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Genetic variants in immune-related pathways and breast cancer risk in African American women in the AMBER consortium

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Abstract

Background—Constitutional immunity shaped by exposure to endemic infectious diseases and parasitic worms in Sub-Saharan Africa may play a role in the etiology of breast cancer among African American (AA) women.

Methods—A total of 149,514 gene variants in 433 genes across 45 immune pathways were analyzed in the AMBER consortium among 3,663 breast cancer cases and 4,687 controls. Gene-based pathway analyses were conducted using the adaptive rank truncated product statistic for overall breast cancer risk, and risk by estrogen receptor (ER) status. Unconditional logistic

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regression analysis was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for single variants.

Results—The top pathways were Interleukin Binding ($p=0.01$), Biocarta TNFR2 ($p=0.005$), and Positive Regulation of Cytokine Production ($p=0.024$) for overall, ER+, and ER– cancers, respectively. The most significant gene was *IL2RB* ($p=0.001$) for overall cancer, with rs228952 being the top variant identified (OR=0.85, 95% CI: 0.79-0.92). Only *BCL3* contained a significant variant for ER+ breast cancer. Variants in *IL2RB*, *TLR6*, *IL8*, *PRKDC*, and *MAP3K1* were associated with ER– disease. The only genes showing heterogeneity between ER– and ER+ cancers were *TRAF1*, *MAP3K1*, and *MAPK3* ($p=0.02$). We also noted genes associated with autoimmune and atopic disorders.

Conclusions—Findings from this study suggest that genetic variants in immune pathways are relevant to breast cancer susceptibility among AA women, both for ER+ and ER– breast cancers.

Impact—Results from this study extend our understanding of how inherited genetic variation in immune pathways is relevant to breast cancer susceptibility.

Keywords

Immune pathways; Genetic variation; breast cancer risk; estrogen receptor; African American

INTRODUCTION

Endemic infectious diseases fundamentally shape the immune systems of populations at the genetic level, selecting for immunity that preserves well-being through the reproductive years(1). The immune system is organized into two central arms of defense; the non-specific innate arm which provides protection against a wide variety of pathogens by activating the inflammatory response, and the more specific adaptive arm that targets specific antigens of pathogens(2). Individuals of African ancestry (AA) show inherited differences in constitutional immune function from European Americans (EAs), partly due to evolutionary responses to infection and endemic helminth exposure in Sub-Saharan Africa, causing both a stronger inflammatory immune response as well as a Type 2 immunosuppressive phenotype(1, 3).

As reviewed by Palmer and colleagues(4), AA women are more likely than EAs to be diagnosed with breast cancer before age 50, and to have more aggressive disease of higher grade, lacking receptors for estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (HER2), which precludes use of targeted therapies, often resulting in poorer survival. Although it is becoming clearer that some reproductive and hormonal factors may, in part, account for some of the disparities in breast cancer aggressiveness(5, 6), it is possible that constitutional immune phenotype could play a role in breast cancer etiology, particularly for ER– disease(7). In the AMBER Consortium, we hypothesized that the higher prevalence of ER– tumors in AAs could arise, in part, from differences in immunological profiles such as those that typically define inflammation and/or suppressive phenotypes. Thus, we examined genetic variation in immune response pathways involved in host defense and/or self-tolerance in relation to overall breast cancer risk and risk of ER– compared to ER+ cancers. Because ER– breast cancers are most prevalent among

premenopausal AAs, and immune biomarkers were previously observed to be more strongly associated with being diagnosed with ER– compared to ER+ breast cancer among premenopausal women(8), we also examined the role of genetic variation in immune pathways and breast cancer risk by menopausal status.

MATERIALS AND METHODS

Study Population

These analyses were conducted in the context of the African American Breast Cancer Epidemiology and Risk (AMBER) Consortium designed to investigate the etiology of breast cancer subtypes among AA women(4). Studies forming the basis for AMBER were the Women’s Circle of Health Study (WCHS), the Carolina Breast Cancer Study (CBCS), the Black Women’s Health Study (BWHS), and the Multi-Ethnic Cohort Study (MEC)(9–13), which are described in Supplementary Methods 1. Research protocols were conducted in accordance with the ethical standards of the Declaration of Helsinki, and were approved by the Institutional Review Board at each participating institution. Informed consent was provided by all study participants. Table 1 shows the total number of cases and controls included in the analysis by age, menopausal status, and ER status.

Gene and SNP Selection

A total of 433 genes in 45 curated pathways involved in immune response were selected from the Molecular Signatures Database (MSigDB) and included BIOCARTA, KEGG, REACTOME, and Gene Ontology (GO) pathways(14). Pathways considered for inclusion were those related to cytokines, Th1 and Th2-related immunity, inflammation, T-cell activation, and immune response regulation. Priority was given to those containing the highest proportion of genes identified in GWAS (full catalog downloaded from the National Genome Research Institute on 03/08/2012) found to be associated with breast cancer, or disease conditions and/or phenotypes associated with dysregulated immune function, hormonal or reproductive factors, obesity-related traits, or circulating vitamin D levels as risk factors for breast cancer. A full list of traits used to generate the list of GWAS genes used to help prioritize immune pathways is provided in Supplementary Table 1. The list of GWAS genes used for prioritization is provided in Supplementary Table 2, with N=74 found within the 45 immune pathways prioritized. Tag single nucleotide polymorphisms (SNPs) were selected for the 433 unique genes \pm 10kb to capture SNPs with minor allele frequency 10% (at $r^2 \geq 0.8$) based on the haplotype structure of the Yoruban population (YRI) in 1000 Genomes Phase I reference panel (<http://www.1000genomes.org>).

Genotyping and QC protocol

Genotyping was performed by the Center for Inherited Disease (CIDR) using the Illumina Human Exome Beadchip v1.1 with additional custom content SNPs, as previously described(15). A total of 13,235 SNPs in the 433 immune-related genes examined passed quality control (QC). Imputation was performed using IMPUTE2 software(16) and the 1000 Genomes Phase I reference panel (5/21/2011 1000G data, December 2013 haplotype release in IMPUTE2 site). Genetic data from 533 cases and 989 controls were available in the MEC, from a previous GWAS on the Illumina Human 1 M-Duo chip, with SNPs imputed to the

same release of 1000 Genomes. Imputed genotypes were combined into a final data set after omitting variants with mismatching alleles in AMBER and MEC, allele frequencies that were different by more than 0.15, and had imputation INFO scores < 0.5 in either study. A total of 149,514 SNPs were included in these analyses. Genotype principal components were computed using the smartpca program in the EIGENSOFT package to examine population structure.

Protein-Protein Interaction (PPI) Network Construction

The Search Tool for the Retrieval of Interacting Genes (STRING, V.10) database was used to construct a PPI network to identify potentially relevant immune pathways. The STRING database provides integrated knowledge on the known and predicted functional relationships between proteins(17). Only associations with high confidence (score 0.7) are presented. Networks were generated for significant genes identified for breast cancer overall, and by ER and menopausal status.

Statistical Analysis

As previously described(15), gene-based pathway analyses were conducted using the adaptive rank truncated product (ARTP) statistic as implemented in the R package PIGE, which allowed us to optimize the number of SNP p-values combined in each pathway and in each gene-level test. To avoid capturing highly correlated signals within a gene, a subset of “pruned-in” SNPs were selected to ensure that all SNP pairs had linkage disequilibrium (LD) $r^2 < 0.8$, using the R2 filter option in the R package AdaJoint. The ARTP gene-level tests combined the optimal number of the most significant SNP p-values from among the top 10 pruned-in SNPs for each gene. Single variant tests were only conducted within genes reaching a nominal significance level of $p=0.05$. Unconditional logistic regression analysis was performed using imputed dosage genotype data. A Bonferroni correction was applied for the number of pruned-in SNPs tested within each gene to identify SNPs with gene-wide significance. We performed case-case analyses to compare odds of being diagnosed with ER – to ER+ disease to identify pathways, genes, and SNPs that may differentially impact ER status. Although none of the genotype principal components tested were strongly associated with risk, we included 3 principal components that were associated at $p<0.10$ in all regression models to control for any potential confounding by population structure. Models were also adjusted for study, age, geographic location, and DNA source (saliva, blood, mouthwash). Lifestyle factors and comorbid conditions that affect immune function and breast cancer risk were not treated as potential confounders in our study because they could be on the causal pathway between genetic variation and breast cancer risk, and adjustments may inappropriately attenuate risk estimates. Functional follow-up was performed in RegulomeDB, PolyPhen-2, HaploReg v4.1, and GTEx databases(18–21).

RESULTS

Pathways associated with Breast Cancer Risk

Pathway analyses yielded nominally or borderline significant associations for overall breast cancer with cytokine-related pathways (Interleukin Receptor Activity, $p=0.05$; Interleukin Binding, $p=0.01$; Cytokine and Chemokine Mediated Signaling, $p=0.04$) as well as the

Immune Effector Process pathway ($p=0.04$) (Supplementary Table 3). When stratifying by ER status, only the Biocarta TNFR2 pathway achieved nominal significance for ER+ cancer ($p=0.005$), and the Positive Regulation of Cytokine Production pathway was associated with ER- disease ($p=0.024$).

When considering menopausal status, pathways most significant for premenopausal breast cancer (Supplementary Table 4) were those associated with regulation of immune system processes (Positive Regulation of Immune System Process, $p=0.005$; Regulation of Immune System Process, $p=0.01$; Immune Effector Process, $p=0.02$) and lymphocyte and T cell activation (Regulation of Lymphocyte activation, $p=0.03$; Regulation of T-cell activation, $p=0.06$). Premenopausal ER+ cancers were most strongly related to Programmed death-1 (PD-1) signaling ($p=0.04$), while ER- cancers were associated with the Adaptive Immune Response pathway ($P=0.03$), and cytokine and inflammation-related pathways (Biocarta cytokine pathway, $p=0.004$; Biocarta Inflammation pathway, $p=0.03$). Immune activation pathways were also important among postmenopausal women (Supplementary Table 5; Regulation of Lymphocyte activation, $p=0.06$; T-cell activation, $p=0.06$), but cytokine-related pathways appeared to play a more prominent role (Interleukin Receptor activity, $p=0.01$; Interleukin Binding, $p=0.02$; Cytokine and Chemokine Mediated Signaling Pathway, $p=0.03$), involved in both ER+ (Cytokine Binding, $p=0.03$; Biocarta IL5 pathway, $p=0.05$) and ER- cancers (Cytokine and Chemokine Mediated Signaling pathway, $p=0.04$).

Genes and SNPs associated with Breast Cancer Risk

In gene-based testing, a number of genes were nominally related to overall breast cancer risk, and by ER status at $p<0.01$ (Table 2, Supplementary Table 3). Gene functions are summarized in Supplementary Table 6. No genes remained significant after correcting for the overall number of genes tested. For overall breast cancer, the most notable genes were those involved in cytokine-related pathways, which included *IL2RB* ($p=0.001$), *CADMI* ($p=0.003$), *ATP6AP2* ($p=0.006$), and *TLR6* ($p=0.006$). Subsequent SNP testing of these genes showed five variants significant at the gene-wide level (Table 3). The top variant was rs228952 in *IL2RB* ($p^{\text{adj}}=0.007$). In SNP-based analyses, several intronic variants in *POU2AF1* were inversely associated with risk, including rs145624147 ($p^{\text{adj}}=0.008$).

Among premenopausal women (Table 2, Supplementary Table 4), genes most strongly related to risk were *IL21*, *SLA2*, and *CFHR1* ($p=0.004$), involved in regulation of immune system processes. An intronic SNP in *SLA2* (rs221310; $p^{\text{adj}}=0.003$) was associated with approximately 30% increased risk for each copy of the *G* allele. Two intergenic SNPs associated with *IL21*, rs115698762 ($p^{\text{adj}}=0.008$) and rs143266239 ($p^{\text{adj}}=0.01$), increased risk by approximately two-fold. Among postmenopausal women, genes associated with overall risk included *IL2RB*, *CYP4F11*, and *POUF2AF1* ($p=0.007$), as well as *IL21* ($p=0.004$). Two genes, *CASP8* ($p=0.006$) and *IRAK2* ($p=0.008$) were specific to overall breast cancer risk in postmenopausal women. When SNPs were examined, several in genes involved in interleukin and inflammatory response pathways, i.e. *IL2RB*, *CYP4F11*, *IRAK2*, and *IL21* were associated with risk. Of these, the most significant were in *IL21* (rs2390350, $p^{\text{adj}}=0.008$; rs17005895, $p^{\text{adj}}=0.01$).

Genes and SNPs associated with Risk or ER+ and ER– Cancers

None of the genes associated with overall breast cancer risk were significantly associated with ER+ cancers at $p < 0.01$. Only *BCL3*, a putative proto-oncogene important for the development, survival, and activity of adaptive immune cells(22) contained a variant (rs34698726) associated with overall breast cancer risk ($p^{\text{adj}}=0.01$) and risk of ER+ cancers ($p^{\text{adj}}=0.01$). Except for *CADMI* ($p=0.006$), none of the genes associated with overall breast cancer risk were associated with ER– cancers. Instead, genes involved in TNFR1 (*PRKDC*, $p=0.002$) and inflammatory response pathways (*IL8*, $p=0.003$; *NFATC3*, $p=0.008$), regulation of cytokine production (*CARD11*, $p=0.007$), and *MAPK* signaling pathways (*MAPK3*, $p=0.006$) were related to risk of ER– breast cancers. Several SNPs in *IL8* (rs188246983, rs113973067), *PRKDC* (rs148411126, rs8178033, rs56411879) and *MAP3K1* (rs863839, rs191188130) were associated with risk of ER– breast cancer.

Genes and SNPs associated with Risk or ER+ and ER– Cancers by Menopausal Status

Among premenopausal women, genes associated with overall breast cancer risk were also associated with ER+ cancers, but not ER– disease (Table 2, Supplementary Table 4). None of the genes associated with overall risk of ER– breast cancer were observed when limited to premenopausal women, except for *IL8*, with two significant variants (rs113976067, rs188246983). The genes *IL10* ($p=0.001$) and *HELLS* ($p=0.008$) were also associated with risk of premenopausal ER– cancer, with ORs for SNPs associated with *IL10* ranging from 1.5 to 1.8 for each additional copy of the variant allele. Several SNPs within the *HELLS* gene were significant (rs200175744, $p^{\text{adj}}=0.02$; rs10882476, $p^{\text{adj}}=0.05$; rs11188009, $p^{\text{adj}}=0.03$).

Similar to the pattern observed in premenopausal women, genes associated with overall breast cancer risk in postmenopausal women were also associated with ER+ disease (*IL2RB*, *CYP4F11*, *CASP8*, *POU2AF1*, $p < 0.01$; Table 2). Several SNP variants in *POU2AF1*, *UBE2N*, *CD274*, and *XCR1* were associated with postmenopausal ER+ risk, including rs75716067 in *POU2AF1* ($p^{\text{adj}}=0.003$). The genes *IL2RB* ($p=0.008$) and *IRAK2* ($p=0.001$), associated with overall breast cancer risk in postmenopausal women, were also associated with ER– cancers. Generally, few SNPs were significantly associated with postmenopausal ER– breast cancer. The most significant were intronic SNPs in *IRAK2* (rs149858020, $p^{\text{adj}}=0.02$) and *PRKDC* (rs8178153, $p^{\text{adj}}=0.03$).

Case-case Analyses

In case-case analyses comparing odds of being diagnosed with ER– versus ER+ breast cancers, the only genes showing heterogeneity between ER– and ER+ cancers for all women combined were *TRAF1* ($p=0.001$) and *MAP3K1* ($p=0.007$). There was also some indication that *MAPK3* ($p=0.02$) plays a more pronounced role in ER– cancers. No heterogeneity were observed in pre- or postmenopausal women at the $p=0.01$ level. Of the above genes, only rs863839 in *MAP3K1* was significantly different in ER– versus ER+ disease among all women combined (OR=0.66, 95% CI: 0.52-0.82, $p^{\text{adj}}=0.04$), with the study having 80% power to detect this level of difference.

PPI Networks—In the PPI network for all significant immunity genes identified at $P < 0.01$ ($N=38$, Supplementary Table 6), there were a total of 22 genes with proteins (nodes) that were connected to at least one other protein (Figure 1). The greatest number of protein-protein interactions were observed with *IL2RB* (node degree = 5), *MAPK3* (node degree = 4), *MAP3K1* (node degree=6), *IL8* (node degree=5) and *IL10* (node degree=5). For ER+ cancers ($N=29$ genes), *MAP3K1* showing the most protein-protein interactions (node degree=4), while *IL2RB* interacted with the most proteins (node degree = 4) for ER– cancers ($N=26$ genes).

DISCUSSION

The immune system plays a critical role in preventing and inhibiting tumor development, but may also act to promote tumor growth and progression(23). In these analyses, we used a comprehensive approach to investigate the role of hereditary immunity on breast cancer risk in AA women by assessing multiple immune response pathways, individual genes within these pathways, and the contribution of specific gene variants. Pathways and genes associated with regulation of immune system processes, immune activation, and inflammation were associated with overall breast cancer, with genes and SNPs in the NF- κ B pathway associated with innate immunity and activation of the inflammatory response playing a role in ER+ breast cancers and pathways associated with *MAP3K1* activation playing a role in ER– cancers. Of the genes identified by these analyses, only *MAP3K1* was previously identified as a breast cancer GWAS locus, and previously reported to be related to ER– cancer in the AMBER consortium [see Haddad *et al.* for discussion(15)]. We also noted that a number of genes related to dysregulation of humoral immunity, and involved in autoimmune and atopic disorders were associated with risk.

Th2-related and PD-1 Immunosuppressive Pathways Relevant to Both ER+ and ER– cancers

Individuals and populations vary in their resistance to infectious disease and pathogens. Much of this variation in phenotype is genetic, with pathogens acting as a selective force on genetic diversity(1, 24). Studies in West Africa show inheritance of immune phenotypes(3). The higher prevalence of bias towards a stronger T-helper type 2 (Th2) immune response among AAs is due in part to evolutionary responses to endemic helminth exposure in Sub-Saharan Africa, and is traditionally viewed as immunosuppressive, favoring tumor growth by inhibiting cell-mediated immunity and promoting angiogenesis. This immune bias contributes to higher incidence of atopic conditions such as asthma, which is characterized by chronic activation of humoral immunity pathways(25) and can lead to allergy-driven inflammatory cytokines (25, 26). In this study, a number of genes identified in relation to breast cancer risk were previously associated with asthma in GWAS studies, including *IL2RB*, *HLA-DQ*, and *SMAD3*, the main mediator of TGF- β signaling, which is important in T-cell activation and immune tolerance(27, 28), and promotes a Th2 immune phenotype(29). The variant rs2289259 in *SMAD3* was strongly related to increased risk of ER– cancer among postmenopausal women and is in high LD with rs2033784 ($r^2=0.81$), which affects *SMAD3* expression in thyroid tissue and blood(18).

IL5 was the Th2-related pathway most strongly related to breast cancer risk in these analyses, and is the most important driver of eosinophil production and host defense against helminth parasite infections. Excessive production of IL-5 and eosinophilia would be expected to dampen anti-tumor immune responses and increase allergy-related inflammation. Among postmenopausal women, the Biocarta IL5 pathway was significantly related to ER+ breast cancer, with significant associations observed for *IL5*, *IL5RA*, and *CCR3* in gene-level analyses ($p < 0.05$). No significant variants, however, were found in any of these genes. These findings were similar to those in a recent pooled analysis of ~40,000 cases and 40,000 controls in the Breast Cancer Association Consortium, which found an association with *IL5* in gene-level analyses, but did not identify individual SNPs associated with risk(30). *PRG3*, coding for proteoglycan 3, an eosinophil protein that is involved in the positive regulation of IL-8, histamine, and leukotriene C4 release was also associated with breast cancer risk, but only among premenopausal women. The *PRG3* variant rs4411290 and rs1867128 are eQTL sites and are associated with increased *PRG2* expression in adipose tissue(31). These findings support the original hypothesis that a Type 2, immunosuppressive phenotype plays a role in breast cancer risk among AA women, although it appears that this phenotype may be relevant for both ER+ and ER- cancers.

In further support of a potential role for immunosuppression and breast cancer risk was the identification of several genes involved in PD-1 signaling, including among postmenopausal women an association between ER+ cancers and CD274, the primary ligand for the PD-1 checkpoint molecule on T cells, as well as an association between ER- cancers and CD247, which is involved in the downregulation of T-lymphocytes due to chronic inflammation, and has been identified as a susceptibility locus for systemic sclerosis and rheumatoid arthritis(32, 33).

Association with Genes Linked to Autoimmune Disease and role of B-cell Pathways

Several of the genes associated with breast cancer risk in our study have previously been associated with autoimmune diseases in GWAS studies, pointing to the possibility that dysregulated immune responses associated with autoimmune conditions, including chronic activation and proliferation of B cells, can alter breast cancer risk in AA women. Genes identified include *IL21*, *HLA-DQA1*, *HLA-DMA*, *TRAF1*, *ICOSLG*, and *SMAD3*. The *IL21* rs2390350 variant associated with increased risk of breast cancer among postmenopausal women is in high LD with rs907715 ($r^2=0.82$), an independent susceptibility locus for systemic lupus erythematosus in both AAs and EAs(34, 35), and was found to be borderline significant in our analyses (OR=0.86, 95% CI: 0.79-0.95, $p^{\text{adj}}=0.072$). The gene *PRKDC* involved in regulation of autoimmune responses(36), as well as T-cell tolerance, inflammatory disease, and DNA repair was the gene most strongly related to risk of ER- breast cancer. The missense variant (rs8178033) identified in this gene, however, was considered to be benign by PolyPhen-2 (Score: 0.29). Given that AA women are more likely to be diagnosed with systemic autoimmune diseases compared to EAs(37), greater understanding is needed of how these pathways might impact breast cancer risk in these women.

Several variants in genes that play a role in B cell activation and development were also noted in this study. This included SNPs in *POU2AF1*, a B cell specific transcriptional co-activator required for B cell maturation(38), and a polymorphism in *SLA2*, which encodes for a SRC-like adaptor protein (SLAP) required for maintaining normal levels of B cell receptor expression and development that is associated with several oncogenic signaling pathways and rheumatoid arthritis(39). The variant rs17848049 in the *CXCR4* gene region was found to be associated with premenopausal breast cancer. The CXCR4 receptor binds to stromal cell-derived factor 1 and controls B-cell development, can activate inflammatory signaling pathways, and is dysregulated in autoimmune conditions(40). A recent finding from the Women's Health Initiative study of a specific pre-diagnostic autoimmune response signature related to humoral immunity in triple negative breast cancers provide some support for B-cell pathways playing a role in breast cancer etiology(41), although our findings support an association for B-cell pathways in both ER+ and ER- cancers among AAs.

Association with Inflammation Related to Innate Immunity

In this study, the associations found between *MAP3K1*, *IL1R1*, *IRAK2*, and *TRAF1* in the PPI network generated for ER+ cancers supports a role for inflammation related to innate immunity and the NF- κ B pathway as a contributor to disease. MAP3K1 activates CHUK and IKKB, the central protein kinases of the NF- κ B pathway, and IL1R1 mediates IL-1 dependent activation of NF- κ B and MAPK, and strongly induces IL-8 expression, a major mediator of the inflammatory response(42). Signaling involves recruitment of adaptor proteins such as IRAK2, and *IRAK2*, *MAPK3*, and *IL-8* were all found to be associated with increased risk of ER- breast cancer. Associations with ER- disease were also noted with other genes in innate-immunity pathways including *HELLS* in premenopausal women. All three variants identified in *HELLS* (rs200175744, rs10882476, rs11188009) are associated with increased gene expression(31). Our findings are consistent with recent results indicating that inflammation is associated with breast cancer development(43).

Role for IL-2, IL-15, and IL-21 signaling pathways

The closely related *IL-2*, *IL-15*, and *IL-21* signaling pathways were found to play a prominent role in breast cancer risk in this study, and are involved in leukocyte development, and immune response activation and cessation. Signal transduction occurs via the Janus Kinase (JAK)-STAT pathway, the phosphoinositide 3-kinase (P13K)-AKT pathway, and the mitogen activated protein kinase (MAPK) pathway [reviewed in (44, 45)]. IL-21 triggers rapid activation of ERK1/2 required for IL-21 induced cytokine production, which includes IL-1, IL-8, CCL3, CCL5, and CCL11(46), and induces differentiation and production of cytotoxic T cells, macrophages, NK, and B cells. This is the first study to observe associations between breast cancer risk and *IL21* variants.

IL2RB, coding for one of 3 subunits in the IL-2 receptor complex, was the most significant gene associated with overall breast cancer risk, and found to be relevant for both ER+ and ER- cancers in postmenopausal women, with rs228952 being the top variant identified. Generally, few studies have examined the role of IL-2 receptors in breast cancer. The high affinity IL-2 receptor composed of a β , γ , and α subunit binds to IL-2 and is highly expressed on Treg cells(47). Increased ratios of Treg cells to total T lymphocytes, an

important determinant of immune suppression, was recently shown in a case-cohort study in EPIC-Heidelberg to be associated with increased risk of ER– breast cancers(7). Dysregulation of IL-15 expression, potentially mediated by altered IL-2R $\beta\gamma$ function, may alternatively contribute to autoimmune diseases by inhibiting Treg-mediated self-tolerance(44, 48). In this study, there were suggestions that the *IL15RA* gene was associated with ER– breast cancer, with significant heterogeneity by ER status ($p=0.02$), but no SNPs were found to be significant.

Study Limitations

Despite this being the first large scale study of AAs that comprehensively examines the role of immune-related pathways, there was still limited power to detect associations by ER and menopausal status, and to observe significant pathway and gene-level associations after correcting for multiple testing. Limited sample size may have also contributed to the observation that specific genes and variants showing the strongest associations differed by menopausal and ER status, similar to previous findings(49). These differences, however, may have been due in part to age-related changes in immune function. Premenopausal women, for instance, are more prone to autoimmunity, while older postmenopausal women are at higher risk of chronic low grade inflammation due to increased activation of the innate immune system(50). Most of the genetic variants of interest identified in this study were imputed, which is a potential study limitation, although we focused only on SNPs with high imputation INFO scores that achieved gene-wide significance. Regardless, this is the largest study to date in AAs that has examined the role of specific sub-components of immunity, such as the adaptive and innate immune response, inflammatory response, and cytokine-related pathways.

Summary

Results from this study represent an important extension of our understanding of how inherited genetic variation in immune pathways is relevant to breast cancer susceptibility and show the importance of pathways involved in innate immune response, immune activation, and immune suppression for both ER+ and ER– cancers among AA women, including a role for Th2, B-cell, and PD-1 related pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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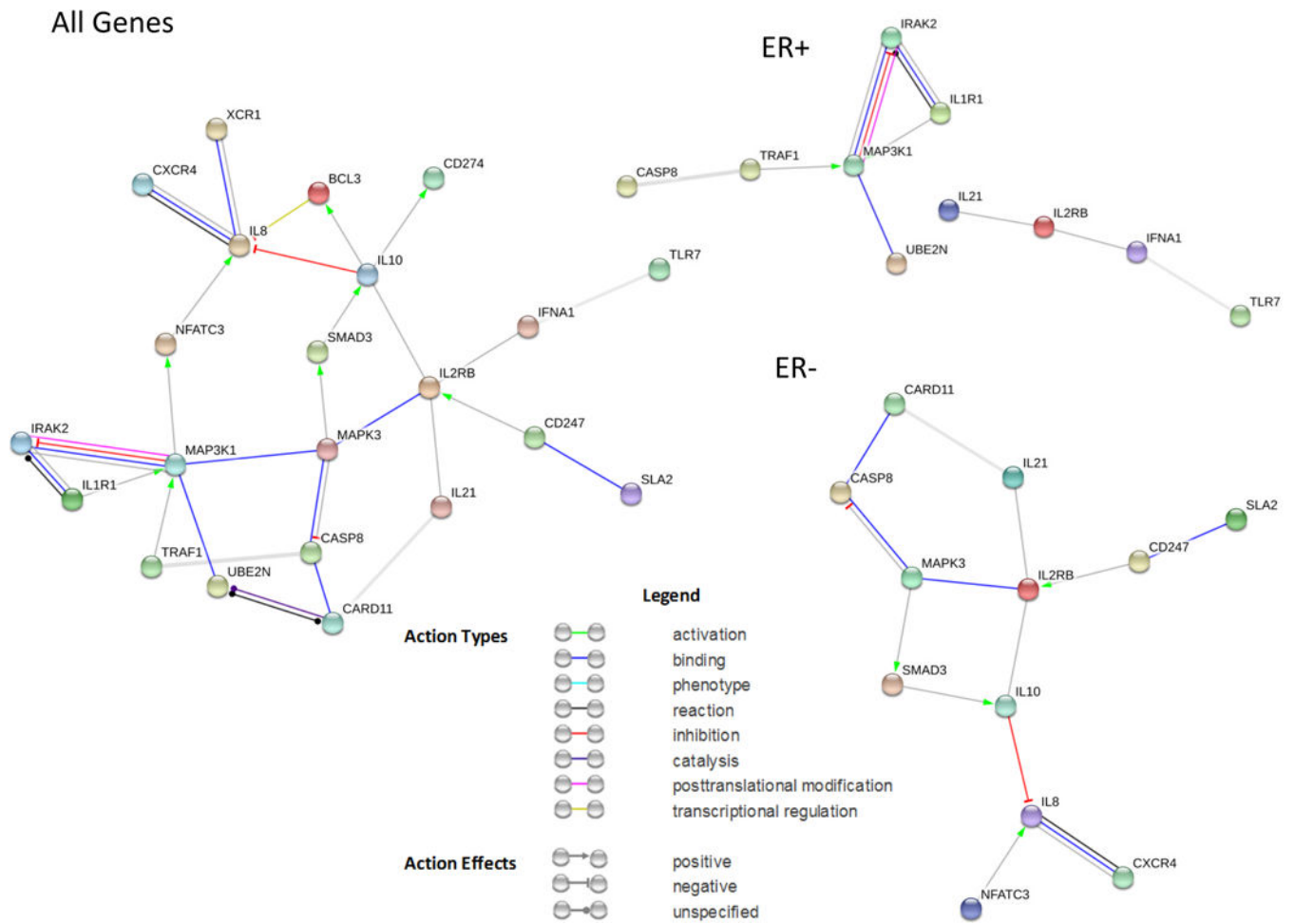


Figure 1. Protein network for all genes associated with breast cancer risk overall (N genes=38), and for ER+ (N genes=29) and ER- (N genes=26) breast cancers, in either all women combined, or in pre- or postmenopausal women only. Gene-gene interactions are represented by interconnecting lines, with edge combined scores ≥ 0.7 . Disconnected nodes are not shown.

Table 1
Descriptive Characteristics of Participants in the AMBER Consortium by Study

	BWHS			CBCS			WCHS			MEC			AMBER		
	Ca	Co	%	Ca	Co	%	Ca	Co	%	Ca	Co	%	Ca	Co	%
Total (N)	901	2249		1408	615		821	834		533	989		3663	4687	
Age, Years, N (%)															
< 40	47 (5.2%)	217 (9.6%)	204 (14.5%)	87 (14.1%)	87 (14.1%)	85 (10.4%)	116 (13.9%)	116 (13.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	336 (9.2%)	420 (9.0%)	
40-49	262 (29.1%)	652 (29.0%)	459 (32.6%)	211 (34.3%)	211 (34.3%)	215 (26.2%)	228 (27.3%)	228 (27.3%)	16 (1.6%)	16 (1.6%)	9 (1.7%)	16 (1.6%)	945 (25.8%)	1107 (23.6%)	
50-59	302 (33.5%)	770 (34.2%)	381 (27.1%)	150 (24.4%)	150 (24.4%)	292 (35.6%)	319 (38.2%)	319 (38.2%)	222 (22.4%)	222 (22.4%)	112 (21.0%)	222 (22.4%)	1087 (29.7%)	1461 (31.2%)	
60-69	204 (22.6%)	442 (19.7%)	267 (19.0%)	114 (18.5%)	114 (18.5%)	173 (21.1%)	142 (17.0%)	142 (17.0%)	288 (29.1%)	288 (29.1%)	175 (32.8%)	288 (29.1%)	819 (22.4%)	986 (21.0%)	
70+	86 (9.5%)	168 (7.5%)	97 (6.9%)	53 (8.6%)	53 (8.6%)	56 (6.8%)	29 (3.5%)	29 (3.5%)	463 (46.8%)	463 (46.8%)	237 (44.5%)	463 (46.8%)	476 (13.0%)	713 (15.2%)	
Menopausal Status, N (%)															
Premenopausal	310 (34.4%)	834 (37.1%)	526 (37.4%)	234 (38.0%)	234 (38.0%)	363 (44.2%)	394 (47.2%)	394 (47.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1199 (32.7%)	1462 (31.2%)	
Postmenopausal	482 (53.5%)	1178 (52.4%)	745 (52.9%)	308 (50.1%)	308 (50.1%)	361 (44.0%)	386 (46.3%)	386 (46.3%)	533 (100.0%)	533 (100.0%)	533 (100.0%)	533 (100.0%)	2121 (57.9%)	2861 (61.0%)	
Unknown	109 (12.1%)	237 (10.5%)	137 (9.7%)	73 (11.9%)	73 (11.9%)	97 (11.8%)	54 (6.5%)	54 (6.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	343 (9.4%)	364 (7.8%)	
ER status, N (%)															
ER+	498 (55.3%)		741 (52.6%)			435 (53.0%)			309 (58.0%)				1983 (54.1%)		
ER-	233 (25.9%)		565 (40.1%)			165 (20.1%)			135 (25.3%)				1098 (30.0%)		
Unknown	170 (18.9%)		102 (7.2%)			221 (26.9%)			89 (16.7%)				582 (15.9%)		

BWHS: Black Women's Health Study; CBCS: Carolina Breast Cancer Study; WCHS: Women's Circle of Health Study; MEC: Multi-Ethnic Cohort; AMBER: African American Breast Cancer Epidemiology and Risk; Ca: Cases; Co: Controls; ER: Estrogen receptor.

Table 2

Nominally Significant genes associated with overall, ER+, ER– breast cancer risk in the AMBER Consortium

Gene	Chromosomal Location	Pathway ^d	N SNPs		P-Value			
			Total	Pruned In	All	ER+	ER– vs ER+	
All Women								
IL2RB	22q13	INTERLEUKIN BINDING ^b	322	220	0.001	0.074	0.018	0.485
CADM1	11q23.2	CYTOKINE PRODUCTION	1605	638	0.003	0.031	0.006	0.957
ATP6AP2	Xp11.4	POSITIVE REGULATION OF CYTOKINE PRODUCTION ^d	283	96	0.006	0.130	0.091	0.689
TLR6	4p16.1	CYTOKINE METABOLIC PROCESS	494	141	0.006	0.142	0.024	0.147
HLA-DQA1	6p21.3	REACTOME PDI SIGNALING (Programmed death-1 (PD-1) is a cell surface molecule that regulates the adaptive immune response)	1986	188	0.007	0.043	0.079	0.361
POU2AF1	11q23.1	HUMORAL IMMUNE RESPONSE	244	94	0.009	0.033	0.338	0.881
TLR7	Xp22.3	BIOCARTA DC PATHWAY	251	159	0.009	0.330	0.563	0.949
CYP4F11	19p13.1	INFLAMMATORY RESPONSE	336	122	0.010	0.068	0.231	0.910
UBE2N	12q22	POSITIVE REGULATION OF IMMUNE RESPONSE	227	66	0.098	0.001	0.378	0.345
IFNA1	9p22	BIOCARTA INFLAM PATHWAY	143	77	0.014	0.002	0.146	0.770
TRAF1	9q33-q34	BIOCARTA TNFR2 PATHWAY ^c	258	62	0.377	0.003	0.725	0.001
CALCA	11p15.2	CYTOKINE PRODUCTION	129	49	0.034	0.005	0.015	0.124
MAP3K1	5q11.2	BIOCARTA TNFR1 PATHWAY	544	141	0.096	0.007	0.015	0.007
BCL3	19q13.32	CYTOKINE PRODUCTION	214	131	0.038	0.007	0.205	0.422
ICOSLG	21q22.3	REGULATION OF LYMPHOCYTE ACTIVATION	264	148	0.018	0.009	0.574	0.225
PRKDC	8q11	BIOCARTA TNFR1 PATHWAY	444	109	0.131	0.488	0.002	0.052
IL8	4q13-q21	BIOCARTA INFLAM PATHWAY	122	49	0.395	0.644	0.003	0.570
MAPK3	16p11.2	KEGG NOD LIKE RECEPTOR SIGNALING PATHWAY	24	19	0.688	0.953	0.006	0.019
CARD11	7p22	REGULATION OF CYTOKINE PRODUCTION ^d	1471	828	0.069	0.665	0.007	0.099
NFATC3	16q22	INFLAMMATORY RESPONSE	660	117	0.898	0.948	0.008	0.147
Premenopausal Women								
IL21	4q26-q27	POSITIVE REGULATION OF IMMUNE SYSTEM PROCESS ^b	75	32	0.001	0.019	0.448	0.954
SLA2	20q11.23	POSITIVE REGULATION OF IMMUNE SYSTEM PROCESS ^b	220	80	0.001	0.076	0.122	0.704

Gene	Chromosomal Location	Pathway ^d	N SNPs		P-Value			
			Total	Pruned In	All	ER+	ER- vs ER+	
TLR7	Xp22.3	CYTOKINE BIOSYNTHETIC PROCESS	251	159	0.003	0.038	0.311	0.500
CFHR1	1q32	REGULATION OF IMMUNE SYSTEM PROCESS ^b	500	123	0.004	0.034	0.210	0.621
PRG3	11q12.1	CYTOKINE BIOSYNTHETIC PROCESS	93	39	0.006	0.004	0.272	0.211
CXCR4	2q21	INFLAMMATORY RESPONSE	90	54	0.008	0.024	0.228	0.060
HLA-DMA	6p21.3	REACTOME PDI SIGNALING (Programmed death-1 (PD-1) is a cell surface molecule that regulates the adaptive immune response) ^c	135	40	0.009	0.002	0.623	0.455
MAP3K1	5q11.2	BIOCARTA TNFR2 PATHWAY	544	141	0.187	0.003	0.595	0.025
LBP	20q11.23	INFLAMMATORY RESPONSE	389	196	0.012	0.006	0.608	0.613
HLA-DOA	6p21.3	REACTOME PDI SIGNALING (Programmed death-1 (PD-1) is a cell surface molecule that regulates the adaptive immune response) ^c	303	136	0.060	0.008	0.261	0.474
IL10	1q31-q32	BIOCARTA CYTOKINE PATHWAY ^d	114	67	0.442	0.498	0.001	0.021
IL8	4q13-q21	KEGG NOD LIKE RECEPTOR SIGNALING PATHWAY	122	49	0.327	0.732	0.001	0.110
HELLS	10q24.2	LYMPHOCYTE ACTIVATION	344	77	0.863	0.982	0.008	0.098
Postmenopausal Women								
IL2RB	22q13	INTERLEUKIN RECEPTOR ACTIVITY ^b	322	220	0.001	0.023	0.008	0.137
CYP4F11	19p13.1	INFLAMMATORY RESPONSE	336	122	0.001	0.005	0.213	0.985
IL21	4q26-q27	REGULATION OF LYMPHOCYTE ACTIVATION	75	32	0.004	0.225	0.237	0.912
CASP8	2q33-q34	KEGG NOD LIKE RECEPTOR SIGNALING PATHWAY	259	122	0.006	0.014	0.100	0.572
POU2AF1	11q23.1	HUMORAL IMMUNE RESPONSE	244	94	0.007	0.012	0.335	0.787
IRAK2	3p25.2	INFLAMMATORY RESPONSE	645	317	0.008	0.478	0.001	0.631
XCR1	3p21.3-p21.1	CYTOKINE BINDING	95	31	0.086	0.002	0.296	0.988
CD274	9p24.1	REACTOME PDI SIGNALING (Programmed death-1 (PD-1) is a cell surface molecule that regulates the adaptive immune response)	130	73	0.113	0.005	0.524	0.532
UBE2N	12q22	POSITIVE REGULATION OF IMMUNE RESPONSE	227	66	0.013	0.006	0.455	0.200
AOAH	7p14.2	INFLAMMATORY RESPONSE	1608	770	0.030	0.006	0.465	0.911
IL1R1	2q12	INTERLEUKIN RECEPTOR ACTIVITY ^b	858	334	0.022	0.009	0.630	0.874
CARD11	7p22	REGULATION OF CYTOKINE PRODUCTION	1471	828	0.554	0.911	0.004	0.011
SMAD3	15q21-q22	CYTOKINE PRODUCTION	837	448	0.094	0.116	0.006	0.051
CD247	1q24.2	REACTOME PDI SIGNALING (Programmed death-1 (PD-1) is a cell surface molecule that regulates the adaptive immune response)	682	367	0.284	0.242	0.008	0.238

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^aPathway associated with the greatest statistical significance for gene as shown in Supplementary Tables 3, 4, or 5.

^bPathway associated with overall breast cancer risk within study population specified (i.e. all women combined, premenopausal women, or postmenopausal women), as shown in Supplementary Tables 3, 4, or 5, $P < 0.05$.

^cPathway associated with risk of ER+ breast cancer risk within study population specified, as shown in Supplementary Tables 3, 4, or 5, $p < 0.05$.

^dPathway associated with ER- breast cancer within study population specified, as shown in Supplementary Tables 3, 4, or 5, $p < 0.05$.

P-values for genes significant at $P < 0.01$ are indicated in bold text.

Table 3

Associations between SNPs reaching gene-wide significance with overall, ER+, and ER- breast cancer risk in the AMBER consortium

Gene	SNP	Function	Ref /variant allele	MAF	PLINK INFO Score	All Cases			ER+		ER-	
						Per allele OR (95% CI)	Gene-wide padj		Per allele OR (95% CI)	Gene-wide padj	Per allele OR (95% CI)	Gene-wide padj
All Women												
IL2RB	rs228952	intronic	G/T	0.28	0.97	0.85 (0.79, 0.92)	0.007	0.88 (0.80, 0.96)	0.967	0.80 (0.71, 0.90)	0.051	
POU2AF1	rs145624147	intronic	CAG/C	0.24	1.01	0.85(0.79,0.92)	0.008	0.88 (0.80, 0.96)	0.549	0.82 (0.73, 0.93)	0.149	
	rs1815948	intronic	G/C	0.14	1.01	0.83 (0.75, 0.92)	0.023	0.85 (0.76, 0.96)	0.868	0.83 (0.71, 0.93)	1.000	
	rs76988807	intronic	T/C	0.08	1.00	0.80 (0.70, 0.90)	0.035	0.79 (0.67, 0.91)	0.180	0.86 (0.71, 1.03)	1.000	
HLA-DQA1	rs12722043	exonic syn SNV	C/T	0.25	0.98	0.85 (0.79, 0.92)	0.010	0.86 (0.78, 0.94)	0.287	0.83 (0.74, 0.94)	0.486	
	rs115976249	intronic	G/A	0.07	0.94	0.76 (0.66, 0.87)	0.012	0.77 (0.65, 0.91)	0.369	0.75 (0.61, 0.92)	1.000	
TLR6	rs141273518	intronic	C/T	0.05	0.86	1.39 (1.18, 1.65)	0.017	1.33 (1.08, 1.63)	1.000	1.63 (1.27, 2.08)	0.015	
	rs141628846	upstream	G/A	0.02	0.97	1.68 (1.28, 2.20)	0.024	1.61 (1.16, 2.22)	0.556	2.02 (1.39, 2.96)	0.037	
ATP6AP2	rs115047524	intronic	G/A	0.09	0.98	0.80 (0.72, 0.90)	0.025	0.82 (0.71, 0.95)	0.639	0.80 (0.67, 0.96)	1.000	
IL21	rs76526843	intergenic	G/T	0.02	0.90	0.64 (0.49, 0.83)	0.028	0.74 (0.54, 1.00)	1.000	0.65 (0.42, 0.98)	1.000	
CADM1	rs73570052	intronic	A/C	0.04	0.97	1.43 (1.20, 1.70)	0.052	1.41 (1.14, 1.74)	0.843	1.58 (1.23, 2.05)	0.274	
BCL3	rs34698726	intergenic	A/T	0.32	0.91	1.16 (1.08, 1.25)	0.010	1.19 (1.09-1.30)	0.013	1.11 (0.99, 1.25)	1.000	
IL8	rs188246983	intergenic	T/C	0.07	0.99	1.17 (1.03, 1.34)	0.914	1.14 (0.97, 1.34)	1.000	1.49 (1.23, 1.81)	0.002	
	rs113976067	intergenic	T/C	0.22	1.00	1.10 (1.01, 1.19)	1.000	1.08 (0.98, 1.19)	1.000	1.23 (1.09, 1.39)	0.047	
MAP3K1	rs863839	intronic	A/G	0.08	1.00	0.96 (0.84, 1.09)	1.000	1.08 (0.93, 1.25)	1.000	0.65 (0.53, 0.81)	0.003	
	rs191188130	intronic	G/T	0.09	0.87	1.03 (0.91, 1.17)	1.000	0.95 (0.81, 1.11)	1.000	1.32 (1.10, 1.58)	0.052	
PRKDC	rs148411126	intronic	C/CT	0.08	0.98	1.16 (1.03, 1.32)	1.000	1.03 (0.89, 1.19)	1.000	1.45 (1.22, 1.72)	0.003	
	rs8178033	exonic nonsyn SNV	G/C	0.08	1.00	1.15 (1.02, 1.30)	1.000	1.02 (0.88, 1.19)	1.000	1.41 (1.19, 1.68)	0.011	
	rs56411879	intronic	T/C	0.02	0.86	1.16 (0.91, 1.48)	1.000	0.93 (0.69, 1.26)	1.000	1.81 (1.31, 2.50)	0.039	
Premenopausal												
SLA2	rs221310	intronic	A/G	0.73	gtyped	1.32 (1.16, 1.51)	0.003	1.26 (1.08, 1.48)	0.354	1.28 (1.06, 1.56)	0.949	
CXCR4	rs17848049	intergenic	G/C	0.09	1.00	0.65 (0.52, 0.80)	0.004	0.59 (0.45, 0.78)	0.009	0.74 (0.54, 1.01)	1.000	
PRG3	rs4411290	intergenic	C/G	0.47	1.00	0.79 (0.70, 0.89)	0.007	0.76 (0.66, 0.89)	0.016	0.84 (0.71, 1.00)	1.000	

Gene	SNP	Function	Ref /variant allele	MAF	PLINK INFO Score	All Cases			ER+		ER-	
						Per allele OR (95% CI)	Gene-wide P _{adj}	Per allele OR (95% CI)	Gene-wide P _{adj}	Per allele OR (95% CI)	Gene-wide P _{adj}	
IL21	rs1867128	intergenic	A/T	0.51	1.00	0.81 (0.72, 0.92)	0.039	0.78 (0.67, 0.91)	0.043	0.86 (0.72, 1.03)	1.000	
	rs115698762	intergenic	C/T	0.02	0.94	2.22 (1.45, 3.41)	0.008	2.19 (1.33, 3.60)	0.064	2.02 (1.07, 3.79)	0.934	
	rs143266239	intergenic	G/A	0.04	0.97	1.77 (1.29, 2.43)	0.013	1.80 (1.25, 2.60)	0.053	1.67 (1.06, 2.65)	0.914	
HLA-DMA	rs580962	intergenic	C/T	0.78	gtyped	1.29 (1.12, 1.49)	0.016	1.42 (1.19, 1.70)	0.004	1.19 (0.96, 1.46)	1.000	
	rs2232587	intronic	T/C	0.11	0.96	0.68 (0.56, 0.83)	0.025	0.64 (0.50, 0.82)	0.079	0.73 (0.55, 0.98)	1.000	
MAP3K1	rs252911	intergenic	A/G	0.83	0.99	1.22 (1.04, 1.43)	1.000	1.45 (1.18, 1.77)	0.043	1.03 (0.82, 1.29)	1.000	
IL10	chr1:206953202:1	intergenic	T/TC	0.11	0.92	1.21 (1.00, 1.48)	1.000	1.06 (0.83, 1.35)	1.000	1.73 (1.32, 2.26)	0.005	
	rs140929284	intergenic	TC/T	0.06	0.88	1.27 (0.98, 1.63)	1.000	1.10 (0.80, 1.52)	1.000	1.88 (1.35, 2.63)	0.015	
	rs74148793	intergenic	C/T	0.15	0.99	1.21 (1.02, 1.42)	0.990	1.12 (0.92, 1.37)	1.000	1.50 (1.19, 1.88)	0.035	
IL8	rs113976067	intergenic	T/C	0.21	1.00	1.20 (1.04, 1.38)	0.660	1.15 (0.97, 1.37)	1.000	1.50 (1.21, 1.85)	0.008	
	rs188246983	intergenic	T/C	0.07	0.99	1.25 (0.99, 1.58)	1.000	1.20 (0.91, 1.60)	1.000	1.78 (1.29, 2.45)	0.022	
CADM1	rs143193835	intronic	G/C	0.03	0.99	1.74 (1.22, 2.47)	0.091	1.45 (0.94, 2.23)	1.000	2.65 (1.69, 4.17)	0.016	
HELLS	rs200175744	intergenic	ACT/A	0.32	0.96	1.11 (0.97, 1.26)	1.000	1.03 (0.88, 1.20)	1.000	1.43 (1.18, 1.72)	0.017	
	rs10882476	intronic	T/G	0.09	0.99	1.05 (0.85, 1.30)	0.990	0.86 (0.66, 1.13)	1.000	1.64 (1.23, 2.17)	0.049	
	rs11188009	intergenic	A/G	0.47	0.98	1.08 (0.96, 1.22)	1.000	1.00 (0.87, 1.16)	1.000	1.38 (1.16, 1.66)	0.032	
Postmenopausal												
POU2AF1	rs75716067	intronic	A/G	0.03	1.02	0.53 (0.40, 0.71)	0.002	0.46 (0.32, 0.66)	0.003	0.60 (0.38, 0.93)	1.000	
HLA-DQA1	rs115976249	intronic	G/A	0.07	0.94	0.66 (0.54, 0.80)	0.004	0.70 (0.56, 0.88)	0.359	0.61 (0.45, 0.83)	0.332	
	rs2390350	intronic	A/G	0.51	1.00	1.19 (1.08, 1.30)	0.008	1.16 (1.04, 1.29)	0.236	1.16 (1.00, 1.33)	1.000	
CYP4F11	rs17005895	intergenic	A/T	0.15	0.97	1.27 (1.11, 1.44)	0.014	1.21 (1.03, 1.41)	0.363	1.32 (1.08, 1.61)	0.160	
	rs4572524	intergenic	A/G	0.65	0.87	0.82 (0.73, 0.90)	0.015	0.84 (0.74, 0.95)	0.560	0.80 (0.68, 0.93)	0.659	
IL2RB	rs73406995	intergenic	C/G	0.16	0.97	0.78 (0.68, 0.88)	0.021	0.77 (0.66, 0.90)	0.186	0.71 (0.58, 0.87)	0.207	
UBE2N	rs76506230	intergenic	T/C	0.02	0.77	2.12 (1.40, 3.22)	0.026	2.52 (1.57, 4.03)	0.008	2.13 (1.18, 3.86)	0.814	
CASP8	rs55637196	intronic	G/A	0.20	0.99	0.81 (0.72, 0.91)	0.034	0.78 (0.68, 0.90)	0.065	0.81 (0.67, 0.97)	1.000	
IRAK2	rs149858020	intronic	C/T	0.13	0.99	1.31 (1.14, 1.50)	0.038	1.23 (1.05, 1.45)	1.000	1.51 (1.24, 1.85)	0.017	
CD274	rs2890657	intronic	G/C	0.04	0.97	0.75 (0.59, 0.95)	1.000	0.56 (0.42, 0.76)	0.016	0.84 (0.59, 1.20)	1.000	
	rs10481593	intronic	G/A	0.24	gtyped	0.87 (0.78, 0.97)	0.655	0.80 (0.70, 0.91)	0.040	0.91 (0.77, 1.07)	1.000	

Gene	SNP	Function	Ref /variant allele	MAF	PLINK INFO Score	All Cases			ER+		ER-	
						Per allele OR (95% CI)	Gene-wide Padj	Per allele OR (95% CI)	Gene-wide Padj	Per allele OR (95% CI)	Gene-wide Padj	
CALCA	rs34587547	exonic nonsyn SNV	C/G	0.01	0.99	2.23 (1.26, 4.29)	0.130	3.20 (1.67, 6.16)	0.024	1.50 (0.54, 4.13)	1.000	
XCRI	rs2373148	intergenic	T/C	0.85	1.01	0.85 (0.75, 0.96)	0.361	0.78 (0.67, 0.90)	0.026	0.89 (0.73, 1.07)	1.000	
	rs2371	upstream	A/G	0.91	0.98	0.82 (0.70, 0.96)	0.382	0.73 (0.61, 0.88)	0.033	0.85 (0.67, 1.08)	1.000	
PRKDC	rs8178153	intronic	C/T	0.08	0.96	1.26 (1.06, 1.50)	1.000	1.09 (0.88, 1.34)	1.000	1.60 (1.24, 2.06)	0.028	
CD247	rs12066323	intronic	G/A	0.13	0.98	1.12 (0.97, 1.28)	1.000	0.98 (0.83, 1.15)	1.000	1.49 (1.22, 1.82)	0.037	
SMAD3	rs2289259	intronic	G/A	0.34	1.00	1.08 (0.98, 1.19)	1.000	1.00 (0.90, 1.12)	1.000	1.34 (1.15, 1.55)	0.049	

Ref: Referent; MAF: Minor allele frequency; SNP: Single nucleotide polymorphism; gtyped: genotyped.

P-values for SNPs with corrected gene-wide significance at p 0.05 are indicated in bold text.

syn SNV: synonymous single nucleotide variant; nonsyn SNV: nonsynonymous single nucleotide variant