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Genetic variation in proinflammatory cytokines IL6, IL6R, TNF-region, and TNFRSF1A and risk of breast cancer

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Abstract

Proinflammatory cytokines are associated with age-related diseases including arthritis and heart disease. IL6 and TNF also play key roles in estrogen modulation in older women. We explored whether variation in *IL6* and *TNF* genes influenced the risk of breast cancer in samples that differed by age group: <44 years (228 cases and 271 controls), 45–64 years (426 cases and 396 controls), and 65+ years (228 cases and 239 controls). Samples were drawn from population-based case–control studies conducted in Seattle. Age-adjusted odds ratios (ORs) were calculated to evaluate the risk associated with variants in *IL6*, *IL6R*, *TNF*, and *TNFRSF1A*. There was a significantly increased risk of breast cancer associated with one or more C>T alleles at IL6 rs2069861 among subjects in the oldest age group (OR 1.8, 95% CI 1.1–2.9), but no overall increased risk of breast cancer associated with any IL6 or IL6R variants in the combined data. There were significantly elevated risks of breast cancer among women 45–64 years old associated with a UTR 5' flanking SNP LTA rs2009658 C>G allele (OR 1.5, 95% CI 1.1–1.9) and a nonsynonymous coding SNP TNFRSF1A rs767455 T>C allele (OR 1.3, 95% CI 1.1–1.6); these two variants were also elevated in the combined data (OR 1.3, 95% CI 1.1–1.5 and OR 1.2, 95% CI 1.1–1.4, respectively). This study supports a modest association between a variant in IL6 and breast cancer among older women and TNF-related variants and breast cancer among middle-aged women. Further evaluation of these genes in other studies is warranted.

Keywords

Breast cancer; Genetic variation; IL6; TNF; Proinflammatory cytokine genes

Introduction

Established risk factors for breast cancer, such as reproductive history, age, family history, and known genetic factors, do not completely account for the high rate of age-related increases in lifetime risk for breast cancer. Although the genesis of breast cancer is heterogeneous and incompletely understood, a role for endogenous sex hormones is clear. Additional age-related factors that may increase risk of breast cancer beyond the risk associated with hormones include cytokines associated with inflammation [33]. Inflammation-associated cytokines may modulate immune response and aid evasion of tumor surveillance for many diseases associated with aging [5, 18,26, 29]. Further support for a role of inflammation in breast carcinogenesis comes from studies that link use of non-steroidal anti-inflammatory medications to decreased risk of breast cancer [20].

Although there are a large number of candidate genes associated with inflammation, we chose to focus here on two cytokines that are known to increase in the circulation with age, IL6, and TNF.

IL6 mediates cell growth and differentiation in addition to its role in immune response. It is produced by a variety of cell types including lymphoid cells, endothelial cells, fibroblasts, and adipose tissue and at sites of acute and chronic inflammation, where it signals to induce an inflammatory response that includes autocrine stimulation and upregulation of other proinflammatory cytokines. Soluble IL6 is rarely detected in young healthy adults, but is elevated in older adults, even in the absence of trauma or infection. Elevated IL6 levels have been associated with age-related diseases, such as Alzheimer's disease, diabetes, and rheumatoid arthritis. In a review of the literature, Pfeilschifter et al. [30] suggest that the decrease in estrogen after menopause is strongly tied to increased activity of proinflammatory cytokines such as IL6 and TNF. IL6 signals through the IL6 receptor, a heterodimer consisting of two membrane-bound glycoproteins; we focus here on the IL6R subunit, which is the soluble form of the cytokine.

The *TNF* region encompasses three pleiotropic proinflammatory cytokine genes: tumor necrosis factor (*TNF*) and lymphotoxin A and B (*LTA* and *LTB*) located in the class III major histocompatibility complex (MHC) region of chromosome 6. These cytokines are mainly (but not exclusively) produced by macrophages and T cells. Initially, these TNF region cytokines were noted only for their anti-tumor and cell killing activities; more recent work has additionally established them as important markers of acute and chronic inflammation. TNF stimulates estrogen synthesis through aromatase induction [31], which could be a key source of estrogen in the postmenopausal breast, and may act as a tumor promoter. *TNFRSF1A* is a major receptor for TNF, and belongs to a superfamily of receptors for TNF. *TNFRSF1A* can activate NF-KappaB, mediate apoptosis, and regulate inflammation.

In this study we investigated whether variants in the immune-modulating cytokine genes *IL6*, *IL6R*, *TNF*, or *TNFRSF1A* contribute to the risk of breast cancer.

Methods

Study populations

Study samples were drawn from prior population-based case-control studies of invasive breast cancer conducted in the Seattle area. Eligible cases for each study were ascertained from the local SEER registry [19], the Cancer Surveillance System (CSS), and samples were restricted to Caucasian women to limit heterogeneity by race. A brief summary of each study is presented here, and more information can be obtained from prior publications.

The first contributing study, the WISH study, was a multicenter case-control study of risk factors for early-onset breast cancer, including oral contraceptive use, developmental history, physical activity, diet, and genetics [13, 38]. Age at diagnosis for cases included in the current study was 34–44 years of age, with diagnosis dates between 1990 and 1992. Controls were ascertained using random digit telephone dialing (RDD). Interviews were completed by the Seattle site on 642 cases and 608 controls (84 and 74% of those eligible, respectively) and blood was collected from 540 and 476 of the interviewed cases and controls. We randomly selected 228 cases and 271 controls for the current study.

The second study that contributed specimens was the Seattle site of the CARE Study, a multi-center case-control study of breast cancer in women aged 45–64 years (diagnosis years between 1994 and 1998). The study was designed to investigate the risk of breast cancer in relation to exogenous hormone use [25, 27, 39]. At the Seattle site, cases were ascertained via the CSS registry and controls were ascertained by RDD. There were 1,066 cases and 1,017 controls interviewed (with 90.4 and 89.1% interview participation at the Seattle site) and over 90% of subjects provided a blood specimen. We randomly selected 426 cases and 396 controls from the CARE study for inclusion in the current study. Sampling fractions were computed to account for over sampling within strata of age, race, and first degree family history of breast cancer as previously described [25, 39].

The oldest group of women in this study was selected from the PACE study [23, 24], which was designed to investigate the etiology of invasive breast cancer in women 65–79 years old (diagnosed between 1997 and 1999). There were 1,210 eligible cases and 975 (80.6%) were interviewed. Controls for the PACE study ($n = 1,365$) were ascertained from lists of Social Security recipients generated by the Centers for Medicare and Medicaid (CMS), and 1,007 (73.8%) of eligible women were interviewed. Cases were required to have also been listed in CMS as Social Security recipients. Blood was collected from 891 (91.4%) cases and 878 (88%) controls. For the current study we randomly selected 228 case and 239 control samples frequency-matched on age from among the Caucasian PACE study participants.

Participants in all three studies completed extensive in-person interviews and donated blood at the time of interview. Information from the CSS registry on estrogen receptor (ER) and progesterone receptor (PR) status was used to categorize tumors as luminal (ER or PR positive) or non-luminal (ER and PR negative), and histology codes were grouped as ductal (ICDO 8500), lobular (ICDO 8520), or other (ICDO 8000–8481, 8501–8510, 8521–8541, 8980). The reference age of interest in this study is the age at diagnosis of breast cancer for cases, and controls for each study were frequency-matched to the age of the cases in 5-year age groups. A total of 882 cases and 906 controls were included in the current study.

SNP selection

We assessed variation in *IL6*, *IL6R*, *TNF* region, and *TNFRSF1A* genes using a haplotype-block tagging approach. Single nucleotide polymorphisms (SNPs) were grouped based on being in high linkage disequilibrium (LD; $r^2 > 0.80$) and TagSNPs were chosen to capture the majority of common variation within each gene [4]. We used the Seattle SNPs Variation

Discovery Resource (www.gvs.gs.washington.edu/GVS) bioinformatics website to identify the TagSNPs based on resequencing of Caucasian populations. We focused on TagSNPs that had minor allele frequencies (MAF) ≥ 0.05 or SNPs that were coding SNPs within a gene of interest and had a MAF ≥ 0.02 . A total of 9 TagSNPs were chosen for the *IL6* gene, 8 for *IL6R*, 7 for *TNF* region, and 5 for *TNFRSF1A*.

Genotyping assay

SNPs were genotyped using the Applied Biosystems SNPLEX® system and GeneMapper software. Each 96-well plate included a mixture of cases and controls as well as positive and negative DNA assay controls. As a quality control measure, 187 blind duplicate samples were included across all plates to assess the reproducibility of the genotype calls. The laboratory personnel were blinded to all sample characteristics. All SNPs were in Hardy–Weinberg equilibrium when we examined the distribution of genotypes among the controls in this study.

Statistical methods

For age-specific analyses, we used logistic regression to estimate the relative risk of breast cancer associated with SNP genotypes using the odds ratio (OR) approximation, after adjusting for linear age at reference. For analyses combining the three age groups, we allowed the logistic-linear association between age and risk to be arbitrarily different from one age group to the next. Data from subjects aged 45–64 were weighted by the inverse probability of being sampled and providing a blood specimen in the CARE study (based on age, race, study site, and family history). The sampling is explained more detail in Marchbanks et al. [27]. Analyses combining the three age groups used the CARE inverse probability of sampling weights for CARE participants, and a weight of one for participants from the PACE and WISH studies.

We fit additive logistic models relating each additional copy of the minor allele to the log odds of being a case for the majority of SNPs. However, in instances when there were fewer than five minor allele carriers among cases or controls, we fit a dominant model. We used a $P_{\min}$ per-mutation-test procedure with 10,000 replications to account for multiple comparisons when evaluating the significance of the most associated SNP within each gene [9]. In secondary analyses, we used logistic regression of the combined study data to examine the association between SNP genotype and breast cancer risk. We fit separate models comparing ductal cases ($n = 640$) and luminal cases ($n = 679$) to all controls after adjusting for age at reference and study, as described above.

Results

A genotyping call rate of $>90\%$ was used as a quality control filter. Of the 29 TagSNPs genotyped, 2 failed genotyping (*IL6R* rs4845617 and *TNFRSF1A* rs2229092), 6 were excluded due to $<90\%$ call rate (*IL6R*: rs4075015, rs6694817, rs2229238, rs8192282; *TNF* region: rs2239704; *TNFRSF1A*: rs4149572), leaving 21 SNPs for the final analysis (see Table 1). The genotyping calls were concordant for 99.47% of the 187 replicate pairs tested; discordance among 21 pairs ranged from 1 to 3 SNP pairs. The only SNP with 3 discordant pairs was *IL6R* rs4509570.

As expected, the characteristics of the cases and controls differ markedly by age group (Table 2). For all case and control subjects, body mass index (BMI) was lowest in the youngest age group, and number of prior pregnancies was highest in the oldest age group. Overall, the youngest age group had the highest percentage of control women without a family history of breast cancer. Tumor characteristics also differed by age group, with the

highest proportion of luminal tumors found in the oldest age group and the highest proportion of ductal tumors in the youngest age group.

In the primary analyses, each SNP was examined separately by age group and then in all ages combined (Table 3). Among the oldest group of women there was a significant increased risk of breast cancer associated with C>T IL6 rs2069861 (OR 1.8, 95% CI 1.1–2.9) and a marginally decreased risk of breast cancer associated with C>T IL6 rs2069849 (OR 0.4, 95% CI 0.2–1.0). There were no significant associations with IL6R variants, although we observed a marginally decreased risk of breast cancer associated with C>G IL6R rs4509570 (OR 0.7, 95% CI 0.5–1.0) among the youngest women. No association was observed between the widely studied SNP IL6 promoter SNP rs1800795 (alias –174) and breast cancer. No SNP in IL6 or IL6R survived the $P_{\min}$ test for multiple comparisons; however, the strongest evidence of association between IL6 and breast cancer was found in the oldest age group of women ($P = 0.09$).

In the TNF region on chromosome 6, which contains the *TNF*, *LTA*, and *LTB* genes, we found an increased risk for breast cancer with C>G allele in *LTA* rs2009658 (OR 1.5, 95% CI 1.1–1.9) among women 45–64 years old. This variant was also associated with increased risk in the aggregate across all women aged 35–79 years old (OR 1.3, 95% CI 1.1–1.5). In the TNF receptor gene *TNFRSF1A*, the T>C allele rs767455 was associated with a significantly increased risk of breast cancer in middle-aged women (OR 1.3, 95% CI 1.1–1.6) and in women of all ages combined (OR 1.2, 95% CI 1.1–1.4). The overall $P_{\min}$ test for the *TNFRSF1A* gene in the combined age groups data was significant ($P = 0.01$).

In Table 4 we examined the association between genetic variation in our SNPs and selected breast cancer subtypes, specifically those with ductal histology ($n = 640$) or luminal tumor markers (ER+ or PR+, $n = 679$). The above findings were similar in these two large subgroups.

Discussion

We found increased risks of breast cancer associated with minor alleles in TNF-family variants *LTA* rs2009568 (OR 1.3, 95% CI 1.1–1.5) and *TNFRSF1A* rs767455 (OR 1.2, 95% CI 1.1–1.4) among all age groups and a marginally increased risk of breast cancer associated with IL6 rs2069861 among women 65–79 years old (OR 1.8, 95% CI 1.1–2.9). The associations for the TNF family SNPs were similar when the case group was restricted to women whose tumors had ductal histology or hormone markers consistent with luminal breast cancer. Associations between these TNF gene family variants and breast cancer risk have not been previously reported. Our TNF findings may point to risk of breast cancer associated with these gene variants or variants with which they are in linkage. We found little evidence of variability in relative risk of breast cancer across age groups at diagnosis.

Location of the TNF region between highly polymorphic human leukocyte antigen (HLA) class I and class II genes, which are important in immune surveillance, is intriguing. Linkage disequilibrium between TNF and HLA class I and II genes contributes to formation of haplotypes or stable combinations in this highly polymorphic region of the genome [8, 11]. It remains to be determined if *LTA* rs2009658 in the TNF region is carried with any HLA haplotypes which may be associated with breast cancer. The only well-powered study to examine this question [7] compared 956 Dutch breast cancer cases to family member controls. They found evidence for a potential association between moderate breast cancer risk and a haplotype in the HLA region.

Although no genome-wide association studies (GWAS) of breast cancer has generated evidence of breast cancer risk associated with variants in IL6 or TNF genes, the potential

relationship warrants consideration. IL6 and TNF stimulate conversion of estrone to estradiol [1, 10]. Through this pathway, both cytokines may contribute to initiation of tumor development, from the very earliest stages of disease, or promote growth of established disease [2, 6]. Over the course of many years, and likely combined with the effects of other genetic factors and the cumulative effects of hormonal and environmental exposures, a proinflammatory cytokine milieu may favor the development of breast cancer in older women. Thus, we hypothesized that common variants in the pro-inflammatory cytokines IL6 and TNF, or their receptors, may be associated with incomplete penetrance and low relative risk, which may make them difficult to detect in a GWAS.

A 2007 candidate gene study by Gaudet et al. [16] used a TagSNP approach similar to ours when they evaluated the role of variation in TNF region genes in two case-control studies conducted in the USA and Poland. They found a marginally significant association between risk of breast cancer and the G>A allele of a TNF promoter SNP, rs361525. In a larger follow-up study of 30,000 cases and 30,000 controls, there was no evidence of association of rs361525 with risk (OR 1.00, 95% CI 0.95–1.06), so that, to date, there has been little evidence for a relationship between this SNP and breast cancer risk [17]. In the current study, TNF rs3093662 was evaluated; it tags the same signal as TNF rs361525 ($r^2 = 1.0$). We saw no evidence that genetic variation in this LD block was associated with risk in the age-specific studies or the combined study.

The minor allele of the TNF promoter SNP rs1800629 (alias -308) was not related to breast cancer risk in a candidate SNP study of 84 breast cancer patients and 110 control women conducted in Italy [3, 34] or in the larger Dutch HLA study described above [7]. The current study supports these findings. However, in a recently published meta-analysis that included 13 studies of TNF variants and breast cancer risk [35], the G>A allele of TNF rs1800629 was associated with decreased risk of breast cancer (OR 0.9, 95% CI 0.879–0.978) in Caucasians; further, the AA genotype may be a risk factor for increased breast cancer risk in Africans.

We also examined the risk of breast cancer associated with variants in IL6 and IL6R. There was a significantly increased risk of breast cancer associated with allele C>T at IL6 rs2069861 (OR 1.8, 95% CI 1.1–2.9) in the oldest group of women. This variant is in the 3' region near the IL6 gene and has not been reported to be associated with breast cancer in other studies. Variation in 3' UTR regions may affect binding site of miRNA and influence gene transcription, as was previously shown for *IL1A* [15]. We examined whether the IL6 rs2069861 variant is a target binding site for miRNAs by searching TargetScanHuman 5.1 database (www.targetscan.org), but did not find any miRNAs targeting this SNP region. In another recent study of IL6 polymorphisms, including rs2069861, there was no difference in plasma levels of IL6 by any of eight SNPs in IL6 among women 30–55 years old from the Nurses Health study [32]. However, it may be of interest to examine IL6 plasma levels and genotype association in older women, since IL6 and other pro-inflammatory marker levels increase with age [14].

Conflicting results of association of IL6 promoter variant rs1800795 with breast cancer have been reported. In an Australian case-only study [22] of 256 breast cancer patients, homozygous carriers of the variant allele had higher-grade breast tumors. However, in a UK study of 129 breast cancer patients and 263 organ donor controls, this variant was not associated with case-control difference in breast cancer risk [36]. Another larger study of 269 cases and 227 controls conducted in European Caucasians described an increased risk of breast cancer associated with the IL6 rs1800795 (OR 1.5, 95% CI 0.4–1.9 for GC and OR 2.0, 95% CI 1.1–3.6 for CC) [21]. The current study did not detect an increased risk of breast cancer associated with this IL6 promoter SNP.

Some limitations of this study should be noted. They include a relatively small sample size for a study of genetic variation. Since some variants likely increase the risk of breast cancer with low penetrance, we may have missed associations that do exist because of our small sample size. This size limitation was potentially made more important by our separate examination of the associations in the three age-specific populations included in this study; this was mitigated by our finding that the significant estimates from the age-specific populations were reflected in the estimates for all populations combined. Another limitation is that some of the variation in the studied genes was missed either due to assay failure, probe failure, or that a specific type of variant (for example, one in a repeat region or a copy number variation) was not detectable on the platform. This problem was particularly acute for IL6R, for which only 3 of the 8 SNPs were successfully genotyped. Future studies will be able to address these limitations by including more samples and using multiple platforms or resequencing to comprehensively cover the genetic variation for each gene.

The strength of this study is that each age group we studied showed a distribution of known risk factors for breast cancer consistent with that shown in other studies. Also, the TagSNP approach to candidate genes can comprehensively address signal variation across the genes and suggest functional roles that an individual SNP might play. LTA rs2009658, for example, is an intronic SNP located near the UTR at the 5' end of the LTA gene, which may influence the level of gene expression for LTA. The other significant association in this study, TNFRSF1A rs767455 is a synonymous coding SNP, Pro12Pro. Despite this synonymous change, a clinical series of Japanese Crohn's disease patients suggested that carriers of the G allele are refractory to treatment for with a monoclonal antibody directed at TNF [28].

Prior studies have implicated IL6 and TNF-region genes a wide variety of diseases including cardiovascular disease, diabetes, and cancer [12, 37, 40]. In this study we have followed up on two hypotheses: (1) that cancer may develop from a long-term proinflammatory response [5] and (2) the common disease, common variant hypothesis. This study suggests that variation in the immune-modulating cytokine TNF and its main receptor TNFRSF1A or genes in linkage with them may be related to the risk of breast cancer, but fails to provide strong evidence that variation in the proinflammatory cytokine IL6 and the IL6R subunit were related to breast cancer risk.

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Table 1
TagSNPs in the IL6, IL6R, TNF-region (TNF, LTA, and LTB), and TNFRSF1A genes

TagSNP	SNP location	Chr. position	Study controls		Hapmap ^a		SNPs in Bin ^d
			MAF		Major/minor allele	MAF	
IL6 (Chr. 7: 22729345–22742141)							
rs12700386	UTR 5', flanking	22748639	0.196	C/G	0.225		Singleton
rs2069825	UTR 5', flanking	22731866	0.408	CT/− <i>b</i>	0.457 ^b		Singleton
rs2069827	UTR 5', flanking	22731981	0.096	G/T	0.100		Singleton
rs1800795	UTR 5', flanking	22733170	0.423	G/C	0.467		rs1474347
							rs1474348
							rs1554606
							rs1800795
							rs1800797
							rs2069832
							rs2069833
							rs2069845
rs2069837	Intron	22734552	0.080	A/G	0.067		Singleton
rs2069840	Intron	22735097	0.343	C/G	0.317		Singleton
rs1554606	Intron	22735232	0.445	G/T	0.458		rs1474347
							rs1474348
							rs1554606
							rs1800795
							rs1800797
							rs2069832
							rs2069833
							rs2069845
							rs2069843
							rs2069844
							rs2069849
							rs7808204
rs2069849	Coding, Phe201Phe	22737681	0.026	C/T	0.017		Singleton
rs2069861	UTR 3', flanking	22738179	0.111	C/T	0.061		Singleton

TagSNP	SNP location	Chr. position	Study controls	Hapmap ^a		SNPs in Bin ^d
			MAF	Major/minor allele	MAF	
IL6R (Chr. 1: 152640293–152710812)						
rs1386821	Intron	152648673	0.197	A/C	0.167	rs1386821
rs4509570	Intron	152703008	0.236	C/G	0.328	rs12083537 rs4240872 rs4341355 rs4509570 rs10752641 rs2228145
rs2228145 ^c	Coding, Asp358Ala	152693594	0.387	A/C	0.350	rs4129267 rs4393147 rs4453032 rs4537545 rs4845622 rs4845623 rs7518199
TNF, LTA, LTβ (Chr. 6: 31640072–31666181)						
rs1800629	UTR 3', flanking (LTA) UTR 5', flanking (TNF)	31651010	0.173	G/A	0.217	Singleton
rs1800610	Intron (TNF)	31651806	0.094	C/T ^b	0.068 ^b	rs769178 ^b rs1799724 rs1800610
rs3093662	Intron (TNF)	31652168	0.081	A/G	0.071	rs3093553 rs3093662
rs2009658	UTR 5', flanking (LTA)	31646223	0.147	C/G	0.158	Singleton
rs2229094	Coding (LTA), Cys13Arg	31648535	0.261	T/C	0.242	Singleton
TNFRSF1A (Chr. 12: 6308184–6321522)						
rs767455	Coding, Pro13Pro	6321206	0.401	T/C	0.492	rs4149576 rs767455 rs1800693 rs1860545

TagSNP	SNP location	Chr. position	Study controls		Hapmap ^a		SNPs in Bin ^d
			MAF	Major/minor allele	MAF		
rs4149578	Intron	6317698	0.106	G/A	0.108		rs11064145 rs4149573 rs4149578
rs1860545	Intron	6317038	0.384	C/T	0.467		rs4149576 rs767455 rs1800693 rs1860545
rs4149584	Coding, Arg121Pro	6312904	0.019	G/A	0.033		rs11064145 Singleton

Chr chromosome

^aDetermined from HapMap-CEU, European population

^bDetermined from PGA-EUROPEAN-PANEL

^crs2228145 was previously rs8192284

^dFrom GVS as of January 11, 2010

Table 2

Selected demographic and tumor characteristics in three Seattle-based breast cancer case-control studies

	Age 34-44				Age 45-64 ^a				Age 65-74			
	Control (n = 271)		Case (n = 228)		Control (n = 396)		Case (n = 426)		Control (n = 239)		Case (n = 228)	
	n	%	n	%	n	%	n	%	n	%	n	%
Education												
<High school	9	3.3	5	2.2	17	4.2	15	3.4	30	12.6	27	11.8
High school	61	22.5	48	21.1	93	23.4	113	26.4	95	39.7	86	37.7
Some college	113	41.7	90	39.5	132	34.1	146	34.8	74	31.0	74	32.5
College graduate	88	32.5	85	37.3	154	38.3	152	35.4	40	16.7	41	18.0
BMI at reference (kg/m ²)												
<18.5	10	3.8	7	3.1	10	2.5	6	1.5	5	2.2	6	2.7
18.5-24.9	159	60.0	139	62.3	215	54.8	258	60.5	96	41.4	63	28.5
25-29.9	56	21.1	46	20.6	106	25.9	102	23.9	72	31.0	86	38.9
30+	40	15.1	31	13.9	65	16.9	59	14.1	59	25.4	66	29.9
Parity												
Nulliparous	47	17.3	47	20.6	62	16.0	72	16.5	18	7.5	21	9.2
Parous	224	82.7	181	79.4	334	84.0	354	83.5	221	92.5	207	90.8
1	55	20.3	42	18.5	42	11.5	52	12.2	19	7.9	25	11.0
2	94	34.7	89	39.2	124	31.6	142	33.1	36	15.1	60	26.3
3	44	16.2	37	16.3	98	24.0	102	24.3	70	29.3	57	25.0
4+	31	11.4	12	5.3	70	16.9	58	13.9	96	40.2	65	28.5
Menopausal status												
Premenopausal	251	92.6	222	97.8	141	40.0	164	37.7	0		0	
Postmenopausal	20	7.4	5	2.2	255	60.0	262	62.3	239	100.0	228	100.0
Family history of breast cancer												
None	134	60.1	87	46.8	132	52.8	129	42.4	77	56.2	47	36.2
1st degree	23	10.3	37	19.9	80	19.8	117	27.3	35	25.5	50	38.5
2nd degree	66	29.6	62	33.3	71	27.4	92	30.3	25	18.2	33	25.4
Histology												
Ductal	0		185	81.1	0		307	72.1	0		148	64.9

	Age 34-44				Age 45-64 ^a				Age 65-74			
	Control (n = 271)		Case (n = 228)		Control (n = 396)		Case (n = 426)		Control (n = 239)		Case (n = 228)	
	n	%	n	%	n	%	n	%	n	%	n	%
Lobular	0		11	4.8	0		35	8.3	0		32	14.0
Ductal/lobular	0		2	0.9	0		26	6.2	0		18	7.9
Other	0		30	13.2	0		58	13.4	0		30	13.2
Estrogen receptor (ER) and progesterone receptor (PR) status												
ER- PR-	0		55	24.7	0		70	17.9	0		28	13.1
ER+ PR-	0		18	8.1	0		40	10.0	0		36	16.9
ER- PR+	0		22	9.9	0		7	1.7	0		1	0.5
ER+ PR+	0		128	57.4	0		279	70.3	0		148	69.5

^aProportions for age 45-64 weighted using inverse probability of sampling weights from CARE

Table 3

Risk of breast cancer associated with SNPs in IL6, IL6R, TNF region (TNF and LTA), and TNFRSF1A by age group and across studies

	Age 34-44				Age 45-64				Age 65-74				Overall			
	Control (n = 271)		Case (n = 228)		Control (n = 396)		Case (n = 426)		Control (n = 239)		Case (n = 228)		OR		95% CI	
	n	%	n	%	n	%	n	%	n	%	n	%				
IL6																
P	0.33				0.95				0.09						0.99	
rs12700386																
CC	166	62.6	146	65.2	262	66.8	268	65.2	148	62.4	140	62.2	Dominant	1.0	0.7-1.5	log-Additive
CG	85	32.1	74	33.0	117	29.8	125	30.4	82	34.6	81	36.0				0.9-1.2
GG	14	5.3	4	1.8	13	3.3	18	4.4	7	3.0	4	1.8				
rs2069825																
CTCT	88	33.0	87	38.7	134	33.9	148	35.3	93	39.1	79	34.8	log-Additive	1.1	0.9-1.5	log-Additive
-CT	126	47.2	107	47.6	197	49.9	191	45.6	111	46.6	111	48.9				0.9-1.1
-	53	19.9	31	13.8	64	16.2	80	19.1	34	14.3	37	16.3				
rs2069827																
GG	214	80.5	196	86.7	323	81.6	339	80.1	201	84.5	180	80.0	Dominant	1.4	0.8-2.2	log-Additive
GT	46	17.3	28	12.4	67	16.9	80	18.9	37	15.5	43	19.1				0.8-1.2
TT	6	2.3	2	0.9	6	1.5	4	0.9	0	0.0	2	0.9				
rs1800795																
GG	85	31.6	77	33.8	128	32.6	141	33.4	86	36.1	73	32.2	log-Additive	1.1	0.9-1.5	log-Additive
CG	126	46.8	117	51.3	199	50.6	195	46.2	114	47.9	114	50.2				0.9-1.2
CC	58	21.6	34	14.9	66	16.8	86	20.4	38	16.0	40	17.6				
rs2069837																
AA	205	84.4	171	81.4	312	84.3	320	82.7	188	84.3	182	86.3	Dominant	0.9	0.5-1.5	Dominant
AG	38	15.6	39	18.6	58	15.7	66	17.1	33	14.8	26	12.3				0.8-1.5
GG	0	0.0	0	0.0	0	0.0	1	0.3	2	0.9	3	1.4				
rs2069840																
CC	117	43.3	101	44.5	162	41.0	189	44.7	104	44.4	90	39.8	log-Additive	1.1	0.8-1.4	log-Additive
CG	123	45.6	101	44.5	191	48.4	178	42.1	100	42.7	111	49.1				0.9-1.2
GG	30	11.1	25	11.0	42	10.6	56	13.2	30	12.8	25	11.1				

	Age 34-44						Age 45-64						Age 65-74						Overall						
	Control (n = 271)			Case (n = 228)			OR	95% CI	Control (n = 396)			Case (n = 426)			OR	95% CI	Control (n = 239)			Case (n = 228)			OR	95% CI	
	n	%		n	%				n	%		n	%				n	%		n	%				n
rs1554606							log-Additive							log-Additive									log-Additive		log-Additive
GG	74	28.0	69	30.9	0.8	0.6–1.1	119	30.4	128	31.6	1.0	0.8–1.3	78	32.9	70	31.1	1.0	0.8–1.3	1.0	0.9–1.1					
GT	133	50.4	119	53.4			200	51.0	188	46.4			115	48.5	114	50.7									
TT	57	21.6	35	15.7			73	18.6	89	22.0			44	18.6	41	18.2									
rs2069849							log-Additive							log-Additive									log-Additive		log-Additive
CC	252	96.2	213	95.5	1.2	0.5–2.9	371	95.6	388	95.1	1.2	0.6–2.4	213	92.2	213	96.8	0.4	0.2–1.0	0.9	0.6–1.5					
CT	10	3.8	10	4.5			17	4.4	20	4.9			18	7.8	7	3.2									
rs2069861							log-Additive							Dominant									Dominant		log-Additive
CC	186	73.8	168	78.1	0.8	0.6–1.2	301	78.8	319	78.6	1.0	0.7–1.4	195	84.8	164	75.6	1.8	1.1–2.9	1.0	0.8–1.3					
CT	61	24.2	42	19.5			78	20.4	86	21.2			33	14.3	52	24.0									
TT	5	2.0	5	2.3			3	0.8	1	0.2			2	0.9	1	0.5									
IL6R																									
P		0.12						0.80						1.00										0.69	
rs1386821							log-Additive							log-Additive									log-Additive		log-Additive
AA	174	64.9	138	61.6	1.2	0.9–1.6	255	65.9	261	64.0	1.1	0.9–1.4	142	61.7	142	64.3	1.0	0.7–1.3	1.1	0.9–1.3					
AC	83	31.0	73	32.6			122	31.5	133	32.6			77	33.5	65	29.4									
CC	11	4.1	13	5.8			10	2.6	14	3.4			11	4.8	14	6.3									
rs4509570							log-Additive							log-Additive									log-Additive		log-Additive
CC	151	56.8	145	65.9	0.7	0.5–1.0	223	56.9	242	58.3	1.1	0.8–1.3	134	57.3	127	58.3	1.0	0.7–1.4	1.0	0.8–1.2					
CG	100	37.6	66	30.0			153	39.0	146	35.2			92	39.3	80	36.7									
GG	15	5.6	9	4.1			16	4.1	27	6.5			8	3.4	11	5.0									
rs8192284							log-Additive							log-Additive									log-Additive		log-Additive
AA	91	34.1	68	30.6	1.2	0.9–1.6	148	37.7	166	39.2	1.0	0.8–1.3	88	37.0	85	37.4	1.0	0.8–1.3	1.1	0.9–1.2					
AC	138	51.7	111	50.0			198	50.4	192	45.3			113	47.5	109	48.0									
CC	38	14.2	43	19.4			47	12.0	66	15.6			37	15.5	33	14.5									
TNF, LTA, LTB																									
P		0.58						0.02						0.80										0.07	
rs2009658							Dominant							log-Additive									log-Additive		log-Additive
CC	199	74.8	171	75.7	1.0	0.6–1.4	290	73.2	281	66.3	1.5	1.1–1.9	165	69.3	154	67.5	1.1	0.8–1.6	1.3	1.1–1.5					

	Age 34-44						Age 45-64						Age 65-74						Overall		
	Control (n = 271)			Case (n = 228)			Control (n = 396)			Case (n = 426)			Control (n = 239)			Case (n = 228)			OR		
	n	%		n	%		n	%		n	%		n	%		n	%		OR	95% CI	OR
CG	61	22.9	52	23.0			101	25.5	126	29.7			67	28.2	64	28.1					
GG	6	2.3	3	1.3			5	1.3	17	4.0			6	2.5	10	4.4					
rs2229094						log-Additive						log-Additive							log-Additive		log-Additive
TT	139	54.9	119	54.6	0.9	0.7-1.3	212	55.5	208	51.0	1.2	1.0-1.5	124	53.7	117	52.7	1.1	0.8-1.5	1.1	1.0-1.3	1.1
CT	93	36.8	87	39.9			146	38.2	157	38.5			95	41.1	85	38.3					
CC	21	8.3	12	5.5			24	6.3	43	10.5			12	5.2	20	9.0					
rs1800629						Dominant						log-Additive							log-Additive		log-Additive
GG	185	69.5	159	71.3	0.9	0.6-1.4	275	69.6	289	68.6	1.0	0.8-1.3	153	64.0	155	68.9	0.8	0.6-1.2	1.0	0.8-1.2	1.0
AG	79	29.7	56	25.1			106	26.8	125	29.7			79	33.1	64	28.4					
AA	2	0.8	8	3.6			14	3.5	7	1.7			7	2.9	6	2.7					
rs1800610						Dominant						log-Additive							Dominant		log-Additive
CC	219	82.0	186	81.9	1.0	0.6-1.6	324	82.4	347	81.8	1.1	0.8-1.5	196	82.7	191	84.1	0.9	0.6-1.5	1.0	0.8-1.3	1.0
CT	44	16.5	37	16.3			64	16.3	69	16.3			39	16.5	33	14.5					
TT	4	1.5	4	1.8			5	1.3	8	1.9			2	0.8	3	1.3					
rs3093662						Dominant						Dominant							Dominant		log-Additive
AA	216	82.4	192	86.9	0.7	0.4-1.2	334	85.2	351	84.0	1.1	0.7-1.6	202	86.0	192	85.3	1.0	0.6-1.8	1.0	0.8-1.3	1.0
AG	44	16.8	27	12.2			56	14.3	67	16.0			32	13.6	30	13.3					
GG	2	0.8	2	0.9			2	0.5	0	0.0			1	0.4	3	1.3					
TNFRSF1A																					
P	0.43						0.03						0.31							0.01	
rs767455						log-Additive						log-Additive							log-Additive		log-Additive
TT	82	34.0	75	36.1	1.0	0.8-1.3	144	39.5	112	29.6	1.3	1.1-1.6	76	33.0	61	29.2	1.3	1.0-1.7	1.2	1.1-1.4	1.2
CT	115	47.7	87	41.8			159	43.6	194	51.3			125	54.3	107	51.2					
CC	44	18.3	46	22.1			62	17.0	72	19.0			29	12.6	41	19.6					
rs4149578						Dominant						Dominant							Dominant		log-Additive
GG	202	78.0	184	83.3	0.7	0.5-1.1	314	80.3	341	82.8	0.8	0.6-1.2	189	79.7	187	84.6	0.7	0.4-1.2	0.8	0.6-1.0	0.8
AG	55	21.2	35	15.8			73	18.7	67	16.3			48	20.3	32	14.5					
AA	2	0.8	2	0.9			4	1.0	4	1.0			0	0.0	2	0.9			log-Additive		log-Additive
rs1860545						log-Additive						log-Additive							log-Additive		log-Additive

	Age 34-44						Age 45-64						Age 65-74						Overall		
	Control (n = 271)			Case (n = 228)			Control (n = 396)			Case (n = 426)			Control (n = 239)			Case (n = 228)			OR		
	n	%		n	%		n	%		n	%		n	%		n	%		OR	95% CI	OR
GG	87	32.8		86	38.6	0.9	159	40.5		142	34.0	1.2	86	36.4		78	34.5		1.2	0.9-1.6	1.1
AG	138	52.1		97	43.5		177	45.0		209	50.0		123	52.1		108	47.8				
AA	40	15.1		40	17.9		57	14.5		67	16.0		27	11.4		40	17.7				
rs4149584																					
GG	253	95.5		217	96.0	0.9	381	96.9		405	95.5	1.4	225	95.7		209	93.3		1.6	0.7-3.7	1.3
AG	12	4.5		9	4.0		12	3.1		18	4.2		10	4.3		15	6.7				
AA	0	0.0		0	0.0		0	0.0		1	0.2		0	0.0		0	0.0				

OR (odds ratio) adjusted for age (continuous) in each age group, and the overall OR was adjusted for age, study, and their interaction.

* Significant result, any 1.0 confidence intervals with an asterisk indicate are due to rounding. The *P* value is for the *P*_{min} permutation test for each gene

Table 4

Risk of ductal or luminal breast cancer associated with SNPs in IL6, IL6R, TNF region (TNF and LTA), and TNFRSF1A across studies

	Control (n = 906)			Ductal (n = 640)			OR	Luminal (n = 679)			OR	95% CI	
	n	%	n	n	%	%		n	%	n		%	
IL6													
rs12700386							log-Additive				log-Additive		
CC	576	64.4	400	63.9			1.0	428	64.6		1.0	0.9–1.2	
CG	284	31.8	207	33.1				216	32.6				0.9–1.2
GG	34	3.8	19	3.0				19	2.9				
rs2069825							log-Additive				log-Additive		
CTCT	315	35.0	233	36.8			0.9	232	34.6		1.0	0.8–1.1	0.9–1.1
–CT	434	48.2	297	46.9				327	48.8				
–	151	16.8	103	16.3				111	16.6				
rs2069827							Dominant				log-Additive		
GG	738	82.0	518	81.8			1.0	547	81.3		1.0	0.8–1.3	0.8–1.3
GT	150	16.7	111	17.5				120	17.8				
TT	12	1.3	4	0.6				6	0.9				
rs1800795							log-Additive				log-Additive		
GG	299	33.2	214	33.6			1.0	215	31.8		1.0	0.8–1.1	0.9–1.2
CG	439	48.8	309	48.6				341	50.4				
CC	162	18.0	113	17.8				120	17.8				
rs2069837							Dominant				Dominant		
AA	705	84.3	487	83.5			1.1	525	84.7		1.0	0.8–1.5	0.8–1.4
AG	129	15.4	93	16.0				91	14.7				
GG	2	0.2	3	0.5				4	0.6				
rs2069840							log-Additive				log-Additive		
CC	383	42.6	275	43.2			1.0	294	43.7		1.0	0.9–1.2	0.9–1.2
CG	414	46.1	282	44.3				298	44.3				
GG	102	11.3	80	12.6				81	12.0				
rs1554606							log-Additive				log-Additive		
GG	271	30.3	196	31.7			1.0	198	30.0		1.0	0.8–1.1	0.9–1.2
GT	448	50.2	305	49.4				335	50.8				

	Control (<i>n</i> = 906)			Ductal (<i>n</i> = 640)			OR	95% CI			OR	95% CI		
	n	%		n	%			n	%			n	%	
TT	174	19.5		117	18.9			127	19.2					
rs2069849							log-Additive				log-Additive			
CC	836	94.9		590	95.8		0.9				0.9			0.6–1.5
CT	45	5.1		26	4.2									
rs2069861							log-Additive				log-Additive			
CC	682	78.9		479	78.9		1.0				1.0			0.8–1.3
CT	172	19.9		121	19.9									
TT	10	1.2		7	1.2									
IL6R														
rs1386821							log-Additive				log-Additive			
AA	571	64.5		401	64.5		1.0				1.1			0.9–1.3
AC	282	31.9		195	31.4									
CC	32	3.6		26	4.2									
rs4509570							log-Additive				log-Additive			
CC	508	57.0		367	58.9		1.0				1.0			0.8–1.2
CG	345	38.7		216	34.7									
GG	39	4.4		40	6.4									
rs8192284							log-Additive				log-Additive			
AA	327	36.4		245	38.6		1.0				1.0			0.9–1.2
AC	449	50.0		285	44.9									
CC	122	13.6		105	16.5									
TNF and LTA														
rs2009658							log-Additive				log-Additive			
CC	654	72.7		435	68.2		1.3				1.3			1.1–1.6
CG	229	25.4		183	28.7									
GG	17	1.9		20	3.1									
rs2229094							log-Additive				log-Additive			
TT	475	54.8		320	51.7		1.2				1.1			1.0–1.4
CT	334	38.6		245	39.6									
CC	57	6.6		54	8.7									

	Control (n = 906)			Ductal (n = 640)			OR	Luminal (n = 679)			OR	95% CI		
	n	%		n	%			n	%			n	%	
rs1800629							log-Additive				log-Additive			
GG	613	68.1		430	68.3		1.0				1.0			0.8–1.2
AG	264	29.3		187	29.7									
AA	23	2.6		13	2.1									
rs1800610							log-Additive				log-Additive			
CC	739	82.4		526	82.7		1.0				1.1			0.8–1.4
CT	147	16.4		104	16.4									
TT	11	1.2		6	0.9									
rs3093662							Dominant				Dominant			
AA	752	84.6		538	85.8		0.9				1.0			0.8–1.4
AG	132	14.8		85	13.6									
GG	5	0.6		4	0.6									
TNFRSF1A														
rs767455							log-Additive				log-Additive			
TT	302	36.1		179	30.8		1.3				1.2			1.0–1.4
CT	399	47.7		289	49.7									
CC	135	16.1		113	19.4									
rs4149578							log-Additive				log-Additive			
GG	705	79.5		515	82.4		0.9				0.8			0.6–1.0
AG	176	19.8		103	16.5									
AA	6	0.7		7	1.1									
rs1860545							log-Additive				log-Additive			
GG	332	37.1		225	35.7		1.1				1.1			0.9–1.3
AG	438	49.0		305	48.3									
AA	124	13.9		101	16.0									
rs4149584							Dominant				Dominant			
GG	859	96.2		598	94.5		1.5				1.3			0.8–2.2
AG	34	3.8		35	5.5									
AA	0	0.0		0	0.0									