4 receptors with a chemical antagonist decreases metastasis and tumor proliferation as effectively as nonspecific COX inhibitors (5).

One study has shown that *TBXAS1* is expressed at significantly lower levels in high-grade and poor prognosis patients. The gene product, thromboxane, is thought to be prometastatic, whereas prostacyclin, which is synthesized by *PTGIS*, is antimetastatic and atheroprotective.

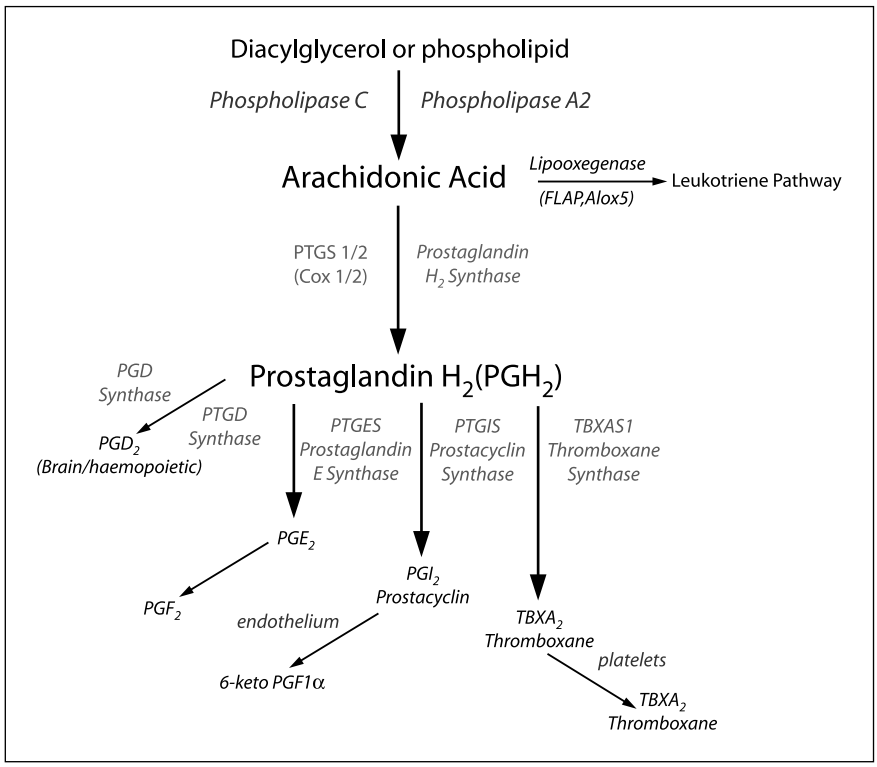

Fig. 1. Prostaglandin pathway. Designed by Philip Ball, M.A., University of Cambridge.

Table 1. Prostaglandin pathway

Protein	Role	Gene
Cyclooxygenase 1/ prostaglandin H2 synthase 1	Involved in the conversion of arachidonic acid to prostaglandin H2. They exist in the following two forms: as COX-1, which is constitutively expressed in most tissues, and COX-2, which is inducible and regulated by mitogens, growth factors, and cytokines.	*COX1/PTGS1*
cyclooxygenase 2/ prostaglandin H2 synthase 2		*COX2/PTGS2*
Prostaglandin E synthase	Converts prostaglandin H2 to prostaglandin E. Its actions are mediated through the EP receptors, which are G-protein-coupled receptors that activate second messenger systems within the cell.	*PTGES*
Prostacyclin synthase	Converts prostaglandin H2 to prostaglandin.	*PTGIS*
Prostaglandin D2 synthase (brain)	Converts prostaglandin H2 to prostaglandin D2.	*PTGDS*
Prostaglandin D2 synthase (hemopoietic)	Converts prostaglandin H2 to prostaglandin D2.	*PGDS*
Thromboxane synthase	Converts prostaglandin H2 to thromboxane.	*TBXAS1*

NOTE: Designed by Phillip Ball, M.A., University of Cambridge.

There is substantial evidence that prostaglandins may play a variety of roles in breast cancer that merits further investigation. This study aims to evaluate the association between common germ-line variants in seven key genes coding for enzymes in the prostaglandin pathway with breast cancer susceptibility and outcome within the SEARCH case control study: *PTGS1*, *PTGS2*, *TBXAS1*, *PTGIS*, *PTGES*, *PTGDS*, and *PGDS* (Fig. 1 and Table 1 for all abbreviations). The SEARCH study was part of a multistage, genome-wide association study published recently (31). No association between common variation in these genes and breast cancer susceptibility was detected. However, the power of the genome-wide association study was limited by the small sample size for the first stage of the study and the limited coverage of common variation in these genes. The proportion of common variants tagged with square of the pairwise correlation coefficient (r^2) >0.8 ranged from 0% to 65%. We have genotyped single nucleotide polymorphisms (SNP) that tag all known common variants for each gene in our population and our power to detect variants with modest effect is much greater.

Materials and Methods

Patients and controls. Cases were drawn from SEARCH, an ongoing population-based study, with cases ascertained through the Eastern Cancer Registration and Information Centre. All women diagnosed with invasive breast cancer at age <55 years since 1991 and still alive in 1996 (prevalent cases, median age 48 years) together with all those diagnosed at age <70 years between 1996 and the present (incident cases; median age, 54 years) were eligible to take part. Of 2,805 potential prevalent cases, 569 (20%) were ineligible for recruitment as they had died before the study started. Sixty-seven percent (4,679) of those eligible (prevalent and incident) returned questionnaires and 64% (4,470) of those eligible provided a blood sample for DNA analysis. Eligible patients who did not take part were similar to responders for age at diagnosis and histopathologic morphology and grade, but the proportion of clinical stage III/IV cases was somewhat higher in nonparticipants. Controls were randomly selected from the Norfolk component of European Prospective Investigation of Cancer (EPIC). EPIC is a European-wide prospective study of diet and cancer. The EPIC Norfolk cohort comprises 25,000 individuals resident in Norfolk, East Anglia, the same region from which the cases have been

Table 2. Tagging efficiency of genes evaluated

Gene	Size (kb)	SNP data source	No. common variants MAF > 0.05	No. tagSNPs selected for r^2 > 0.8*	No. (%) common variants tagged r^2 > 0.8 (pairwise)	No. (%) common variants tagged r^2 > 0.5	No. (%) SNPs tagged with r^2s > 0.8	No. common variants tagged by multimarker tags with r^2s > 0.8
PTGS1	24.7	Seattle SNPs	39	6	35 (90)	38 (97)	35 (90)	NIL
PTGS2	8.6	Seattle SNPs	18	7	14 (78)	15 (83)	14 (78)	NIL
PTGES	14.7	HapMap	16	5	14 (88)	15 (94)	15 (94)	NIL
PTGIS	64.3	HapMap	41	16	38 (93)	38 (93)	38 (93)	NIL
PTGDS	10.7	HapMap	18	3	17 (94)	18 (100)	18 (100)	1†
PGDS	44.3	HapMap	10	2	9 (90)	9 (90)	9 (90)	NIL
TBXAS1	241.9	HapMap	148	25	79 (53)	109 (74)	100 (68)	7†

Abbreviations: r^2, pairwise correlation coefficient; r^2s, correlation between a SNP and haplotype.
*Successfully assayed.
†See Supplementary Table S2.

Table 3. Breast cancer risks associated with prostaglandin tagSNPs examined

Gene and SNPs*	MAF †	OR (95% CI) (heterozygotes vs common homozygotes)	OR (95% CI) (rare homozygotes vs common homozygotes)	Heterogeneity *P* (2 *df*)	Trend test *P* (1 *df*)
PTGS1					
rs1330344(01)	0.21	0.92 (0.81-1.05)	1.01 (0.77-1.33)	0.47	0.44
rs10306122(04)	0.07	0.98 (0.82-1.18)	0.93 (0.38-2.30)	0.97	0.83
rs10306194(05)	0.16	0.90 (0.79-1.03)	0.93 (0.63-1.39)	0.3	0.15
rs4836887(07)	0.13	0.93 (0.81-1.06)	0.96 (0.59-1.58)	0.56	0.31
rs10306108(08)	0.07	1.03 (0.87-1.22)	0.97 (0.44-2.12)	0.9	0.77
rs10306146(15)	0.13	0.92 (0.80-1.06)	0.98 (0.58-1.67)	0.49	0.28
PTGS2					
rs4648310(01)	0.04	0.80 (0.62-1.02)	1.03 (0.26-4.11)	0.2	0.1
rs689467(02)	0.07	1.09 (0.92-1.29)	1.29 (0.53-3.11)	0.55	0.28
rs2206593(03)	0.05	1.05 (0.86-1.27)	2.27 (0.86-5.98)	0.21	0.28
rs5275(04)	0.34	1.05 (0.92-1.19)	1.08 (0.89-1.31)	0.66	0.36
rs5277(09) set 1 only	0.16	0.85 (0.75-0.98)	0.90 (0.61-1.33)	0.07	0.03
rs5277(09) ‡	**0.16**	**0.95 (0.87-1.05)**	**1.13 (0.86-1.50)**	**0.37**	**0.71**
rs20424(11)	0.02	0.84 (0.61-1.15)	1.03 (0.00-1.13)	0.54	0.27
rs4648276(17)	0.12	0.98 (0.85-1.14)	1.16 (0.73-1.84)	0.79	0.91
PTGIS					
rs6090990(03)	0.25	1.02 (0.90-1.15)	1.05 (0.82-1.35)	0.9	0.66
rs5602(04) set 1 only	0.49	0.87 (0.75-1.00)	1.06 (0.90-1.25)	0.01	0.55
rs5602(04) ‡	***0.49***	***0.98 (0.88-1.08)***	***1.14 (1.01-1.28)***	***0.02***	***0.04***
rs6090996(05) set 1 only	0.21	0.81 (0.71-0.92)	1.08 (0.80-1.45)	0.004	0.04
rs6090996(05) ‡	**0.21**	**0.91 (0.83-0.99)**	**1.04 (0.85-1.29)**	**0.07**	**0.18**
rs508757(06)	0.2	0.96 (0.84-1.09)	1.11 (0.82-1.49)	0.62	0.95
rs707528(08)	0.49	0.93 (0.80-1.07)	1.06 (0.90-1.25)	0.17	0.49
rs477627(11) set 1 only§	0.16	1.05 (0.92-1.20)	0.61 (0.41-0.91)	0.03	0.47
rs477627(11) ‡ §	***0.16***	***1.03 (0.94-1.14)***	***0.66 (0.50-0.87)***	***0.01***	***0.3***
rs693649(12)	0.2	0.92 (0.81-1.04)	0.88 (0.64-1.19)	0.34	0.15
rs6012696(13)	0.07	1.07 (0.90-1.27)	1.15 (0.49-2.72)	0.7	0.4
rs556731(14) set 1 only	0.05	0.74 (0.60-0.92)	0.51 (0.13-2.03)	0.02	0.003
rs556731(14) ‡	**0.05**	**0.92 (0.79-1.06)**	**1.22 (0.55-2.74)**	**0.45**	**0.36**
rs6095543(15)	0.16	1.05 (0.92-1.19)	1.37 (0.95-1.96)	0.2	0.15
rs1393343(16)	0.22	0.89 (0.78-1.01)	1.14 (0.87-1.50)	0.08	0.48
rs574113(17)	0.34	0.97 (0.86-1.10)	1.04 (0.86-1.26)	0.76	0.92
rs501908(18)	0.1	1.07 (0.93-1.25)	0.98 (0.58-1.67)	0.64	0.43
rs8183919(19) set 1 only	0.29	0.99 (0.87-1.12)	1.28 (1.03-1.58)	0.05	0.14
rs8183919(19) ‡	***0.29***	***1.01 (0.93-1.10)***	***1.23 (1.06-1.43)***	***0.02***	***0.04***
rs1066894(20)	0.31	0.91 (0.80-1.03)	0.95 (0.78-1.17)	0.35	0.28
rs476496(21)	0.24	1.00 (0.88-1.13)	1.03 (0.80-1.33)	0.97	0.92
PTGES					
rs2302821(03)	0.08	0.86 (0.73-1.01)	1.19 (0.55-2.57)	0.17	0.14
rs4837404(04)	0.35	0.98 (0.86-1.11)	1.02 (0.84-1.23)	0.89	0.99
rs10739757(05)‖	0.1	1.05 (0.91-1.22)	1.62 (0.87-2.99)	0.25	0.21
rs10448290(06) set 1 only	0.09	1.00 (0.85-1.17)	2.92 (1.36-6.28)	0.02	0.27
rs10448290(06) ‡	**0.09**	**1.01 (0.91-1.14)**	**1.78 (1.09-2.90)**	**0.06**	**0.24**
rs12553596(13)‖	0.07	1.02 (0.86-1.21)	2.96 (1.16-7.52)	0.06	0.27
PTGDS					
rs11787588(01)	0.27	0.9 (0.8-1.0)	0.8 (0.7-1.1)	0.32	0.13
rs10781530(03)‖	0.31	1.1 (1.0-1.2)	1.0 (0.8-1.2)	0.31	0.44
rs908839(04)‖	0.47	1.0 (0.9-1.2)	1.1 (0.9-1.3)	0.63	0.38
PGDS					
rs10516950(02)	0.44	1.00 (0.88-1.15)	1.05 (0.89-1.25)	0.82	0.6
rs1045435(03)	0.39	0.91 (0.80-1.03)	0.8 9 (0.74-1.06)	0.24	0.11
TBXAS1					
rs6971207(01)	0.01	1.24 (0.84-1.81)	209.5 (0.0-7155E)	0.2	0.15
rs2267681(02)‖	0.42	0.97 (0.85-1.11)	0.92 (0.77-1.09)	0.62	0.34
rs7810415(03)§,‖	0.48	0.94 (0.82-1.09)	1.09 (0.92-1.29)	0.14	0.34
rs3779134(04)	0.12	1.05 (0.91-1.22)	1.05 (0.67-1.67)	0.78	0.49
rs194149(05)‖	0.28	1.04 (0.91-1.17)	0.93 (0.75-1.16)	0.63	0.9
rs11973494(08)‖	0.22	0.90 (0.79-1.02)	0.93 (0.71-1.22)	0.26	0.16
rs2284205(09)‖	0.16	1.03 (0.90-1.18)	0.87 (0.60-1.25)	0.65	0.96
rs2107901(11)	0.17	0.94 (0.82-1.07)	1.08 (0.76-1.53)	0.57	0.64
rs10487665(12)‖	0.09	0.96 (0.82-1.13)	1.10 (0.54-2.23)	0.85	0.73
rs2190087(13)	0.23	1.04 (0.92-1.17)	1.03 (0.79-1.35)	0.85	0.61
rs1557967(14)	0.2	0.99 (0.87-1.13)	1.13 (0.83-1.53)	0.72	0.72

(*Continued on the following page*)

Table 3. Breast cancer risks associated with prostaglandin tagSNPs examined (Cont'd)

Gene and SNPs*	MAF †	OR (95% CI) (heterozygotes vs common homozygotes)	OR (95% CI) (rare homozygotes vs common homozygotes)	Heterogeneity *P* (2 *df*)	Trend test *P* (1 *df*)
rs1003816(15)	0.1	1.01 (0.87-1.18)	1.89 (1.09-3.28)	0.07	0.23
rs41728(16)	0.12	0.97 (0.84-1.12)	1.77 (1.08-2.88)	0.06	0.44
rs41727(17) set 1 only	0.1	0.96 (0.82-1.12)	3.71 (1.77-7.80)	0.0008	0.3
rs41727(17) ‡	***0.1***	***1.0 1 (0.91-1.13)***	***1.83 (1.23-2.74)***	***0.01***	***0.12***
rs2267701(20)	0.14	0.99 (0.86-1.14)	1.58 (1.00-2.50)	0.14	0.42
rs740150(21)	0.19	1.10 (0.97-1.26)	0.88 (0.65-1.21)	0.19	0.5
rs10487667(23)	0.25	1.12 (0.99-1.27)	1.05 (0.83-1.33)	0.2	0.16
rs2267706(25)	0.23	1.07 (0.94-1.21)	1.07 (0.82-1.40)	0.55	0.31
rs22861987(28)	0.38	1.13 (1.00-1.29)	1.11 (0.93-1.34)	0.15	0.11
rs2286196(29)	0.19	1.06 (0.93-1.21)	0.85 (0.62-1.17)	0.36	0.89
rs2267692(30)	0.35	1.02 (0.90-1.16)	1.07 (0.89-1.29)	0.75	0.47
rs1015572(31)‖	0.05	1.00 (0.82-1.23)	0.52 (0.16-1.72)	0.55	0.75
rs1015570(32)‖	0.39	1.09 (0.96-1.24)	1.03 (0.86-1.23)	0.43	0.5
rs2299887(37)‖	0.4	1.04 (0.91-1.19)	1.00 (0.84-1.19)	0.81	0.84
rs10544451(38)	0.24	1.02 (0.90-1.16)	1.07 (0.82-1.39)	0.86	0.59

NOTE: Bold italic: results significant at $P < 0.05$ or $P = 0.05$ in the combined data for sets 1 and 2 (4,470 cases and 4,560 controls).
*All genes assessed in set 1 (2,270 cases and 2,280 controls) only, unless in bold text.
†MAF is among controls in set 1.
‡SNPs in bold text were taken through into set 2. Results refer to the combined data for sets 1 and 2 unless specified.
§Controls are out of Hardy-Weinberg.
‖Multi-SNP haplotypes used to increase tagging efficiency.

recruited. Controls were not matched to cases but were broadly similar in age at blood draw (age, 42-81 years; median age, 63 years). The ethnic background of both cases and controls, as reported on the questionnaires, is similar with >98% being White. The study is approved by the Eastern Region Multicentre Research Ethics Committee, and all participants provided written informed consent.

The total number of cases available for analysis was 4,470, of whom 3,263 (73%) cases enrolled as incident cases and 1,207 (27%) as prevalent cases. The samples have been split into two sets to save DNA and reduce genotyping costs: the first set (n = 2,270 cases and 2,280 controls) is genotyped for all SNPs and the second set (n = 2,200 cases and 2,280 controls) is then tested for those SNPs that show marginally significant associations in set 1 ($P_{heterogeneity}$ or $P_{trend} < 0.05$). Cases were randomly selected for set 1 from the first 3,500 recruited, with set 2 comprising the remainder of these plus the next 970 incident cases recruited. As the prevalent cases were recruited first, the proportion of these is higher in set 1 than in set 2 (33% versus 20%). Median age at diagnosis was similar in both sets (ages 51 and 52 years, respectively). There was no significant difference in the morphology, histopathologic grade, or clinical stage of the cases by set or by prevalent/incident status.

Clinical follow-up. The Eastern Cancer Registration and Information Centre has active follow-up at 3 and 5 years after diagnosis and then at 5-year intervals. Follow-up information and all-cause mortality are obtained by searching hospital information systems for recent visits. If a patient has not had a recent visit, the patient's general practitioner is contacted to obtain the vital status. Death certificate flagging through the Office of National Statistics also provides the registries with notification of deaths. The lag time with this process is a few weeks for cancer deaths and 2 months to 1 year for noncancer deaths. Cause-specific mortality was obtained from part I of the death certificate.

Selection of tagging SNPs. The aim of SNP tagging is to identify a set of SNPs (tagSNP) that efficiently tags all the common variation in a given gene. Where the common variation has not been systematically identified, we postulate that a set of SNPs that tags the known common variation will also tag any hitherto unidentified SNPs in the gene. The HapMap and Seattle SNPs databases were used to select tagSNPs (32, 33). To maximize coverage (Table 2), the database providing the greatest SNP density for each gene was used.

Data from the International HapMap Project European (CEU) samples of 30 parent-offspring trios were used to select tagSNPs for *TBXAS1*, *PTGIS*, *PTGES*, *PTGDS*, and *PTGDS*.

Resequencing data from Seattle SNPs were used to select tagSNPs for *PTGS1* and *PTGS2*. It focuses on candidate genes and pathways that underlie inflammatory responses in humans in a panel of 90 individuals representative of U.S. ethnicities including 24 European Americans, 24 African Americans, 12 Mexican Americans, 6 Native Americans, and 24 Asian Americans (PDR90). We excluded the samples with African American-specific variants, as it is known that there is greater genetic diversity in individuals of African origin. Data from the remaining 62 individuals were used to identify the tagSNPs.

We aimed to define a set of tagSNPs such that all known common SNPs in a gene had an estimated r^2 of >0.8 with at least one tagSNP. Some SNPs are poorly correlated with a single SNP but may be efficiently tagged by a haplotype defined by multiple SNPs. We used the aggressive 2- and 3-SNP tagging option of the program Tagger implemented in Haploview to select tagSNPs (34). The best measure of the extent to which one SNP tags another is the square of the pairwise correlation coefficient (r^2). The loss in power incurred by using a marker SNP in place of a true causal SNP is directly related to r^2 value; therefore, we aimed for the correlation between each SNP and tagSNP or haplotype of tagSNPs to be >0.8.

TaqMan genotyping. Genotyping was carried out using TaqMan according to the manufacturer's instructions. Primers and FAM- and VIC-labeled probes were supplied directly by Applied Biosystems as Assays-by-Design. All assays were carried out in 384-well plates. Each plate included negative controls (with no DNA) and positive controls duplicated on a separate quality-control plate. Plates were read on the ABI Prism 7900 using the Sequence Detection Software (Applied Biosystems). Failed genotypes were not repeated. Assays in which the genotypes of duplicate samples did not show >95% concordance were discarded and replaced with alternative assays with the same tagging properties. Call rates for each assay were >95%.

Statistical methods. For each polymorphism, deviation of the genotype frequencies from those expected under Hardy-Weinberg

Table 4. Global haplotype analysis of tagSNPs

Gene*†	Haplotype‡	Frequency	*P*	OR (95% CI)§	Global *P*
PTGS1‖	h000000	0.53	0.12		0.54
08, 01, 04, 15, 07, 05	h000001	0.15	0.09		
	h000110	0.09	0.79		
	h010000	0.06	0.34		
	h011000	0.04	0.55		
	h110000	0.07	0.77		
	Combined rare¶	0.05	0.72		
PTGS2‖	h0000000	0.44	0.99		0.55
01, 02, 03, 04, 17, 09, 11	h0000010	0.11	0.36		
	h0001000	0.15	0.77		
	h0001100	0.12	0.9		
	h0010000	0.05	0.27		
	h0101000	0.07	0.31		
	h1000010	0.03	0.1		
	Combined rare¶	0.02	0.6		
PTGIS ‖	h000000	0.12	0.19		0.06
block 1: 14, 15, 03, 16, 17, 04	h000001	0.02	0.4		
	h000010	0.34	0.94		
	h000101	0.22	0.45		
	h001001	0.09	0.33		
	h011001	0.16	0.15		
	h100000	0.03	0.33		
	Combined rare¶	0.02	0.01	0.65 (0.48-0.89)	
PTGIS ¶	h0000000000	0.05	0.95		0.01
block 2: 05, 06, 08, 18, 19, 20, 21, 11, 12, 13					
	h0000000010	0.05	0.34		
	h0000001100	0.04	0.04	1.28 (1.01-1.63)	
	h0000010000	0.04	0.37		
	h0000011100	0.03	0.005	0.65 (0.48-0.88)	
	h0010000000	0.04	0.03	0.74 (0.56-0.96)	
	h0010010000	0.07	0.23		
	h0010011100	0.02	0.24		
	h0010100000	0.14	0.03	1.16 (1.02-1.32)	
	h0010100001	0.02	0.52		
	h0010100010	0.05	0.89		
	h0010101000	0.04	0.34		
	h1100000000	0.03	0.16		
	h1100000010	0.03	0.85		
	h1101010000	0.03	0.81		
	Combined rare¶	0.33	0.25		
PTGES ‖	h00000	0.52	0.57		0.44
03, 13, 04, 05, 06	h00010	0.03	0.47		
	h00100	0.29	0.6		
	h01011	0.06	0.1		
	h10000	0.03	0.38		
	h10100	0.04	0.32		
	Combined rare¶	0.04	0.27		
PTGDS ‖	H000	0.03	0.98		0.74
04, 01, 03	h001	0.23	0.51		
	h010	0.26	0.11		
	h100	0.4	0.49		
	h101	0.07	0.66		
	Combined rare¶	0.01	0.73		
PGDS ‖	h00	0.18	0.22		0.42
02, 03	h01	0.38	0.13		
	h10	0.43	0.58		
	Combined rare¶	0.01	0.99		
TBXAS1‖	h0000000000000	0.24	0.72		0.4
block 1: 01, 02, 03, 04, 05, 37, 31, 32, 30, 08, 09, 11, 12					
	h0000000000010	0.04	0.54		
	h0000000011101	0.05	0.23		
	h0010000000000	0.02	0.06		
	h0010010110000	0.06	0.12		
	h0100000000000	0.05	0.34		
	h0110000000000	0.09	0.59		
	h0111110100000	0.08	0.97		
	Combined rare¶	0.36	0.81		

(*Continued on the following page*)

Table 4. Global haplotype analysis of tagSNPs (Cont'd)

Gene*†	Haplotype‡	Frequency	*P*	OR (95% CI)§	Global *P*
TBXAS1‖	h0000000	0.57	0.66		0.28
block 2: 38, 13, 14, 15, 16, 17, 20	h0000001	0.09	0.19		
	h0010000	0.05	0.5		
	h0010001	0.04	0.78		
	h1100000	0.11	0.92		
	h1111110	0.09	0.17		
	Combined rare¶	0.05	0.06		
TBXAS1‖	h00000	0.43	0.31		0.4
block 3: 21, 23, 25, 28, 29	h00010	0.1	0.57		
	h00011	0.06	0.85		
	h00100	0.04	0.3		
	h00111	0.07	0.46		
	h01000	0.03	0.61		
	h01111	0.03	0.07		
	h11000	0.07	0.42		
	h11010	0.02	0.22		
	h11111	0.02	0.98		
	Combined rare¶	0.14	0.1		

*SNPs used for haplotype analysis are arranged in chromosome order: 0, major allele; 1, minor allele.
†All genes assessed in set 1 (2,270 cases and 2,280 controls) only.
‡The baseline for each test is the frequency of all the other haplotypes combined.
§OR (95% CI) given for haplotypes showing a difference between cases and controls at a significance level of $P \leq 0.05$.
‖See Table 3 for study assay numbers and their related rs numbers.
¶Rare haplotypes (≤5%) were pooled.

equilibrium was assessed in set 1 controlled by a χ^2 test. Genotype frequencies in cases and controls were compared using a χ^2 test with 2 *df* ($P_{heterogeneity}$) and the Cochrane-Armitage trend test (χ^2 on 1 *df*) for the trend in breast cancer risk with number of rare alleles (P_{trend}). The relative risks of breast cancer for heterozygotes and rare homozygotes, relative to common homozygotes, were estimated as odds ratios (OR) with associated 95% confidence intervals (95% CI). Any SNP with a P_{trend} or $P_{heterogeneity} \leq 0.05$ in set 1 was subsequently genotyped in set 2. Rather than treating set 2 as an independent replication set, the results for sets 1 and 2 were combined, as the power of joint analysis in a staged study design has been shown to be superior to that of replication-based analysis (35).

In addition to the univariate analyses, we carried out tests of association for the specific haplotype combinations of alleles that tagged specific SNPs. We also carried out a general comparison of common haplotype frequencies in each haplotype block using the data from all the tagSNPs in that block. Haplotype blocks were defined such that the haplotypes with frequency >5% accounted for at least 80% of the haplotype diversity. We considered haplotypes with >2% frequency to be "common" and rare haplotypes were pooled. For both specific haplotype marker tests and the general comparison of haplotype frequencies by haplotype block, haplotype frequencies and subject-specific expected haplotype indicators were calculated separately for each study using an in-house program. This implements an expectation substitution approach to account for the haplotype uncertainty given unphased genotype data. Subjects missing 50% genotype data in each block were excluded from haplotype analysis. We then used unconditional logistic regression to test the null hypothesis of no association between specific multimarker tagging haplotype and cancer by comparing a model with terms for subject specific-haplotype indicator with the intercept-only model.

The global null hypothesis of no association between haplotype frequency (by haplotype block) and breast cancer was tested by comparing a model with multiplicative effects for each common haplotype (treating the most common haplotype as the reference) to the intercept-only model. Haplotype-specific ORs were also estimated with their associated 95% CIs. We used the admixture maximum likelihood test (36) as a single experiment-wise test. In brief, the admixture maximum likelihood tests the null hypothesis that none of the SNPs are associated with disease compared with the alternative that one or more of SNPs are associated.

Cox regression analysis was used to test for an association between SNP genotype and survival. The proportional hazards assumption was evaluated by visual inspection of log-log plots as well as tested analytically using Schöenfeld residuals.

Time at risk began on the date of blood sample receipt and ended on the date of death from any cause or, if death did not occur, on November 30, 2006. This allows for the difference in ascertainment of incident and prevalent cases and provides an unbiased estimate of the relative hazard provided that the proportional hazards assumption is correct. Follow-up was censored at 10 years after diagnosis, as follow-up became less reliable for each individual after 10 years. A hazard ratio was estimated for heterozygous and rare homozygous genotypes relative to the common genotype. Primary tests used were a likelihood ratio test (2 *df*) for heterogeneity of risk among the three genotypes (common homozygote, heterozygote, and rare homozygote) and a trend test (1 *df*) based on the number of rare alleles carried. All analyses were done in Intercooled Stata version 8.2.

The statistical power of the study depends on the susceptibility allele frequency, the risks conferred, and the genetic mode of action (dominant, recessive, or codominant). The staged approach substantially reduces genotyping costs without significantly affecting statistical power. For example, assuming that the causative SNP is tagged with $r^2 = 0.8$, a type I error rate of 0.0001, and genotyping success rate of 0.95, the staged study has 86% power to detect a dominant allele with minor allele frequency (MAF) of 0.05 that confers a relative risk of 1.5. Power to detect a dominant allele with MAF of 0.25 that confers a relative risk of 1.3 is 87% (full study = 89%). The power to detect recessive alleles is less in the staged study, e.g. an allele with MAF of 0.25 and risk 1.5 has 53% power in a staged study but 60% power in a full study. For an allele with MAF 0.5 and risk 1.3, the power is 71% in a staged study but 75% in a full study.

Table 5. Prostaglandin tagSNP survival analysis

Gene/SNP*	MAF	P_{trend}	Hazard ratio per allele (95% CI†)	$P_{heterogeneity}$	Heterozygote risk (95% CI)	Homozygote risk (95% CI)
PTGDS						
PTGDS(01)rs11787588	0.27	0.92	1.01 (0.85-1.20)	0.29	1.13 (0.90-1.40)	0.79 (0.48-1.30)
PTGDS(03)rs10781530	0.31	0.43	1.07 (0.91-1.25)	0.6	1.01 (0.81-1.27)	1.2 (0.85-1.71)
PTGDS(04)rs908839	0.47	0.68	0.97 (0.83-1.13)	0.73	0.9 (0.70-1.16)	0.95 (0.70-1.27)
PTGES						
PTGES(03)rs2302821	0.08	0.08	1.27 (0.98-1.65)	0.11	1.18 (0.87-1.58)	2.61 (1.08-6.33)
PTGES(04)rs4837404	0.35	0.51	0.95 (0.81-1.11)	0.72	0.91 (0.73-1.14)	0.93 (0.66-1.31)
PTGES(05)rs10739757	0.1	0.27	0.87 (0.68-1.12)	0.53	0.89 (0.68-1.17)	0.66 (0.21-2.05)
PTGES(06)rs10448290	0.09	0.06	0.77 (0.58-1.02)	0.07	0.84 (0.62-1.13)	0.22 (0.03-1.57)
PTGES(13)rs12553596 (set 1)	0.07	0.04	0.73 (0.53-1.00)	0.1	0.76 (0.54-1.06)	0.32 (0.05-2.26)
PTGES(13)rs12553596 (set 1 and 2 combined)	***0.07***	***0.07***	***0.81 (0.65-1.02)***	***0.19***	***0.82 (0.64-1.05)***	***0.6 (0.19-1.88)***
PTGIS						
PTGIS(03)rs6090990	0.25	0.71	0.97 (0.81-1.15)	0.86	0.94 (0.75-1.18)	0.99 (0.64-1.53)
PTGIS(04)rs5602	0.49	0.86	0.99 (0.85-1.14)	0.14	1.23 (0.94-1.60)	0.98 (0.72-1.33)
PTGIS(05)rs6090996	0.21	0.56	0.95 (0.78-1.14)	0.84	0.96 (0.75-1.21)	0.87 (0.51-1.50)
PTGIS(06)rs508757	0.2	0.26	1.11 (0.93-1.33)	0.53	1.12 (0.89-1.41)	1.2 (0.73-1.96)
PTGIS(08)rs707528	0.49	0.91	1.01 (0.87-1.17)	0.76	0.93 (0.72-1.21)	1.02 (0.76-1.36)
PTGIS(11)rs477627	0.16	0.51	1.07 (0.87-1.32)	0.76	1.05 (0.83-1.33)	1.28 (0.63-2.59)
PTGIS(12)rs693649	0.2	0.38	1.09 (0.9-1.31)	0.33	1 (0.79-1.26)	1.47 (0.91-2.37)
PTGIS(13)rs6012696	0.07	0.94	0.99 (0.74-1.32)	0.95	0.97 (0.71-1.32)	1.22 (0.30-4.89)
PTGIS(14)rs556731†	0.05	0.2	0.75 (0.48-1.17)			
PTGIS(15)rs6095543	0.16	0.68	0.96 (0.79-1.17)	0.24	0.85 (0.66-1.09)	1.22 (0.77-2.18)
PTGIS(16)rs1393343	0.22	0.28	1.1 (0.93-1.31)	0.51	1.14 (0.91-1.42)	1.14 (0.72-1.81)
PTGIS(17)rs574113	0.34	0.31	1.08 (0.93-1.26)	0.23	1.22 (0.97-1.53)	1.07 (0.76-1.51)
PTGIS(18)rs501908	0.1	0.44	1.1 (0.87-1.38)	0.74	1.09 (0.84-1.42)	1.24 (0.51-3.01)
PTGIS(19)rs8183919	0.29	0.88	1.01 (0.86-1.19)	0.98	1.02 (0.82-1.28)	1.01 (0.70-1.46)
PTGIS(20)rs1066894	0.31	0.81	1.02 (0.87-1.20)	0.97	1.02 (0.82-1.28)	1.04 (0.72-1.49)
PTGIS(21)rs476496	0.24	0.77	1.03 (0.86-1.22)	0.74	0.97 (0.77-1.22)	1.16 (0.76-1.79)
PTGS1						
PTGS1(01)rs1330344	0.21	0.35	1.09 (0.91-1.3)	0.36	1.18 (0.94-1.47)	1 (0.61-1.63)
PTGS1(04)rs10306122†	0.07	0.2	0.81 (0.58-1.12)			
PTGS1(05)rs10306194	0.16	0.74	0.96 (0.78-1.20)	0.87	0.99 (0.77-1.27)	0.81 (0.36-1.82)
PTGS1(07)rs4836887	0.13	0.19	0.86 (0.68-1.09)	0.38	0.88 (0.68-1.14)	0.57 (0.18-1.77)
PTGS1(08)rs10306108	0.07	0.59	1.08 (0.82-1.42)	0.47	1.15 (0.86-1.54)	0.49 (0.07-3.47)
PTGS1(15)rs10306146	0.13	0.14	0.84 (0.65-1.07)	0.34	0.84 (0.64-1.10)	0.66 (0.21-2.06)
PTGS2						
PTGS2(01)rs4648310†	0.04	0.89	1.03 (0.67-1.58)			
PTGS2(02)rs689467	0.07	0.55	0.91 (0.68-1.23)	0.75	0.94 (0.68-1.28)	0.56 (0.08-3.98)
PTGS2(03)rs2206593†	0.05	0.43	1.13 (0.84-1.51)			
PTGS2(04)rs5275	0.34	0.52	1.05 (0.90-1.23)	0.55	1.13 (0.9-1.42)	1.04 (0.73-1.48)
PTGS2(09)rs5277	0.16	0.79	1.03 (0.84-1.26)	0.77	1.07 (0.84-1.37)	0.86 (0.41-1.83)
PTGS2(11)rs20424 (set 1)†	0.02	0.05	0.41 (0.17-0.99)			
PTGS2(11)rs20424 (set 1 and 2 combined)†	***0.02***	***0.27***	***0.75 (0.45-1.25)***			
PTGS2(17)rs4648276	0.12	0.06	1.23 (1.00-1.52)	0.13	1.29 (1.01-1.64)	1.26 (0.59-2.67)
PGDS						
PGDS(02)rs10516950	0.44	0.54	1.05 (0.90-1.22)	0.59	0.97 (0.76-1.24)	1.12 (0.83-1.50)
PGDS(03)rs1045435	0.39	0.96	0.7 (0.86-1.16)	0.98	1.01 (0.80-1.27)	0.98 (0.71-1.36)
TBXAS1						
TBXAS1(01)rs6971207†	0.01	0.7	1.12 (0.63-2.00)			
TBXAS1(02)rs2267681 (set 1)	0.42	0.15	1.12 (0.96-1.30)	0.02	1.4 (1.10-1.78)	1.16 (0.83-1.61)
TBXAS1(02)rs2267681 (set 1 and 2 combined)	***0.42***	***0.15***	***1.09 (0.97-1.21)***	***0.11***	***1.21 (1.01-1.46)***	***1.14 (0.90-1.44)***
TBXAS1(03)rs7810415	0.48	0.97	1 (0.86-1.16)	0.91	1.05 (0.81-1.35)	0.99 (0.73-1.35)
TBXAS1(04)rs3779134	0.12	0.52	1.08 (0.86-1.34)	0.41	1.16 (0.90-1.48)	0.73 (0.27-1.96)
TBXAS1(05)rs194149	0.28	0.33	1.09 (0.92-1.28)	0.29	1.19 (0.96-1.49)	1.02 (0.67-1.55)
TBXAS1(08)rs11973494	0.22	0.19	1.13 (0.95-1.34)	0.13	1 (0.80-1.27)	1.56 (1.03-2.36)
TBXAS1(09)rs2284205	0.16	0.22	1.14 (0.93-1.39)	0.2	1.04 (0.82-1.32)	1.74 (1.00-3.04)
TBXAS1(11)rs2107901	0.17	0.31	1.11 (0.91-1.34)	0.59	1.1 (0.86-1.39)	1.26 (0.72-2.21)
TBXAS1(12)rs10487665	0.09	0.57	1.08 (0.83-1.39)	0.84	1.07 (0.82-1.41)	1.27 (0.41-3.96)
TBXAS1(13)rs2190087	0.23	0.63	1.04 (0.88-1.25)	0.56	1.11 (0.89-1.39)	0.93 (0.56-1.54)
TBXAS1(14)rs1557967	0.2	0.99	1 (0.83-1.20)	0.98	1.01 (0.81-1.27)	0.96 (0.56-1.65)
TBXAS1(15)rs1003816	0.1	0.81	1.03 (0.82-1.30)	0.89	1 (0.76-1.32)	1.21 (0.57-2.56)
TBXAS1(16)rs41728	0.12	0.64	1.05 (0.85-1.31)	0.87	1.07 (0.83-1.39)	1.03 (0.48-2.17)

(*Continued on the following page*)

Table 5. Prostaglandin tagSNP survival analysis (Cont'd)

Gene/SNP*	MAF	P_{trend}	Hazard ratio per allele (95% CI†)	$P_{heterogeneity}$	Heterozygote risk (95% CI)	Homozygote risk (95% CI)
TBXAS1(17)rs41727	0.1	0.82	1.03 (0.81-1.31)	0.7	0.97 (0.73-1.28)	1.38 (0.65-2.92)
TBXAS1(20)rs2267701	0.14	0.93	1.01 (0.82-1.25)	0.72	0.96 (0.74-1.23)	1.28 (0.66-2.49)
TBXAS1(21)rs740150	0.19	0.55	0.94 (0.78-1.14)	0.57	1 (0.79-1.25)	0.71 (0.37-1.39)
TBXAS1(23)rs10487667	0.25	0.4	1.08 (0.91-1.27)	0.68	1.09 (0.87-1.36)	1.13 (0.75-1.71)
TBXAS1(25)rs2267706	0.23	0.8	1.02 (0.86-1.22)	0.43	1.12 (0.90-1.39)	0.85 (0.51-1.41)
TBXAS1(28)rs22861987	0.38	0.15	1.12 (0.96-1.31)	0.1	1.3 (1.02-1.65)	1.18 (0.84-1.64)
TBXAS1(29)rs2286196	0.19	0.41	1.08 (0.90-1.31)	0.21	1.19 (0.96-1.49)	0.8 (0.41-1.56)
TBXAS1(30)rs2267692	0.35	0.16	1.12 (0.96-1.30)	0.35	1.08 (0.85-1.36)	1.27 (0.92-1.73)
TBXAS1(31)rs1015572	0.05	0.91	1.02 (0.72-1.45)	0.89	0.99 (0.69-1.44)	1.7 (0.24-12.13)
TBXAS1(32)rs1015570	0.39	0.49	0.95 (0.81-1.11)	0.27	0.83 (0.66-1.05)	0.96 (0.71-1.32)
TBXAS1(37)rs2299887	0.4	0.72	0.97 (0.83-1.13)	0.42	0.87 (0.69-1.10)	1 (0.74-1.36)
TBXAS1(38)rs10544451	0.24	0.48	1.07 (0.90-1.27)	0.54	1.13 (0.91-1.41)	0.99 (0.61-1.61)

NOTE: Bold italic: significant at $P < 0.05$ or $P = 0.05$ for either P_{trend} or $P_{heterogeneity}$ results show combined sets 1 and 2 data (4,470 cases and 4,560 controls).
*All genes assessed in set 1 (2,270 cases and 2,280 controls) only, unless $P \leq 0.05$ for either P_{trend} or $P_{heterogeneity}$; see bold italic areas.
†Values for these SNPs have been calculated individually due to their low frequency.

Results

The tagSNP selection databases included a total of 290 common SNPs in the seven genes under investigation. We selected 64 tagSNPs, which, together with eight multimarker tags, tagged 229 of these 290 (79%) with $r^2 > 0.8$. Details of the databases used and the SNP tagging efficiency for each gene are summarized in Table 1. We were unable to tag the remaining 61 common SNPs, with similarly high r^2 as assays could not be designed for them.

The selected tagSNPs were genotyped in breast cancer case-control set 1 and the results are shown in Table 3. Eight SNPs showing evidence of association with breast cancer in set 1 ($P < 0.05$) were further genotyped in breast cancer case-control set 2. The results for the combined analysis of sets 1 and 2 are also presented in Table 3. The genotype distributions in controls conformed to expected values under Hardy-Weinberg equilibrium ($P \geq 0.05$) for all except two of these SNPs. The genotype clustering for these two SNPs was good and it is likely that these deviations from Hardy-Weinberg equilibrium represent chance findings. (Supplementary Table S1 provides details of genotype frequencies in cases and controls.).

The admixture likelihood global test, using all the tagSNPs assessed in this study, indicated borderline evidence for an association with breast cancer susceptibility ($P_{heterogeneity} = 0.01$ and $P_{trend} = 0.3$), suggesting that one or more of the SNPs across all the genes investigated is disease-associated.

In the univariate analyses for breast cancer susceptibility, four SNPs showed modest associations ($P < 0.05$), three of these were in the *PTGIS* gene and one in the *TBXAS1* gene. No association with breast cancer was observed with individual tagSNPs for the *PTGS1*, *PTGS2*, *PTGDS*, or *PGDS* genes. The three *PTGIS* SNPs associated with breast cancer risk were *PTGIS*(04)rs5602 ($P_{heterogeneity} = 0.02$), *PTGIS*(11)rs477627 ($P_{heterogeneity} = 0.01$), and *PTGIS*(19)rs8183919 ($P_{heterogeneity} = 0.02$). The genotype-specific risks are shown in Table 3. The best-fitting genetic model for all three was recessive. Homozygotes for the minor alleles of *PTGIS*(04)rs5602 and *PTGIS*(19)rs8183919 are associated with an increase in risk compared with common allele carriers (OR, 1.15; 95% CI, 1.04-1.27; $P = 0.005$ and OR, 1.22; 95% CI, 1.06-1.41; $P = 0.006$ respectively). For *PTGIS*(11)rs477627, a protective effect was observed for the minor allele homozygotes (OR, 0.66; 95% CI, 0.5-0.86; $P = 0.002$). These three SNPs are very weakly correlated with each other ($r^2 = 0.004$ and 0.02 for rs5602 with rs477627 and rs8183919, respectively, and $r^2 = 0.04$ for rs477627 with rs8183919, respectively) and all three were significant in a multivariate logistic regression model.

TBXAS1(17)rs41727 was associated with a modest increase in breast cancer risk ($P_{heterogeneity} = 0.01$). Again, the best-fitting model was recessive with OR of 1.83 (95% CI, 1.22-2.73) and $P = 0.003$ for the rare homozygotes compared with common allele carriers. None of the tested SNPs were associated with age in controls and age-adjusted results were similar to the unadjusted results (data not shown).

None of the eight multimarker tags were associated with breast cancer (Supplementary Table S2), indicating that none of the known common variants were associated with breast cancer risk.

To investigate whether other, as yet undiscovered, variants in these genes could be associated with susceptibility, we carried out a global haplotype analyses. The haplotype frequencies for each gene or haplotype block were estimated from the tagSNPs used and compared between cases and controls. The haplotype-specific risks are presented in Table 4. No significant differences in haplotype frequencies were seen between cases and controls for the *PTGS1*, *PTGS2*, *PTGES*, *PTGDS*, or *PGDS* genes, each of which lie in a single haplotype block. The *PTGIS* gene fell into two blocks and the *TBXAS1* gene into three blocks before analysis. Haplotypes in each block were analyzed separately. *PTGIS* block 1 showed marginally significant results ($P = 0.01$; OR, 0.65; 95% CI, 0.48-0.89; global test $P = 0.06$). However, this was entirely due to the combined rare haplotype group and it was unclear which individual rare haplotype is responsible for this effect. In *PTGIS* block 2, four separate haplotypes showed frequency

differences between cases and controls at the $P = 0.05$ level and together these generated a global test $P = 0.01$ (Table 4). One of these haplotypes, carrying the minor alleles of SNPs *PTGIS*(11)rs477627, *PTGIS*(21)rs476496, and *PTGIS*(20)rs1066894, confers a reduced susceptibility to breast cancer ($P = 0.005$; OR, 0.65; 95% CI, 0.48-0.88). For the *TBXAS1* blocks, neither the global test nor the individual haplotype frequency differences were significant.

In survival analysis, univariate Cox regression analysis was used to assess association of tagSNP genotype with all-cause mortality among the breast cancer cases. The initial data in set 1 suggested that the minor allele carriers of *PTGES*(13)rs12553596 and *PTGS2*(11)rs20424 have improved survival in a dose-dependant manner (Table 5). However, after further analyses in set 2, the combined results (combined sets 1 and 2; Table 5) suggested that neither variant was associated with improved survival. None of the tagSNPs in the other genes were significantly associated with survival. The global survival analysis was not significant ($P_{heterogeneity} = 0.68$ and $P_{trend} = 0.86$).

Discussion

We have carried out a comprehensive tagSNP analysis of common genetic variation in seven genes in the prostaglandin pathway and susceptibility to breast cancer and all-cause mortality after diagnosis. We found little evidence that common variants are associated with modest risks of breast cancers. However, we cannot exclude the possibility that the alleles investigated are associated with smaller risks. Common SNPs or haplotypes tagging the *PTGIS* and *TBXAS1* genes were associated (at the 5% level) with breast cancer risk. There may be other susceptibility variants in these genes that are not strongly correlated with the polymorphisms examined, particularly for *TBXAS1*, which was not well tagged [$r^2 > 0.8$ (53%) and $r^2 > 0.5$ (74%)].

These results need to be interpreted with some caution. There are a multitude of candidate breast cancer susceptibility polymorphisms and the prior probability of association of any one is low. None of the associations reported here have yet reached the level of significance suggested as appropriate for candidate genes studies (at least $P < 10^{-4}$), but they merit further investigation in other case-control sets. This study did not have the required power to detect rare low-penetrance allelic associations and these were not attempted.

The SNPs studied were selected because they were the best tagSNPs for the known variants in these genes available at the time of this study. The *TBXAS1*, *PTGIS*, *PTGES*, *PTGDS*, and *PGDS* genes have not been fully resequenced and it is therefore not known how many common variants exist in them. However, because SNPs have been identified at high densities, in the majority of these genes, it is anticipated that most undiscovered variants will have been adequately tagged by the tagSNPs we have selected.

The *PTGS1* and *PTGS2* genes have been substantially resequenced, in 23 European subjects, by the Seattle SNPs project. More than 75% of the gene footprint for *PTGS1* and >95% of that for *PTGS2* have been resequenced, meaning that most of the common variants in these genes will have been identified. Neither the individual SNP genotypes nor the haplotype association tests showed any statistically significant differences between cases and controls at $P < 0.05$, therefore suggesting that neither play a significant role in breast cancer susceptibility.

Previous breast cancer susceptibility studies investigating *PTGS1* and *PTGS2* have been inconclusive. Langsenlehner et al. (28) showed a positive association between *PTGS2*(04)rs5275 SNP and breast cancer risk (rare homozygote, CC; $P = 0.002$; OR, 2.05; 95% CI, 1.30-3.25), whereas Cox et al. (29) excluded an increase in breast cancer susceptibility but raised the possibility of an inverse association between this polymorphism and breast cancer risk (rare homozygote, CC; $P = 0.05$; OR, 0.80; 95% CI, 0.62-1.03). In our study, this SNP was not significantly associated with breast cancer susceptibility ($P_{heterogeneity} = 0.66$ and $P_{trend} = 0.36$; OR, 1.0; 95% CI, 0.8-1.3) or survival (hazard ratio, 1.05; 95% CI, 0.9-1.23; $P_{trend} = 0.52$). Furthermore, a meta-analysis of the three data sets found no evidence for association, although there was significant heterogeneity between studies (Supplementary Table S3).

Both univariate SNP and haplotype analyses indicate that the *PTGIS* gene may play a role in breast cancer susceptibility. The haplotype analysis uses a trend test with limited power to detect haplotypes with a recessive effect; nonetheless, it revealed that certain haplotypes are associated with increased or decreased risks, which were not detected by the univariate SNP analysis. A haplotype marked by the minor alleles of a combination of SNPs *PTGIS*(11)rs477627, *PTGIS*(21)rs476496, and *PTGIS*(20)rs1066894 confers a reduced susceptibility to breast cancer, but it is unclear which variants within the haplotype are responsible for this effect. They are probably acting as markers for other, unidentified SNPs, which are the causal variants. *PTGIS*(19)rs8183919, *PTGIS*(20)rs1066894, *PTGIS*(21)rs476496, and *PTGIS*(11)rs477627 all lie within the 5′ region and intron 1 (block 2) of the *PTGIS* gene.

TagSNPs for the *PGDS*, *PTGDS*, and *PTGES* genes were selected using the HapMap database, which has good coverage but is not comprehensive. Therefore, although no evidence for an association was found with variants in these genes, such an association cannot be categorically excluded.

Overall, there was no evidence for association of genotypes of any of the tagSNPs studied here with all-cause mortality. The global survival test, with all the tagSNPs in the pathway, was not significant. The use of all-cause mortality as an endpoint may result in some loss of power, as a small proportion of women will have died from causes other than breast cancer. However, most women in the age range of participants in SEARCH who die within 10 years will have died from disease recurrence and the misclassification error will be small.

It is possible that these SNPs alter risk in subgroups of the population that have been exposed to specific environmental and lifestyle factors, for example, if an association of *PTGIS* with breast cancer risk was limited to women with high nonsteroidal anti-inflammatory drug consumption. However, where no main effect has been detected (for either gene or environment), such subgroup effects must be small. The at-risk subgroup must represent a small proportion of the population under study, or there must be true crossover effects (genotype associations of different directions among subgroups), which are unlikely.

Prostaglandin-targeted therapies (e.g., COX inhibitors) are presently under investigation for chemoprevention of a variety of cancers including colorectal, lung, and breast cancers (37).

Although there is evidence for the efficacy of these inhibitors in colorectal cancer (38), their role, if any, in breast cancer is uncertain. A recent review, which looked at >20 studies, concluded that nonsteroidal anti-inflammatory drugs, which act via the prostaglandin pathway, may offer protection against developing breast cancer, but it is unclear if this benefit is confined to aspirin alone or all nonsteroidal anti-inflammatory drugs (39). Equally, it is unclear what the clinical cost would be in terms of toxicities, secondary to regular aspirin use. Our findings that common variants in two genes within this pathway may affect breast cancer susceptibility leaves open the possibility that only individuals with particular genotypes may benefit from these therapies, thus affecting their clinical utility. The study sizes required to confirm such findings would be large. Future clinical studies looking at the viability of prostaglandin pathway-targeted therapies should consider correlating variables such as toxicity and outcome with the relevant SNP profile of any individuals recruited.

In the absence of such data, it will be difficult to ascertain who is likely to benefit from such treatment and, more importantly, for whom such treatments maybe deleterious. In addition, the precise role and contribution of each of these genes to the modulation of the effects of therapy requires further investigation as does defining the relevant SNP profile. Further analyses in larger studies would be required to establish this. It is plausible that other members of the pathway (e.g., *PTGIS* or *TBXAS1*) might be better therapeutic targets than *PTGS2*.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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