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Cytokine and cytokine receptor genes of adaptive immune response are differentially associated with breast cancer risk in American women of African and European ancestry

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Abstract

Disparities in breast cancer biology are evident between American women of African ancestry (AA) and European ancestry (EA), and may be due, in part, to differences in immune function. To assess the potential role of constitutional host immunity on breast carcinogenesis, we tested associations between breast cancer risk and 47 single nucleotide polymorphisms (SNPs) in 26 cytokine-related genes of the adaptive immune system using 650 EA (n=335 cases) and 864 AA (n=458 cases) women from the Women's Circle of Health Study (WCHS). With additional participant accrual to the WCHS, promising SNPs from the initial analysis were evaluated in a larger sample size (1307 EAs and 1365 AAs). Multivariate logistic regression found SNPs in genes important for T helper type 1 (Th1) immunity (*IFNGR2* rs1059293, *IL15RA* rs2296135, *LTA* rs1041981), Th2 immunity (*IL4R* rs1801275), and T regulatory cell-mediated immunosuppression (*TGFB1* rs1800469), associated with breast cancer risk, mainly among AAs. The combined effect of these five SNPs was highly significant among AAs (*P*-trend=0.0005). When stratified by estrogen receptor (ER) status, *LTA* rs1041981 was associated with ER positive breast cancers among EAs and marginally among AAs. Among AA women only, *IL15* rs10833 and *IL15RA* rs2296135 were associated with ER positive tumors, and *IL12RB1* rs375947, *IL15* rs10833 and *TGFB1* rs1800469 were associated with ER negative tumors. Our study systematically identified genetic variants in the adaptive immune response pathway associated with breast cancer risk, which appears to differ by ancestry groups, menopausal status and ER status.

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Keywords

Breast Cancer; Estrogen Receptor; African-American; Cytokine; Adaptive Immune Response; Disparity

Introduction

In 2012, approximately 226,870 new cases of breast cancer and 39,510 breast cancer deaths were expected to occur among US women [1]. Breast cancer is a heterogeneous disease with numerous genetic, molecular and cellular characteristics. Disparities in breast cancer biology are evident between American women of African ancestry (AA) and those of European descent (EA). AA women are more likely than EA women to be diagnosed with breast cancer before age 50, and to have tumors with more aggressive features, such as negative estrogen receptor (ER) status [2]. Such tumors cannot be treated with anti-estrogen therapy and often result in poorer clinical outcomes[3]. Although the mechanisms underlying such disparities are yet unknown, it has been reported that there is a similarly high proportion of ER negative breast cancers in Africa [4, 5]. This indicates that genetic factors related to African ancestry may, in part, account for the early-onset and aggressiveness of breast cancers among AA women.

Immune response pathways have been associated with the development of cancer [6-8]. Acute tumor-directed immune responses involving cytolytic T cells, type 1 helper T (Th1) cells and natural killer (NK) cells appear to prevent tumor development, whereas chronic activation of humoral immunity and Th2 polarized responses are likely to promote tumor development. T-regulatory (Treg) cells are considered to promote tumor progression by limiting anti-tumor immunity[7]. Over millennia of evolution in Africa, indigenous populations adapted immune profiles to withstand endemic infectious disease, which may modify breast cancer risk. Such immune profiles consist of both the non-specific innate arm that protects against a wide variety of pathogens by activating inflammatory responses, and the specific adaptive arm that targets specific families of pathogens such as protozoa and helminthes [9, 10].

Cytokines are crucial players regulating host immune responses and are important constituents of the tumor microenvironment[7]. Circulating cytokines levels as well as single nucleotide polymorphisms in genes coding for cytokines have both been associated with the stage and progression of breast cancer[6, 11-15]. However, most studies have focused on a small number of cytokines within the innate immune response pathway, including *IL1* (interleukin 1), *IL6*, *IL8* and *TNFα* (tumor necrosis factor α), and have not included AA women. Cytokines involved with the adaptive immune response have not been defined with respect to breast cancer risk despite their importance for cancer control, and their potential to differ between EA and AAs due to disparate evolutionary pressures [16].

In a large case-control study, we systematically examined associations between genetic variants in cytokine and cytokine receptor genes of the adaptive immune response pathway and risk of breast cancer in AA and EA women, including associations by menopausal and ER status.

Materials and Methods

Study Participants

The Women's Circle of Health Study (WCHS), a case-control study designed to evaluate risk factors for aggressive breast cancer in AA women, was conducted in the metropolitan New York City area and seven counties in New Jersey, and has been previously described in detail[17, 18]. Eligible participants included English-speaking AA and EA women ages 20 to 75 years, with no previous history of cancer other than non-melanoma skin cancer, who were diagnosed with primary, histologically confirmed breast cancer. Controls without a history of any cancer diagnosis other than non-melanoma skin cancer were identified by random-digit dialing (RDD) and matched to cases on race and 5-year age group. Controls were recruited and interviewed using the same standardized method and during the same time period as the cases at both sites. Our Stage I analysis involved data and samples from 650 EA (n=335 cases) and 864 AA (n=458 cases) women. With additional participant accrual in WCHS, our stage II analysis involved a total of 1307 EA (n=658 cases) and 1365 AA (n=621 cases) women (Suppl. Figure 1).

Data Collection

This study was approved by the Institutional Review Boards at Roswell Park Cancer Institute (RPCI), the Cancer Institute of New Jersey (CINJ), Mount Sinai School of Medicine (MSSM), and participating hospitals in New York. Informed consent was obtained from each participant. Permission to obtain pathology data, including ER status, and tumor tissue blocks was included in the informed consent form. In-depth in-person interviews were conducted to collect demographic information, medical history, family history of cancer, and information on lifestyle factors. Anthropometry measures and biospecimens were also collected during the interview. Formalin-fixed paraffin-embedded blocks and corresponding pathology reports from patients who signed the pathology and tissue release consent form were retrieved from hospitals at which patients were diagnosed. Information on ER status was available for 254 EA cases (n=52 ER negative) and 332 AA cases (n=101 ER negative) in stage I analysis, and 468 EA cases (n=82 ER negative) and 473 AA cases (n=150 ER negative) in the entire dataset.

Sample Collection and Genotyping

Initially, blood samples were collected from study participants. We later transitioned to non-invasive collection of saliva for DNA collection. Genomic DNA was extracted in batches from whole blood using the FlexiGene DNA protocol (Qiagen Inc, Valencia, CA, US) and from saliva using the Oragene protocol (DNA Genotek Inc., Ottawa, ON, Canada) following the manufacturers' instructions. Quality and quantity of purified DNA were evaluated using Nanodrop UV-spectrometer (Thermo Fisher Scientific InC., Wilminton, DE, US) and PicoGreen–based fluorometric assays (Invitrogen Inc., Carslbad, CA, US). DNA samples were stored at -80°C until analysis.

We included in our analysis all major cytokines and cytokine receptors of the adaptive immune response pathway, including interleukins (IL), chemokines, interferons (IFN), Lymphotoxin alpha (LTA) and transforming growth factor beta (TGFβ). We then surveyed

the Human Genome Epidemiology (HuGE) Navigator for the selected genes to identify SNPs within these genes that were previously associated with risk of any cancer or cancer outcome, with a focus on SNPs that were previously shown to be functional[19]. In stage I of the study, 49 SNPs in 26 genes were included in our analyses. Genomic DNA was plated and genotyped at the Genomics Core Facility at RPCI using MassARRAY technology and iPLEX Gold Assay (Sequenom Inc., San Diego, CA, US). Five percent duplicates and two sets of in-house trio samples of European and African ancestry were included for quality control purposes. The concordance among blind duplicate pairs was >99.9%. The average successful genotyping rate for each sample and SNP was 95.94%. Samples or SNPs with call rate <90% were excluded, as well as monomorphic SNPs or SNPs with an MAF<5% in both AA and EA populations. Clustering plots of SNPs that were significant in the statistical analysis were manually re-inspected post-hoc to ensure that the calls were robust. Accruals in WCHS continued after this initial genotyping effort. Stage II of the study included significant SNPs (p or p for trend ≤ 0.05) from our stage I analysis. Five SNPs, i.e. *IL15* rs10833, *IL15RA* rs2296135, *LTA* rs1041981, *LTA* rs746868, and *IL4R* rs1801275, were genotyped in the larger WCHS sample set using the Illumina GoldenGate assay (Illumina Inc., San Diego, CA). Three significant SNPs, however, i.e. *TGFB1* rs1800469, *IL12RB1* rs375947 and *IFNGR2* rs1059293, were not genotyped due to multiplexing issues using the Illumina GoldenGate platform. To account for the potential inaccuracy of self-reported race/ethnicity and to assess ancestry quantitatively, all DNA samples were also genotyped for a panel of 100 ancestry informative markers (AIMs) using the GoldenGate Assay[20].

Statistical Analysis

All analyses were conducted using SAS 9.2 (SAS Institute, Cary CA) separately for EA and AA women, according to self-reported race. Descriptive variables were compared between cases and controls using Chi-square tests for categorical variables and Wilcoxon rank-sum test for continuous variables. Proportions of European and African ancestry in individual EA and AA women were estimated quantitatively based on AIM genotypes using the Bayesian Markov Chain Monte Carlo clustering algorithm implemented in STRUCTURE 2.3[21]. Since the sum of two ancestral proportions in each individual is always one, we used only the proportion of European Ancestry in all analyses. For each SNP, Hardy-Weinberg equilibrium was assessed among controls. Two SNPs that deviated from Hardy-Weinberg disequilibrium, *TGFB1* rs11466314 and *IFNG* rs2069709, were excluded. Therefore, 47 SNPs were included in the final analyses (Suppl. Table1). Genotype frequencies of each SNP were compared between EA and AA controls using Chi-square test or Fisher's Exact test where appropriate. To compare allele frequencies obtained with our study to those previously observed, frequencies for Caucasians (CEU), African Americans of the American Southwest (ASW) and Yoruban in Ibadan, Nigeria (YRI) were obtained from HapMap release #28 (phase 1, 2 and 3 merged). Odds ratios (ORs) and 95% confidence intervals (CIs) for each SNP were derived from multivariable logistic regression models with adjustment for accepted risk factors for breast cancer: age at diagnosis (continuous), body mass index (continuous), education (less than high school, high school, college and graduate school), family history of breast cancer (yes, no), history of benign breast disease (yes, no), proportion of European ancestry (continuous), smoking (current, former, never), number of full pregnancy (continuous), breast feeding (yes, no, nulliparous), HRT(yes, no) and

menopausal status (premenopausal, postmenopausal). The fully adjusted model would control for any potential confounding caused by associations between these common risk factors for breast cancer and alterations in immune function. None of the individual covariates modified regression coefficients by greater than 10%. Using the fully adjusted model based on risk factors rather than a more parsimonious model based on statistical significance facilitates comparison of results across studies. *P* values were calculated assuming a co-dominant model. *P* for trend was calculated by coding SNPs as 0, 1, 2 and testing whether there was a linear dose-response effect of the variant alleles when it was analyzed as an ordinal variable (*p*-trend). *P* values from the co-dominant model were adjusted for multiple comparisons using a modified false discovery rate (FDR) method[22]. Since the genotype frequencies of rare homozygotes were low for some SNPs, OR and 95% CIs were also calculated for each significant SNP after collapsing the rare homozygotes and heterozygotes. OR and 95% CIs were further calculated for each SNP after stratification by menopausal status. For significant SNPs, potential interactions between a SNP and self-reported race was tested by including a self-reported race*SNP term in the logistic regression model without estimates of ancestry (P interaction). Using the method described above, we also tested whether a SNP specifically contributed to the risk of ER negative or ER positive breast cancers using cases by their ER status and all controls.

Results

Participant Characteristics

Participant characteristics are shown in Table 1. Among self-reported EAs and AAs, the mean proportion of European Ancestry was 97-98% among EAs and 14% in AAs, respectively. Among EAs, cases were significantly more likely than controls to have a family history of breast cancer in a first-degree relative, as were AAs, although differences were not statistically significant in the latter. Among both EA and AA women, cases were less likely to have attended college and graduate school and more likely to have a history of benign breast disease. Among AAs, current smokers were more common among controls than cases. Cases did not differ significantly from controls in age, BMI, number of full-term pregnancy, menopausal status, breast feeding, and the use of hormone replacement therapy (HRT) in either group.

Breast Cancer risk in EA and AA women

For 41 of 47 SNPs analyzed, genotype frequencies differed significantly between AA and EA controls (Suppl. Table 1, $p < 0.05$ after correction for multiple testing). Six SNPs, i.e. *IL4* rs2243250, *IL4R* rs1801275, *IL10RA* rs9610, *IL13* rs1295686, *IFNGR2* (interferon gamma receptor 2) rs1058293 and *LTA* (Lymphotoxin alpha) rs1041981, had ‘flipped’ genotypes, where the minor genotype among EA controls was the major genotype among AA controls (Suppl. Table 1). Genotype frequencies obtained from HapMap for each ancestry were very similar to those in our study (Suppl. Table 1). Because of notable differences in allele distributions between EA and AA women, all analyses were stratified by self-reported race.

ORs and 95% CIs for associations between overall breast cancer risk and all 47 analyzed SNPs are shown in Suppl. Table 2. Significant associations with a *p* or *p* for trend < 0.05 before correction for multiple testing are shown in Table 2. The 'A' allele of Th1-related SNP, *IL15RA* rs2296135, was associated with an increased risk among AA women (Table 2, AA vs CC, OR=1.93, 95% CI= 1.11-3.32). The 'T' allele of another Th1-related SNP, *IFNGR2* rs1059293, was also associated with an increased risk among AA women (CT/TT vs CC, OR=1.90, 95% CI= 1.06-3.82). *LTA* rs1041981 was associated with a decreased risk of breast cancer among EA women (CA/AA vs CC, OR=0.71, 95% CI=0.50-0.99), and with non-significant OR estimates below unity among AAs. For Th2-related SNP *IL4R* rs1801275, the 'G' allele was associated with an increased risk of breast cancer among AA women (GG vs AA, OR=1.78, 95% CI=1.03-3.07). A SNP involved in Treg immunity, *TGFB1* rs1800469 (transforming growth factor beta 1), was associated with decreased risk of breast cancer among AA women (CT/TT vs CC, OR=0.74, 95% CI=0.59-0.97), although primarily among postmenopausal AA women following stratification by menopausal status (CT/TT vs CC, OR=0.58, 95% CI=0.38-0.87) (Suppl. Table 2).

Cumulative effects of SNPs associated with breast cancer risk among AA women

Five of the SNPs shown in Table 2, i.e. *IL15RA* rs2296135, *IFNGR2* rs1059293, *LTA* rs1041981, *IL4R* rs1801275 and *TGFB1* rs1800469, were associated with breast cancer risk among AA women. Since none of these associations were statistically significant after correction for multiple testing (data not shown), we examined the potential cumulative effects of these five SNPs among AA women. Based on odds ratio estimates shown in Table 2, we decided qualitatively whether a genotype was 'protective', which was broadly interpreted to include genotypes that were not associated with increased breast cancer risk. For example, the IL15RA rs2296135 'AA' genotype was associated with increased breast cancer risk compared to the referent 'CC' genotype (OR=1.93), with the 'CA' genotype (OR=0.93) being of similar risk compared to 'CC'. Therefore both 'CA' and 'CC' were considered 'protective' compared to 'AA' (Suppl. Table 3). The following eight genotypes were considered 'protective' among AA women: 'CC' and 'CA' for *IL15RA* rs2296135, 'CC' for *IFNGR2* rs1059293, 'CA' and 'AA' for *LTA* rs1041981, 'AA' for *IL4R* rs1801275 and 'CT' and 'TT' for *TGFB1* rs1800469. We found a highly significant inverse association between the number of 'protective' genotypes and breast cancer risk among AA women (Table 3, *p* for trend=0.0005). Compared to the referent group who carried zero or one 'protective' genotype, AA women who carried three or more 'protective' genotypes both showed ∼50% reduced breast cancer risk. When stratified by menopausal status, inverse associations were evident among both premenopausal (*p* for trend=0.08) and postmenopausal AA women (*p* for trend=0.0008). We also tested the combined effects of the five SNPs among EA women. Only a few EA women carried zero or one 'protective' genotype (postmenopausal: 15 cases, 7 controls; premenopausal: 19 cases, 13 controls), which was more commonly observed among AAs. Due to markedly differential genotype distributions by race, we used different referent groups for EA and AA women, as detailed in the footnote of Table 3. There were no associations between number of 'protective' genotypes and breast cancer risk among EA women (Table 3, *p* for trend=0.65).

Risk for Breast Cancer by ER status

We next examined if SNPs of the adaptive immune response pathway are differentially associated with risk by ER status among EA and AA women. ORs and 95% CIs for all findings are shown in Suppl. Table 4, with significant findings shown in Table 4. Th1-related SNP, *IL12RB1* rs375947, was associated with decreased risk of ER negative breast cancer among AA women (AG/GG vs AA, OR=0.61, 95% CI 0.38-0.98). *IL15* rs10833 was associated with decreased risk of ER positive breast cancers (Table 4, GA/AA vs GG, OR=0.60, 95% CI=0.39-0.92), and increased risk of ER negative breast cancer among AA women (AA vs GG, OR=4.98, 95%=CI 1.54-16.08), although estimates of the latter are based on small numbers. This potential association was supported by findings from a case-only analysis modeling the OR of being diagnosed with ER negative breast cancer versus ER positive cancer: AA women carrying the *IL15* rs10833 'A' allele were over two-fold more likely to be diagnosed with ER-negative than ER positive disease (GA/AA vs GG, OR=2.19, 95% CI=1.24-3.87, p=0.007, data not shown). In addition, *IL15RA* rs2296135 was associated with increased risk of ER positive breast cancer among AA women (AA vs CC, OR=1.93, 95% CI=1.05-3.67). Another Th1-related SNP, *LTA* rs1041981, was associated with decreased risk of ER positive cancers among both EA women (CA/AA vs CC, OR=0.60, 95% CI: 0.40-0.89) and AA women (AA vs CC, OR=0.64, 95% CI: 0.40-1.05), although results were not significant in the latter group. Another SNP in the *LTA* gene, rs746868, was associated with increased risk of ER positive breast cancer among EA women (GG vs CC, OR=2.02, 95% CI=1.17-3.47) and increased risk of ER negative breast cancer among AA women (GG vs CC, OR=2.69, 95% CI=1.14-6.32). Among Treg-related SNPs, only *TGFB1* rs1800469 among AAs was found to be inversely associated with ER negative breast cancers (CT/TT vs CC, OR=0.64, 95% CI=0.40-1.00).

Replication Studies

As noted in Methods, accruals in WCHS continued after our initial genotyping effort (Suppl. Figure 1). Promising SNPs arising from these initial analyses were genotyped using a larger sample size, with 486 additional cases and 672 additional controls (see participant characteristics in Suppl. Table 5). As shown in Table 5, five SNPs that were associated with overall breast cancer risk or with either ER-negative or ER-positive breast cancers, i.e. *IL15* rs10833, *IL15RA* rs2296135, *LTA* rs1041981, *LTA* rs746868, and *IL4R* rs1801275, were genotyped in the larger sample set. Most associations were replicated in the larger dataset, although some relationships were attenuated. *LTA* rs1041981 was most strongly replicated and remained inversely associated with breast cancer risk, primarily among premenopausal EA women. The SNP was also inversely associated with ER positive breast cancers among both EA and AA women, although not significant among the latter group. *IL4R* rs1801275 remained associated with an overall increased risk for breast cancer among AA women, although the p-value was marginal and not significant. *IL15* rs10833 continued to be associated with an increased risk of ER negative breast cancer and a borderline decreased risk of ER positive breast cancers among AA women. Findings with *IL15RA* rs2296135 were slightly attenuated showing non-significant increased risks for both overall and ER positive breast cancers among AA women. Associations between breast cancer risk and *LTA* rs746868 by ER status did not replicate and therefore might not be true associations (Table

5). Unfortunately, we were not able to test other promising SNPs in the larger sample set, such as *TGFB1* rs1800469, IL12RB1 *rs375947* and *IFNGR2* rs1059293, due to multiplexing issues using the Illumina GoldenGate platform.

Discussion

Studies in monozygotic or dizygotic twins suggest a genetic component to breast cancer[23], and genome-wide association (GWA) studies have provided some evidence for a role of common variants[24]. Detecting common variants in candidate pathway genes provides a useful approach to complement GWA studies in defining populations at high risk. In this study, we systematically examined the association between cytokine and cytokine receptor genes of the adaptive immune response pathways and risk of breast cancer in EA and AA women. To our knowledge, this is the first study to examine relationships between the adaptive immune response pathway and breast cancer risk, and also the first which includes a large number of AA women.

In general, we found that genotype frequencies in the cytokine and cytokine receptor genes varied markedly between AAs and EAs. Among the 47 SNPs in 26 genes examined, the genotype frequencies of 41 SNPs (87%) differed significantly between EA and AA controls. We also found different relationships between SNPs and breast cancer risk between AAs and EAs, for both overall breast cancer risk and after stratification by menopausal or ER status. Although the SNPs identified were not significant after correction for multiple comparisons, the combined effect of the five SNPs associated with overall risk of breast cancer among AA women, i.e. *IL15RA* rs2296135, *IFNGR2* rs1059293, *LTA* rs1041981, *IL4R* rs1801275 and *TGFB1* rs1800469, was highly significant (Table 3, *P* for trend=0.0005). It is possible that a concert of cytokine and cytokine receptor genes, each with moderate impact, affect breast cancer risk synergistically[25]. Most of the associations observed were replicated in a second stage with a larger sample size, despite slight attenuation of some findings, reducing the probability that the majority of our findings are spurious (Table 5).

Associations between cytokine genes of the adaptive immune pathway and cancer risk have not been widely studied, particularly for breast cancer. *IL15RA* rs2296135 and *IFNGR2* rs1059293 were not associated with non-Hodgkin lymphoma among 1433 EA women[26], but have not been examined with respect to breast cancer risk. The CA and AA genotypes of *LTA* rs1041981 have been associated with a decreased risk of cancer among males (OR=0.72, 95% CI=0.53-0.99), mainly for stomach, lung and colon cancer[27]. Our findings show that *LTA* rs1041981 is associated with breast cancer in the same direction. Similar to our findings, a study in white Caucasians from UK (n= 775 cases and 767 controls) did not detect any association between *IL4R* rs1801275 and risk or severity of breast cancer[28]. It points to the possibility that this SNP may be associated with breast cancer only among AA women as indicated by our results. *TGFB1* rs1982037 were shown to be associated with breast cancer risk in Caucasian women but not in Asians or Africans[29]. We did not identify any association of breast cancer with this SNP in either EA or AA women, probably due to small sample number. Previous meta-analysis did not identify an association between breast cancer risk and *TGFB1* rs1800469[30]. However, Niu. *et. al.* suggested that only the initial

studies showed opposite effects to other studies in the meta-analysis. They reported a moderate reduced risk for breast cancer associated with the T allele after removing the initial study (OR=0.94, 95% CI= 0.88-1.00)[30], which is in the same direction as we have observed.

The adaptive immune system, as compared to the nonspecific innate immune system, is comprised of highly specialized B and T lymphocytes that eliminate or prevent specific types of pathogenic growth. Presence of B and T lymphocytes has been frequently observed in breast tumors[7]. The presence of a high percentage of CD4+ T helper cells at primary tumor sites or axillary lymph nodes correlates with disease progression [31, 32]. There are two types of CD4+ T helper cells, designated as type 1 helper T (Th1) cell and type 2 helper T (Th2) cell. Following an activating stimulus, Th1-polarized CD4+ cells produce INFγ, TNFα (tumor necrosis factor alpha) and IL2 to activate the cytotoxic activities of CD8+ T lymphocytes, M1 macrophages, and natural killer (NK) cells, which are important in mounting an effective anti-tumor immune response. More recently, it has been found that Th1 responses may also activate innate immune cells that contribute to chronic inflammation, supporting tumor development [33].

Th2 responses are characterized by the release of IL4, IL5, IL6, IL10 and IL13, which results in suppression of T-cell-mediated cytotoxicity while enhancing B-cell-mediated humoral immunity[34]. Clinical data have shown that a decreased ratio of circulating Th1 cells to circulating Th2 cells and their corresponding cytokines are associated with an increased risk for several types of cancer[35]. Moreover, increased numbers of Treg cells, with TGFβ and IL4 recognized as key regulators in the generation of Tregs from CD4+ CD25- precursors[36], have been associated with poor survival in many solid tumor cancers, including breast cancer[37].

Differences in distributions of SNPs related to immune functions between AAs and EAs, and differing associations with breast cancer risk, may reflect adaptation over millennia. Endemic infectious diseases in tropical Africa fundamentally shaped the immune systems of populations at the genetic level, selecting for immunity that preserves well-being. Malaria is considered to have the most profound influence on the selection of genetic variation in the adaptive immune response pathway in African populations [9]. Many variants in the Th1-related cytokine genes, such as *IL12B* and *IFNG*, likely occurred in African populations either because they decreased the severity of malaria or imparted some level of resistance against the disease[9, 38].

We identified several important SNPs within Th1-related cytokine and cytokine receptor genes associated with breast cancer risk among AA women, i.e. *IL15*, *IL15RA* and *IFNGR2*. IL15 is a cytokine that shares structural similarity with IL2 and exhibits broad activities, along with its receptor IL15Rα. It regulates activation and proliferation B, T and natural killer (NK) cells, enhances the cytolytic activity of CD8+ T cells and provides survival signals for memory T cells in the absence of antigen. Most importantly, IL15 does not stimulate immunosuppressive Tregs[39]. We found that *IL15* rs10833 was associated with decreased risk of ER positive breast cancers and increased risk of ER negative breast cancers among AA women (Table 4). Such effects were confirmed by case-case analysis (data not shown) and replication with a larger sample size (Table 5). We also found that

IL15RA rs2296135 was associated with increased risk of overall and ER positive breast cancers among AA women (Tables 2, 4 &5). Recently, several clinical trials have been initiated to boost IL15 activity in the treatment of melanoma and pediatric cancers[40]. These studies, if successful, would broaden the applications of our finding in a clinical setting to develop novel preventive or treatment strategies for breast cancer, especially in AA women, utilizing existing IL15 immunotherapy. The different associations discovered when stratified by ER status indicated an interesting relationship between cytokine genes, estrogen function and breast cancer risk. However, it is worth noting that the number of ER negative cases was limited in our study and some associations were marginal. Future studies with larger sample sizes are required to confirm our results.

IFNGR2 encodes IFNγR2, which is part of the IFNγ receptor complex. Besides Th1 polarization, IFNγ and other pro-inflammatory cytokines induced by IL12 also have a direct toxic effect on tumor cells and activate anti-angiogenic mechanisms[41]. Patients with breast tumors containing high amounts of *IFNG* mRNA exhibit prolonged recurrence free survival [42]. Previous studies showed that IFNγR2 play a role in apoptosis regulation as a signal transduction molecule of IFNγ[43]. We observed a 1.9-fold increased breast cancer risk among AA women who carry the *IFNGR2* rs1059293 'T' allele (Table 2), which further supports a function for *IFNGR2* in breast cancer.

Differences in immune function between EA and AA populations were also, in part, shaped by endemic exposure to parasitic worms (helminths) in Africa[10, 44]. Exposure to helminths primarily elicits a Th2 immune response, broadly encompassing the activation of eosinophils, basophils and mast cells, the production of immunoglobulin E (IgE), and T cell proliferation with secretion of IL4, IL5, IL9, IL10, and IL13. Studies in West Africa and in Asia provide support for the selection of genetic variants in Th2 immunity by helminths, since individuals carrying these variants would have diminished intensity of infection with helminths and better health [45]. The first genome scan for intensity of infection in Brazil identified the chromosomal region 5q31-q33 as a risk locus, which was later confirmed in a Senegalese population. This region contains several Th2-related genes, i.e. *IL3*, *IL4*, *IL5*, *IL9*, and *IL13*[46]. Persistent humoral immune responses aided by Th2 cytokines can activate innate immune cells that contribute to chronic inflammation and suppress anti-tumor immune responses[35]. Upregulation of Th2 cytokines has been linked to worse prognosis in a number of cancers, although its role in breast cancer has not been previously studied[47, 48]. As discussed above, IL4 is a central effector of Th2 responses, and IL4R has been found significantly expressed in breast cancers[49]. In our study, *IL4R* rs1801275 was associated with overall breast cancer risk among AA women (Table 2, Table 5).

We also found that a SNP in the central Treg gene, *TGFB1* rs1800469, was associated with risk of overall breast cancer, as well as ER negative disease among AA women. *TGFB1* encodes transforming growth factor beta (TGFβ), a pleiotropic cytokine produced by Treg cells and well-known for its dual role in tumor development. At early stages of carcinogenesis, TGFβ acts as a tumor suppressor through its anti-inflammatory activity and growth inhibition of epithelial proliferation; whereas at later stages it acts as a tumor promoter by inducing angiogenesis and blocking tumor-specific CD8+ T cells[50]. Studies on the role of *TGFB1* rs1800469 in breast cancer susceptibility have yielded inconsistent

results. However, as Niu et al suggested, variation between different populations may account for the inconsistency of findings across studies[30].

Our study has several strengths. We conducted in-person interviews to assure data accuracy. In-depth information on medical history, family history of cancer, hormone-related, and lifestyle factors were collected, allowing us to adjust for potential confounders. We included in the study a large number of cases and controls, both EA and AA, enabling us to stratify by ancestry group and address racial differences. Data on ER status was available for a large proportion of cases, so we were able to identify SNPs specifically associated with ER negative breast cancer. Importantly, we were able to systematically test a large number of cytokine and cytokine receptor genes within adaptive immune pathways and assess the potential role of underlying constitutional host immunity on breast carcinogenesis, while previous studies have been more focused on tumor-related immune changes.

We are aware that SNPs identified as related to breast cancer in our study may not be 'causal'. It is possible that they are in linkage disequilibrium with other SNPs that are functional, but were not tested in our study. None of the SNPs identified remained statistically significant after adjustment for multiple comparisons; however, the combined effects of the SNPs detected among AA women were highly significant (Table 3, *P* trend=0.0005). Our findings are supported by a strong biological rationale that the adaptive immune pathway may play an important role in breast cancer risk, particularly among AAs, as indicated by clinical, epidemiological, and evolutionary evidence.

In conclusion, our study revealed genetic variants within the host adaptive immune response pathway associated with breast cancer risk among EA and AA women. The role of constitutional immune function in the development of breast cancer appears to differ between EAs and AAs, and this is true after stratification by menopausal or ER status. Future work is warranted to validate these findings in a larger sample size, to assess whether adaptive immune pathways contribute to the development of early onset aggressive breast cancers, and to explore immunotherapy, such as IL15 therapy, which may be a promising strategy for prevention or treatment of aggressive breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What is new

This is the first study that specifically examines relationships between breast cancer risk among African American women and genetic variants in cytokine genes involved in adaptive immunity. Our findings suggest that host adaptive immunity plays a more prominent role in breast carcinogenesis among African Americans compared to European Americans, possibly due to evolutionary pressures rendered by endemic pathogens over millennia in Africa. It improves our understanding of breast cancer and opens possibilities to novel treatments.

Table 1

Participant characteristics in the Women's Circle of Health Study (WCHS).

Characteristics	European American			African American		
	Cases	Controls	p[a]	Cases	Controls	p[a]
N	335	315		458	406	
Age (y), mean (SD)	50.52 (8.37)	50.44 (8.31)	0.91	50.92 (10.10)	50.33 (8.89)	0.59
European Anstry, mean (SD)	0.97 (0.09)	0.98 (0.03)	0.002	0.14 (0.16)	0.14 (0.15)	0.28
Menopausal status, n (%)						
Premenopausal	186 (55.52)	167 (53.02)	0.52	242 (54.84)	196 (48.28)	0.18
Postmenopausal	149 (44.48)	148 (46.98)		216 (47.16)	2108 (51.72)	
BMI, Kg/m^2, mean (SD)	26.99 (6.31)	27.63 (7.39)	0.26	31.17 (6.76)	31.70 (7.71)	0.45
BMI, Kg/m^2						
<25	153 (45.67)	135 (42.86)	0.77	85 (18.56)	76 (18.72)	1.00
25-30	87 (25.97)	85 (26.98)		129 (28.17)	114 (28.08)	
>30	95 (28.36)	95 (30.16)		244 (53.28)	216 (53.20)	
History of Benign Breast Disease, n (%)						
No	193 (58.66)	208 (66.24)	0.05	312 (68.42)	311 (76.79)	0.006
Yes	136 (41.34)	106 (33.76)		144 (31.58)	94 (23.21)	
Family history of BrCa, n (%)						
No	247 (73.73)	265 (84.13)	0.001	392 (85.59)	358 (88.18)	0.26
Yes	88 (26.27)	50 (15.87)		66 (14.41)	48 (11.82)	
Education, n (%)						
Less than high school	7 (2.09)	4 (1.27)	0.0002	63(13.76)	47 (11.58)	0.05
High school	73 (21.79)	32 (10.16)		149 (32.53)	107 (26.35)	
College and graduate school	255 (76.12)	279 (88.57)		246 (53.71)	252 (62.07)	
Number of full-term preganancy, mean (SD)	1.47 (1.39)	1.66 (1.67)	0.11	2.17 (1.69)	2.07 (1.57)	0.65
Breast Feeding						
Nulliparous	108 (32.24)	86 (27.30)	0.31	80 (17.47)	70 (17.24)	0.97
No	72 (21.49)	66 (20.95)		170 (37.12)	154 (37.93)	
Yes	155 (46.27)	163 (51.75)		208 (45.41)	182 (44.83)	
Smoking status, n (%)						

Characteristics	European American			African American		
	Cases	Controls	p[a]	Cases	Controls	p[a]
Never Smoker	182 (54.33)	187 (59.37)	0.40	304 (66.38)	246 (60.59)	0.005
Former Smoker	118 (35.22)	96 (30.48)		103 (22.49)	83 (20.44)	
Current Smoker	35 (10.45)	32 (10.16)		51 (11.14)	77 (18.97)	
HRT, n (%)						
No	249 (74.33)	243 (77.14)	0.40	390 (85.90)	353 (86.95)	0.66
Yes	86 (25.67)	72 (22.86)		64 (14.10)	53 (13.05)	

Abbreviations: BMI, body mass index; BMIcat, categorical BMI; HRT, hormone replacement therapy.

[a]*P* values were calculated by Wilcoxon rank-sum test for continuous variables and by Chi-square test for categorical variables.

Table 2

Cytokine and cytokine receptor SNPs and risk of breast cancer in the Women's Circle of Health Study (WCHS).

Gene	SNP	Genotype	European American				African American				P interaction
			Case/Control	OR (95%CI) [a]	P	P-trend	Case/Control	OR (95%CI) [a]	P	P-trend	
Th1-Related											
IL15RA	rs2296135	CC	92/89	1.00	0.98	0.87	256/230	1.00	0.05	0.27	0.15
		CA	166/157	1.03 (0.71-1.51)			157/153	0.93 (0.68-1.23)			
		AA	70/65	1.04 (0.65-1.66)			41/21	**1.93 (1.11-3.32)**			
		CA/AA	236/222	1.03 (0.72-1.49)	0.85		198/174	1.02 (0.77-1.36)	0.87		
IFNGR2	rs1059293	CC	90/83	1.00	0.89	0.68	15/23	1.00	0.06	0.32	0.45
		CT	168/166	1.09 (0.74-1.61)			143/122	**1.95 (1.07-4.00)**			
		TT	70/64	1.10 (0.68-1.78)			296/259	**1.88 (1.03-3.81)**			
		CT/TT	238/230	1.10 (0.76-1.59)	0.63		439/381	**1.90 (1.06-3.82)**	0.05		
LTA	rs1041981	CC	159/133	1.00	0.11	0.11	121/95	1.00	0.23	0.10	0.36
		CA	124/143	0.68 (0.48-0.98)			229/202	0.90 (0.64-1.27)			
		AA	29/29	0.81 (0.45-1.46)			102/107	0.72 (0.48-1.06)			
		CA/AA	153/172	**0.71 (0.50-0.99)**	0.04		331/309	0.84 (0.61-1.16)	0.28		
Th2-Related											
IL4R	rs1801275	AA	215/215	1.00	0.45	0.62	28/42	1.00	0.09	0.03	0.76
		AG	100/83	1.23 (0.85-1.77)			191/178	1.45 (0.85-2.49)			
		GG	14/13	0.80 (0.34-1.90)			233/185	**1.78 (1.03-3.07)**			
		AG/GG	114/96	1.17 (0.82-1.66)	0.39		424/363	1.60 (0.95-2.69)	0.08		
Treg-Related											
TGFB1	rs1800469	CC	124/134	1	0.27	0.11	268/215	1.00	0.19	0.06	0.12
		CT	147/135	1.22 (0.85-1.73)			164/165	**0.75 (0.59-0.98)**			
		TT	56/43	1.47 (0.90-2.40)			22/25	0.72 (0.39-1.33)			
		CT/TT	203/178	1.28 (0.91-1.78)	0.15		186/190	**0.74 (0.59-0.97)**	0.04		

Abbreviations: OR, odds ratio; 95%CI, 95% confidence interval; P, *p* value calculated using a genotypic (co-dominant) model or when heterozygotes and rare homozygotes were combined ; P-trend, *p* value for genetic dose-response by coding genotypes as 0, 1, 2 according to the frequency of variant allele. Potential interactions between a SNP and self-reported race was tested by including a self-reported race*SNP term in the logistic regression model without estimates of ancestry (P interaction).

[a] Adjusted for age at diagnosis (continuous), body mass index (continuous), education (less than high school, high school, college and graduate school), family history of breast cancer (yes, no), history of benign breast disease (yes, no), proportion of European ancestry (continuous), smoking (current, former, never), number of full pregnancy (continuous), breast feeding (yes, no, nulliparous), HRT(yes, no) and menopausal status (premenopausal, postmenopausal).

Table 3

Combined effects of the five SNPs associated with breast cancer risk among AA women in the Women's Circle of Health Study (WCHS).

Total number of 'protective' genotypes[b]	All Women		Premenopasual		Postmenopasual	
	Ca/Co	OR (95%CI)[a]	Ca/Co	OR (95%CI)[a]	Ca/Co	OR (95%CI)[a]
EA Women						
0, 1 or 2[c]	111/99	1.00	65/48	1.00	46/51	1.00
3	117/127	0.81 (0.55-1.20)	67/68	0.68 (0.40-1.18)	50/59	1.02 (0.57-1.83)
4 or 5	82/77	0.93 (0.60-1.43)	45/42	0.78 (0.42-1.47)	37/35	1.14 (0.60-2.17)
P for linear trend		0.65		0.40		0.70
AA Women						
0 or 1[c]	80/48	1.00	42/26	1.00	38/22	1.00
2	228/183	0.72 (0.47-1.09)	122/93	0.70 (0.39-1.26)	106/90	0.77 (0.41-1.44)
3	125/154	0.48 (0.31-0.75)	62/67	0.51 (0.27-0.95)	59/87	0.41 (0.21-0.78)
4 or 5	15/17	0.47 (0.21-1.04)	10/8	0.79 (0.26-2.38)	5/9	0.28 (0.08-1.04)
P for linear trend		**0.0005**		0.08		**0.0008**

Abbreviations: OR, odds ratio; 95%CI, 95% confidence interval. P for linear trend, *p* value for dose-response by coding groups as 1, 2, 3, 4 according to the number of protective genotypes carried.

[a] Adjusted for age at diagnosis (continuous), body mass index (continuous), education (less than high school, high school, college and graduate school), family history of breast cancer (yes, no), history of benign breast disease (yes, no), proportion of European ancestry (continuous), smoking (current, former, never), number of full pregnancy (continuous), breast feeding (yes, no, nulliparous), HRT (yes, no) and menopausal status when not stratified by it (premenopausal, postmenopausal).

[b] Number of genotypes associated with decreased breast cancer risk in 5 genes as described in detail in the text.

[c] Referent groups for EA and AA women differed, because of the differing genotype distributions between EA and AA women. Among EAs, the referent group included women carrying ≤2 protective genotypes, compared to ≤1 protective genotypes in AAs, because there were too few EA women carrying ≤1 protective genotypes to serve as referent group (postmenopausal: 15 cases, 7 controls; premenopausal: 19 cases, 13 controls). If we use ≤2 protective genotypes as the referent group in AA women, the p values would remain largely unchanged (P-trend=0.002 for all AA women and 0.0009 for postmenopausal AA women, data not shown).

Table 4

Association between SNPs and risk of breast cancer by ER status in the Women's Circle of Health Study (WCHS).

Gene	SNP	Genotype	European American						African American					
			ER Positive			ER Negative			ER Positive			ER Negative		
			Case/Cont[a]	OR(95%CI)[b]	P	Case/Cont	OR(95%CI)[b]	P	Case/Cont	OR(95%CI)[b]	P	Case/Cont	OR(95%CI)[b]	P
Th1 Cytokin and Cytokine Receptors														
IL12RB1	**rs375947**	AA	92/146	1.00	0.90	30/146	1.00	0.19	120/205	1.00	0.75	60/205	1.00	0.11
		AG	87/138	1.09 (0.73-1.63)		18/138	0.63 (0.32-1.23)		95/170	1.02 (0.71-1.45)		32/170	0.58 (0.37-1.12)	
		GG	20/29	1.10 (0.56-2.17)		2/29	0.30 (0.06-1.59)		13/29	0.77 (0.37-1.58)		6/29	0.82 (0.31-2.15)	
		AG/GG	107/167	1.09 (0.75-1.6)	0.65	20/167	0.57 (0.30-1.10)	0.10	108/199	0.98 (0.7-1.37)	0.90	38/199	**0.61 (0.38-0.98)**	0.04
			P-trend=0.67			P-trend=0.07			P-trend=0.69			P-trend=0.10		
IL15	**rs10833**	GG	87/141	1.00	0.62	19/141	1.00	0.17	184/295	1.00	0.04	64/295	1.00	0.03
		GA	83/131	1.23 (0.81-1.85)		25/131	1.52 (0.76-3.05)		42/100	**0.64 (0.41-0.97)**		27/100	1.19 (0.70-2.01)	
		AA	20/28	1.07 (0.53-2.14)		2/28	0.38 (0.08-1.9)		1/8	0.18 (0.02-1.55)		6/8	**4.98 (1.54-16.08)**	
		GA/AA	103/159	1.20 (0.81-1.77)	0.37	27/159	1.26 (0.65-2.47)	0.49	43/108	**0.60 (0.39-0.92)**	0.02	33/108	1.40 (0.85-2.29)	0.19
			P-trend=0.52			P-trend=0.89			P-trend=0.01			P-trend=0.05		
IL15RA	**rs2296135**	CC	61/89	1.00	0.79	14/89	1.00	0.21	133/230	1.00	0.05	52/230	1.00	0.55
		CA	97/157	0.93 (0.6-1.43)		21/157	0.80 (0.37-1.74)		73/153	0.81 (0.56-1.17)		39/153	1.12 (0.69-1.82)	
		AA	40/65	0.83 (0.48-1.43)		15/65	1.60 (0.69-3.71)		22/21	**1.93 (1.05-3.67)**		8/21	1.65 (0.66-4.13)	
		CA/AA	137/222	0.90 (0.59-1.35)	0.60	36/222	1.03 (0.51-2.08)	0.94	95/174	0.94 (0.66-1.32)	0.71	47/174	1.18 (0.74-1.88)	0.48
			P-trend=0.50			P-trend=0.30			P-trend=0.54			P-trend=0.33		
LTA	**rs1041981**	CC	100/133	1.00	0.04	25/133	1.00	0.18	64/95	1.00	0.18	24/95	1.00	0.93
		CA	70/143	**0.61 (0.40-0.92)**		18/143	0.54 (0.27-1.10)		114/202	0.90 (0.60-1.35)		48/202	0.93 (0.53-1.63)	
		AA	14/29	0.56 (0.27-1.15)		7/29	1.12 (0.42-3.01)		50/107	0.64 (0.40-1.05)		25/107	0.88 (0.46-1.69)	
		CA/AA	84/172	**0.60 (0.40-0.89)**	0.01	25/172	0.64 (0.34-1.23)	0.19	164/309	0.81 (0.55-1.19)	0.28	73/309	0.91 (0.53-1.55)	0.73
			P-trend=0.02			P-trend=0.56			P-trend=0.08			P-trend=0.70		
LTA	**rs746868**	CC	57/103	1.00	0.04	22/103	1.00	0.39	131/226	1.00	0.93	54/226	1.00	0.04
		CG	95/157	1.21 (0.78-1.89)		21/157	0.62 (0.31-1.23)		85/161	0.98 (0.68-1.40)		33/161	0.87 (0.53-1.44)	
		GG	47/52	**2.02 (1.17-3.47)**		7/52	0.85 (0.32-2.25)		12/18	1.14 (0.52-2.50)		11/18	**2.69 (1.14-6.32)**	
		CG/GG	142/209	1.41 (0.93-2.13)	0.11	28/209	0.67 (0.35-1.27)	0.22	97/179	1.00 (0.70-1.41)	0.98	44/179	1.05 (0.66-1.67)	0.85

Gene	SNP	Genotype	European American						African American					
			ER Positive			ER Negative			ER Positive			ER Negative		
			Case/Cont[a]	OR(95%CI)[b]	P	Case/Cont	OR(95%CI)[b]	P	Case/Cont	OR(95%CI)[b]	P	Case/Cont	OR(95%CI)[b]	P
			P-trend=0.02			P-trend=0.44			P-trend=0.91			P-trend=0.24		
Treg Cytokin and Cytokine Receptors														
TGFB1	**rs1800469**	CC	70/134	1.00	0.24	23/134	1.00	0.91	132/215	1.00	0.66	64/215	1.00	0.17
		CT	92/135	1.30 (0.86-1.97)		21/135	0.88 (0.44-1.73)		84/165	0.85 (0.60-1.21)		30/165	**0.64 (0.39-0.98)**	
		TT	35/43	1.57 (0.89-2.77)		6/43	0.86 (0.31-2.35)		12/25	0.90 (0.43-1.91)		5/25	0.62 (0.22-1.74)	
		CT/TT	127/178	1.36 (0.92-2.01)	0.12	27/178	0.87 (0.46-1.65)	0.67	96/190	0.86 (0.61-1.20)	0.37	35/190	**0.64 (0.40-1.00)**	0.05
			P-trend=0.09			P-trend=0.69			P-trend=0.44			P-trend=0.08		

Abbreviations: OR, odds ratio; 95%CI, 95% confidence interval; P, *p* value calculated using a genotypic (co-dominant) model or when heterozygotes and rare homozygotes were combined ; P-trend, *p* value for genetic dose-response by coding genotypes as 0, 1, 2 according to the frequency of variant allele.

[a] Sum of ER negative and ER positive cases was lower than the total case number since ER status was not available for all cases.

[b] Adjusted for age at diagnosis (continuous), body mass index (continuous), education (less than high school, high school, college and graduate school), family history of breast cancer (yes, no), history of benign breast disease (yes, no), proportion of European ancestry (continuous), smoking (current, former, never), number of full pregnancy (continuous), breast feeding (yes, no, nulliparous), HRT(yes, no) and menopausal status (premenopausal, postmenopausal).

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Table 5

Replication study using the entire sample set of the Women's Circle of Health Study (WCHS).

Gene	SNP	Genotype	European American								African American								European American				African American			
			ALL Women			Premenopausal Women			Postmenopausal Women			ALL Women			Premenopausal Women			Postmenopausal Women		ER Positive		ER Negtive		ER Positive		ER Negtive	
			Case/Cont	OR(95%CI)[a]	P	Case/Cont	OR(95%CI)[a]	P	Case/Cont	OR(95%CI)[a]	P	Case/Cont	OR(95%CI)[a]	P	Case/Cont	OR(95%CI)[a]	P	Case/Cont	OR(95%CI)[a]	P	Case/Cont	OR(95%CI)[a]	P	Case/Cont	OR(95%CI)[a]	P	Case/Cont	OR(95%CI)[a]	P
Replicated SNPs																												
IL15	rs10833	GG	290/303	1.00	0.22	146/154	1.00	0.65	144/149	1.00	0.21	466/553	1.00	0.67	234/304	1.00	0.52	232/249	1.00	0.93	170/303	1.00	0.17	35/303	1.00	0.38	254/553	1.00	0.16	103/553	1.00	0.02
		GA	300/284	1.24 (0.97-1.57)		158/163	1.14 (0.82-1.60)		142/121	1.39 (0.96-2.00)		137/178	0.91 (0.69-1.18)		65/102	0.87 (0.6-1.27)		72/76	0.96 (0.65-1.42)		177/285	1.31 (0.99-1.73)		40/285	1.27 (0.77-2.12)		67/179	**0.79 (0.57-1.10)**		39/179	1.17 (0.77-1.78)	
		AA	60/57	1.18 (0.77-1.80)		39/36	1.22 (0.71-2.10)		21/21	1.10 (0.54-2.26)		11/11	1.24 (0.51-2.99)		6/5	1.71 (0.49-5.91)		5/6	0.81 (0.23-2.89)		35/57	1.20 (0.74-1.97)		5/57	0.67 (0.24-1.89)		2/11	0.34 (0.07-1.63)		7/11	**4.28 (1.55-11.81)**	
		GA/AA	360/341	1.23 (0.97-1.55)	0.09	197/199	1.16 (0.84-1.60)	0.37	163/142	1.35 (0.95-1.91)	0.10	148/189	0.92 (0.71-1.20)	0.55	71/107	0.91 (0.63-1.31)	0.61	77/82	0.95 (0.65-1.39)	0.79	212/342	1.29 (0.98-1.69)	0.07	45/342	1.16 (0.71-1.90)	0.54	69/190	**0.76 (0.55-1.05)**	0.08	46/190	1.32 (0.88-1.96)	0.20
			P-trend=0.14			P-trend=0.37			P-trend=0.20			P-trend=0.69			P-trend=0.83			P-trend=0.74		P-trend=0.13			P-trend=0.99			P-trend=0.07			P-trend=0.04		
IL15RA	rs2296135	CC	172/186	1.00	0.62	90/105	1.00	0.43	82/81	1.00	0.65	343/425	1.00	0.14	167/237	1.00	0.33	176/188	1.00	0.42	108/186	1.00	0.87	22/186	1.00	0.75	182/425	1.00	0.16	80/425	1.00	0.55
		CA	329/323	1.11 (0.85-1.46)		173/163	1.28 (0.88-1.86)		156/160	0.96 (0.64-1.45)		223/276	1.03 (0.81-1.31)		114/151	1.17 (0.84-1.63)		109/125	0.91 (0.64-1.29)		185/323	1.00 (0.73-1.36)		39/323	1.09 (0.61-1.94)		113/277	0.96 (0.72-1.29)		59/277	1.21 (0.83-1.77)	
		AA	149/136	1.17 (0.84-1.62)		80/85	1.17 (0.75-1.82)		69/51	1.20 (0.71-2.00)		48/41	**1.54 (0.98-2.42)**		24/23	1.54 (0.82-2.91)		24/18	1.44 (0.73-2.85)		89/137	1.09 (0.75-1.59)		19/137	1.30 (0.65-2.57)		28/41	**1.62 (0.95-2.74)**		10/41	1.30 (0.61-2.76)	
		CA/AA	478/459	1.13 (0.87-1.46)	0.36	253/248	1.24 (0.88-1.77)	0.22	225/211	1.02 (0.69-1.50)	0.92	271/317	1.09 (0.87-1.37)	0.46	138/174	1.22 (0.89-1.68)	0.21	133/143	0.97 (0.70-1.36)	0.87	274/460	1.02 (0.76-1.38)	0.8	58/460	1.15 (0.66-1.98)	0.29	141/318	1.05 (0.79-1.38)	0.61	69/318	1.22 (0.85-1.76)	0.65
			P-trend=0.34			P-trend=0.44			P-trend=0.55			P-trend=0.20			P-trend=0.14			P-trend=0.72		P-trend=0.69			P-trend=0.47			P-trend=0.31			P-trend=0.29		
LTA	rs1041981	CC	315/284	1.00	0.04	172/142	1.00	0.02	143/142	1.00	0.32	167/185	1.00	0.41	86/109	1.00	0.16	81/76	1.00	0.65	196/284	1	0.05	36/284	1.00	0.18	95/186	1.00	0.22	34/186	1.00	0.94
		CA	261/302	**0.77 (0.61-0.98)**		135/177	**0.62 (0.44-0.86)**		126/125	1.03 (0.71-1.50)		296/363	0.91 (0.69-1.19)		149/188	0.99 (0.69-1.44)		147/175	0.83 (0.56-1.25)		147/303	**0.72 (0.54-0.95)**		32/303	0.80 (0.47-1.35)		152/363	0.83 (0.60-1.15)		70/363	1.01 (0.64-1.60)	
		AA	67/52	1.19 (0.79-1.80)		34/32	0.89 (0.51-1.56)		33/20	1.64 (0.86-3.14)		142/187	0.81 (0.59-1.11)		68/110	0.69 (0.45-1.07)		74/77	0.94 (0.58-1.5)		35/52	1.00 (0.62-1.63)		11/52	1.67 (0.77-3.65)		73/187	0.71 (0.48-1.05)		40/187	1.08 (0.65-1.81)	
		CA/AA	328/354	0.83 (0.66-1.04)	0.08	169/209	**0.66 (0.48-0.91)**	0.01	159/145	1.12 (0.79-1.59)	0.54	438/550	0.87 (0.68-1.13)	0.30	217/298	0.88 (0.62-1.25)	0.48	221/252	0.87 (0.59-1.27)	0.46	182/355	**0.76 (0.58-0.99)**	0.04	43/355	0.92 (0.56-1.50)	0.75	225/550	0.79 (0.58-1.07)	0.13	110/550	1.04 (0.67-1.59)	0.91
			P-trend=0.60			P-trend=0.08			P-trend=0.25			P-trend=0.18			P-trend=0.11			P-trend=0.78		P-trend=0.21			P-trend=0.62			P-trend=0.08			P-trend=0.76		
IL4R	rs1801275	AA	427/445	1.00	0.58	216/249	1.00	0.27	211/196	1.00	0.68	45/76	1.00	0.18	28/35	1.00	0.64	17/41	1.00	0.24	252/446	1.00	0.67	52/446	1.00	0.56	24/76	1.00	0.37	10/76	1.00	0.60
		AG	188/178	1.10 (0.85-1.42)		105/90	1.31 (0.91-1.87)		83/88	0.85 (0.58-1.26)		268/318	1.39 (0.91-2.12)		132/143	1.14 (0.63-2.04)		136/175	1.66 (0.88-3.13)		108/178	1.01 (0.75-1.37)		24/178	1.19 (0.69-2.05)		142/319	1.37 (0.81-2.32)		69/319	1.45 (0.70-3.01)	
		GG	33/19	1.32 (0.70-2.46)		22/13	1.41 (0.65-3.02)		11/6	1.17 (0.38-3.65)		299/344	**1.50 (0.98-2.30)**		149/152	1.29 (0.71-2.33)		150/192	1.74 (0.91-3.31)		20/19	1.38 (0.68-2.79)		4/19	1.76 (0.55-5.64)		156/344	1.47 (0.86-2.50)		69/344	1.38 (0.66-2.87)	
		AG/GG	221/197	1.12 (0.87-1.43)	0.38	127/103	1.32 (0.94-1.85)	0.11	94/94	0.88 (0.60-1.28)	0.49	567/662	1.44 (0.96-2.17)	0.08	281/295	1.20 (0.69-2.11)	0.52	286/367	1.69 (0.91-3.14)	0.10	128/197	1.05 (0.79-1.40)	0.88	28/197	1.25 (0.74-2.10)	0.62	298/663	1.42 (0.85-2.35)	0.81	138/663	1.42 (0.70-2.87)	0.39
			P-trend=0.31			P-trend=0.12			P-trend=0.61			P-trend=0.11			P-trend=0.20			P-trend=0.34		P-trend=0.56			P-trend=0.31			P-trend=0.22			P-trend=0.67		
SNP Not Replicated																																
LTA	rs746868	CC	177/157	1.00	0.33	86/87	1.00	0.37	91/70	1.00	0.21	288/358	1.00	0.56	151/191	1.00	0.95	137/167	1.00	0.28	96/157	1.00	0.17	25/157	1.00	0.57	154/359	1.00	0.38	69/359	1.00	0.68
		CG	322/351	0.85 (0.64-1.12)		178/194	1.03 (0.70-1.51)		144/157	0.69 (0.45-1.05)		254/304	1.06 (0.84-1.35)		125/171	0.99 (0.71-1.38)		129/133	1.16 (0.82-1.64)		188/352	0.95 (0.68-1.31)		40/352	0.75 (0.43-1.32)		128/304	1.03 (0.77-1.38)		61/304	1.09 (0.74-1.60)	
		GG	124/113	1.04 (0.73-1.47)		66/59	1.36 (0.84-2.22)		58/54	0.74 (0.43-1.25)		45/45	1.28 (0.81-2.04)		16/26	0.89 (0.44-1.8)		29/19	1.66 (0.87-3.18)		84/113	1.32 (0.88-1.97)		11/113	0.73 (0.33-1.60)		27/45	1.46 (0.85-2.50)		11/45	1.38 (0.66-2.89)	
		CG/GG	446/464	0.90 (0.69-1.17)	0.42	244/253	1.10 (0.76-1.60)	0.60	202/211	0.70 (0.47-1.05)	0.08	299/349	1.09 (0.87-1.37)	0.46	141/197	0.98 (0.71-1.35)	0.91	158/152	1.22 (0.87-1.71)	0.24	272/465	1.04 (0.76-1.42)	0.78	51/465	0.74 (0.43-1.28)	0.40	155/349	1.08 (0.82-1.43)	0.18	72/349	1.12 (0.77-1.63)	0.32
			P-trend=0.97			P-trend=0.24			P-trend=0.19			P-trend=0.32			P-trend=0.83			P-trend=0.13		P-trend=0.21			P-trend=0.35			P-trend=0.31			P-trend=0.42		

Abbreviations: OR, odds ratio; 95%CI, 95% confidence interval; P, *p* value calculated using a genotypic (co-dominant) model or when heterozygotes and rare homozygotes were combined ; P-trend, *p* value for genetic dose-response by coding genotypes as 0, 1, 2 according to the frequency of variant allele.

[a]Adjusted for age at diagnosis (continuous), body mass index (continuous), education (less than high school, high school, college and graduate school), family history of breast cancer (yes, no), history of benign breast disease (yes, no), proportion of European ancestry (continuous), smoking (current, former, never), number of full pregnancy (continuous), breast feeding (yes, no, nulliparous), HRT(yes, no) and menopausal status when not stratified by it (premenopausal, postmenopausal).