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Hormone-Related Pathways and Risk of Breast Cancer Subtypes in African American Women

Stephen A. Haddad[1], **Kathryn L. Lunetta**[2], **Edward A. Ruiz-Narváez**[1], **Jeannette T. Bensen**[3], **Chi-Chen Hong**[4], **Lara E. Sucheston-Campbell**[4], **Song Yao**[4], **Elisa V. Bandera**[5], **Lynn Rosenberg**[1], **Christopher A. Haiman**[6], **Melissa A. Troester**[3], **Christine B. Ambrosone**[4], and **Julie R. Palmer**[1]

[1]Slone Epidemiology Center at Boston University, Boston, MA

[2]Department of Biostatistics, Boston University School of Public Health, Boston, MA

[3]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC

[4]Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY

[5]Cancer Prevention and Control, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ

[6]Department of Preventive Medicine, Keck School of Medicine, University of Southern California/ Norris Comprehensive Cancer Center, Los Angeles, CA

Abstract

Purpose—We sought to investigate genetic variation in hormone pathways in relation to risk of overall and subtype-specific breast cancer in women of African ancestry (AA).

Methods—Genotyping and imputation yielded data on 143,934 SNPs in 308 hormone-related genes for 3663 breast cancer cases (1098 ER-, 1983 ER+, 582 ER unknown) and 4687 controls from the African American Breast Cancer Epidemiology and Risk (AMBER) Consortium. AMBER includes data from four large studies of AA women: the Carolina Breast Cancer Study, the Women's Circle of Health Study, the Black Women's Health Study, and the Multiethnic Cohort Study. Pathway- and gene-based analyses were conducted, and single SNP tests were run for the top genes.

Results—There were no strong associations at the pathway level. The most significantly associated genes were *GHRH, CALM2, CETP,* and *AKR1C1* for overall breast cancer (gene-based nominal p ≤0.01); *NR0B1, IGF2R, CALM2, CYP1B1,* and *GRB2* for ER+ breast cancer (p ≤0.02); and *PGR, MAPK3, MAP3K1,* and *LHCGR* for ER- disease (p ≤0.02). Single-SNP tests for SNPs with pairwise linkage disequilibrium r^2 <0.8 in the top genes identified 12 common SNPs (in *CALM2, CETP, NR0B1, IGF2R, CYP1B1, PGR, MAPK3,* and *MAP3K1)* associated

Corresponding author: Stephen A. Haddad, Slone Epidemiology Center at Boston University, 1010 Commonwealth Avenue, Boston, MA 02215. Phone: 617-206-6177; Fax: 617-738-5119; sahaddad@bu.edu.

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with overall or subtype-specific breast cancer after gene-level correction for multiple testing. Rs11571215 in *PGR* (progesterone receptor) was the SNP most strongly associated with ER- disease.

Conclusion—We identified eight genes in hormone pathways that contain common variants associated with breast cancer in AA women after gene-level correction for multiple testing.

Keywords

breast cancer; genetics; pathways; hormones; African Americans

Introduction

Women of African ancestry (AA) have been under-represented in genetic studies of breast cancer to date. At the same time, AA women experience higher mortality from breast cancer compared to women of European ancestry [1] and are more likely to be diagnosed with estrogen receptor negative (ER-) tumors, which carry a poor prognosis [2–6]. Given these racial disparities, it is critical that more studies be conducted in AA women, taking advantage of their greater genetic variability to identify risk variants.

There is extensive evidence that steroid hormones affect breast cancer risk. In vitro studies have shown that estrogens and other hormones promote breast cell proliferation [7, 8], and estrogen metabolites may initiate DNA damage and mutations [9–13]. Also, increased rates of mammary tumor development have been reported in rodents given estrogens [7, 14]. In humans, reproductive and hormonal factors such as age at menarche, parity, lactation, and use of exogenous estrogens and progestogens are associated with the risk of breast cancer [15–23]. Prospective studies in humans have shown that low blood concentrations of sex-hormone-binding globulin (SHBG) [24, 25] and increased estrogen [24–27] and androgen [24–28] concentrations are associated with an increased risk of breast cancer. Hormonal profiles vary by race [29], and polymorphisms in steroid hormone pathway genes have been linked to hormone levels [30–34]. Therefore, differences in population allele frequencies at these loci may contribute to racial disparities in breast cancer.

At least three breast cancer GWAS loci, *ESR1* [35], *MAP3K1* [36], and *ITPR1* [37], fall within biological pathways related to steroid hormone metabolism. *ESR1* and *MAP3K1* associations have also been replicated in AA populations [38–42]. Candidate gene studies conducted by the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3) [7] in more than 6,000 breast cancer cases and 8,000 controls found no significant associations with 37 steroid hormone metabolism genes [7, 34, 43–48]. However, the BPC3 subjects were mostly of European ancestry, and the list of assayed genes was limited. Therefore, the present study in an African American population was initiated to evaluate a more comprehensive set of steroid hormone metabolism genes for associations with overall, ER+, and ER- breast cancer. Given the small effect sizes seen for common susceptibility variants, the present analyses utilized pathway- and gene-based testing approaches in an attempt to identify important biological pathways and genes with multiple risk variants that might otherwise be missed in a SNP-based approach.

Methods

Study Population

The present analyses were conducted using data from the African American Breast Cancer Epidemiology and Risk (AMBER) Consortium, a collaboration of four of the largest studies of breast cancer in African American women. The AMBER Consortium [49] and the four individual studies – the Carolina Breast Cancer Study (CBCS) [50], the Women's Circle of Health Study (WCHS) [51, 52], the Black Women's Health Study (BWHS) [53], and the Multiethnic Cohort (MEC) [54] – have been described previously, and each was granted Institutional Review Board approval. All study subjects provided informed consent.

Briefly, the CBCS is a population-based case-control study of women aged 20 to 74 years that began in North Carolina in 1993. Cases were identified through the North Carolina Central Cancer Registry's rapid case ascertainment system, and controls were enrolled through 2001 using Division of Motor Vehicles lists (age <65 years) and Health Care Financing Administration lists (age ≥65). Questionnaire data and samples for DNA analysis were obtained by interviewers in home visits.

The WCHS is an ongoing case-control study that began in 2002 with ascertainment of cases aged 20 to 75 years from New York City hospitals, later expanding to several counties in New Jersey with case identification using the New Jersey State Cancer Registry's rapid case ascertainment system. Controls have been recruited through random digit dialing as well as community-based efforts [52]. In-person interviewers collect risk factor data and obtain samples for DNA analysis.

The BWHS is a prospective cohort study that began in 1995 when 59,000 African American women 21-69 years of age from across the United States completed a postal health questionnaire. Breast cancer cases are identified by self-report in biennial follow-up questionnaires, and cases are confirmed by medical records or from state cancer registry data and the National Death Index. Approximately 27,000 BWHS participants provided saliva samples for DNA analysis.

The MEC study is a prospective cohort study in Hawaii and California that began in 1993 with the enrollment of men and women aged 45-75 years. Data is collected through questionnaires mailed at 5-year intervals, and breast cancer cases are confirmed by linkage with the California and Hawaii state cancer registries and the National Death Index. Blood samples were obtained from study subjects for DNA analysis.

Eligible cases were women with incident invasive breast cancer or ductal carcinoma in situ, with available DNA for genotyping. For BWHS and MEC, controls were selected from among participants who did not have breast cancer, and were frequency matched to cases on 5-year age group and geographical region. Determination of ER status for cases was based on pathology data from hospital records or cancer registry records.

SNP Selection

We selected eight pathways related to steroid hormone synthesis from the Molecular Signatures Database (MSigDB) [55]. These pathways contained a total of 261 genes. We also added some specific genes (N=47) that were not in those eight pathways but had been associated with reproductive traits in candidate gene studies. Tag SNPs were then selected for all 308 genes +/- 10kb flanking regions in order to capture (at $r^2 \geq 0.8$) as many SNPs as possible with minor allele frequency ≥10%, based on the haplotype structure of the Yoruban population (YRI) in 1000 Genomes [56] (http://www.1000genomes.org/).

Genotyping and QC

Genotypes were attempted for 6,936 study subjects from the BWHS, CBCS, and WCHS, and were completed with call rate >98% for 6,828 participants, which included 3,130 cases (963 ER-, 1,674 ER+, 493 ER unknown) and 3,698 controls. The variants considered in the present study had been included as part of >159,000 custom content SNPs added to an Illumina Exome Beadchip in line with the aims of the AMBER project. SNPs that were monomorphic, had Hardy-Weinberg Equilibrium $p < 1 \times 10^{-4}$, call rate <0.98, or had >1 Mendelian error in trios from HapMap [57], or >2 discordant calls in duplicate samples, were excluded. A total of 9,576 SNPs in the 308 genes of interest for this study were successfully genotyped and passed quality control. The University of Washington performed imputation using the IMPUTE2 software [58] and the 1000 Genomes Phase I reference panel (5/21/2011 1000 Genomes data, December 2013 haplotype release).

Genetic data from 533 cases (135 ER-, 309 ER+, 89 ER unknown) and 989 controls in the MEC were available from a previous GWAS on the Illumina Human 1M-Duo chip [59], with SNPs imputed to the same release of 1000 Genomes. Imputed genotypes from MEC were combined with imputed data for the BWHS, CBCS, and WCHS into a final data set. After excluding variants with mismatching alleles or allele frequencies that were different by more than 0.15 in MEC vs. the other three studies, and variants with allele frequencies <0.5% or imputation score INFO <0.5 in either study, the final data set for analysis contained 143,934 genotyped and imputed SNPs in the 308 genes of interest. The final sample size for analysis was 8,350 total subjects: 3,663 cases (1,098 ER-, 1,983 ER+, 582 ER unknown) and 4,687 controls.

Genotype principal components were computed using the smartpca program in the EIGENSOFT package [60]. Relationship checking using PLINK version 1.07 [61] identified several related subjects across and within the individual studies. Relatives (N=156) and those with more extreme principal components (N=35) were flagged so that sensitivity analyses could be performed. The principal components of genotype were tested for association with case status after accounting for the study covariates: study, DNA source (blood, saliva[Oragene], saliva[mouthwash]), and the matching variables age and geographic region. No principal components were strongly associated with case status after controlling for these covariates. For case status and subtype association analyses, we included principal components that were associated in the multivariable model with p <0.1.

Association Analysis

Pooled, gene-based pathway analyses were conducted using the adaptive rank truncated product (ARTP) statistic [62] as implemented in the R package PIGE [63]. The ARTP method was chosen for its ability to optimize the number of SNP p-values combined in each gene-level test and the number of gene p-values combined in each pathway test. Prior to implementing this approach, a subset of SNPs was selected such that all SNP pairs had linkage disequilibrium (LD) r^2 <0.8 using the filter.R2 option in the R package AdaJoint [64]. We call this the "pruned-in" set of SNPs. This pruning process was done to avoid capturing only one or two association signals for some genes due to correlations between their top SNPs. Based on the program parameters chosen, the ARTP gene-level tests combined the optimal number of most significant SNP p-values from among the top 10 pruned-in SNPs for each gene. The ARTP pathway tests combined the optimal percentage (in 5% increments) of the most significant gene p-values in each pathway, without exceeding 50%.

The single SNP association tests, required as input to do the ARTP analyses, were performed using logistic regression analyses of the imputed dosage genotype data. All statistical models were adjusted for study, age, geographic region, DNA source, and genotype principal components 5, 6, and 8.

Results

Pathway analyses yielded one nominal association, for the Steroid Biosynthetic Process Pathway with ER+ breast cancer (p = 0.046). Given the eight pathways tested, this result was not considered to be significant or borderline. Because of the null results seen at this level of analysis (Supplementary Table S1) and our objective of implicating specific genes and SNPs, we turned our focus to the gene-level test results.

Gene-level testing produced a number of nominally significant associations with overall, ER +, and ER- breast cancer (Table 1), although none survived a Bonferroni correction based on the 308 genes tested (Supplementary Table S1). The top four genes for overall, ER+, and ER- breast cancer were selected for follow-up. For overall breast cancer, the most significant gene was *GHRH,* with p = 0.001; the other top genes were *CALM2, CETP,* and *AKR1C1,* which all had p ≤0.01. For ER+ breast cancer, *NR0B1* was the most significant gene, with p = 0.001, and the other top genes, all with p ≤0.02, were *IGF2R, CYP1B1,* and *GRB2* (as well as *CALM2,* which was also a top gene for overall breast cancer). For ER- disease, *PGR* was the top result with p = 0.003, and the other top genes were *MAPK3, MAP3K1,* and *LHCGR,* each with p ≤0.02.

Individual SNP associations within the 12 prioritized genes were then examined, and 17 SNPs across 11 of the 12 genes (none in *GRB2*) survived a within gene correction for multiple testing based on the number of SNPs pruned in for each gene. We refer to these SNPs as "gene-wide significant". Five of these SNPs (two in *GHRH* and one in each of *AKR1C1, MAP3K1,* and *LHCGR*) were imputed SNPs with allele frequencies <1%; thus, we did not consider those SNPs further (or genes *GHRH, AKR1C1,* and *LHCGR,* which contained no other SNPs of interest). Of the 12 remaining gene-wide significant SNPs, three

were associated with overall breast cancer, four with ER+ breast cancer, and five with ER- disease (Table 2).

CALM2 was the most significant gene of interest for overall breast cancer. The most significant SNP in *CALM2* was rs13032512 ($p = 1.3\times10^{-4}$). The A allele at this SNP had a frequency of 5.5% in AMBER controls and was associated with an increased risk of breast cancer (OR 1.33, 95% CI 1.15, 1.54). The most significant SNP in the top ER+ gene, *NR0B1,* was rs138860909 ($p = 3.4\times10^{-4}$). The A allele at this SNP had a frequency of 16.6% in AMBER controls and was associated with a decreased risk of ER+ breast cancer (OR 0.80, 95% CI 0.71, 0.90). The most significant SNP in the top ER- gene, *PGR*, was rs11571215 ($p = 1.0\times10^{-5}$). The C allele at this SNP had a frequency of 9.2% in AMBER controls and was associated with a decreased risk of ER- breast cancer (OR 0.64, 95% CI 0.52, 0.78). In addition, a common nonsynonymous coding SNP in gene *CYP1B1* (rs10012) was associated with an odds ratio of 0.85 (95% CI 0.79, 0.92; $p = 1.1\times10^{-4}$) for ER+ breast cancer.

Given that the BWHS, WCHS, and CBCS subjects were genotyped and imputed together, separately from the MEC subjects, we checked and confirmed that the allele frequencies for the 12 common gene-wide significant SNPs were similar in the two groups (MEC vs. non-MEC). Also, effect estimates were in the same direction and most were of similar magnitude (data not shown).

As expected, the most significant genes – *CALM2* for overall breast cancer, *NR0B1* for ER+ breast cancer, and *PGR* for ER- disease – each contained multiple SNPs with gene-wide significance. The two SNPs in *CALM2* had $r^2 = 0.53$, while the two SNPs in *NR0B1* were not correlated ($r^2 = 0.07$). For *PGR*, two SNPs were strongly correlated ($r^2 = 0.74$), but neither was strongly correlated with the third ($r^2 < 0.5$).

Allele frequencies in AMBER controls and 1000 Genomes African samples were similar. However, the minor allele frequencies for 10 of the 12 SNPs of interest were lower in 1000 Genomes Europeans vs. Africans (Table 2). Furthermore, 5 of the 12 SNPs of interest had 1000 Genomes European allele frequencies <1%, and 2 of these were monomorphic in 1000 Genomes Europeans.

In a sensitivity analysis, we reanalyzed these 12 SNPs with exclusion of 156 first-degree or second-degree relatives (identified via the genotypes), as well as 35 PCA outliers who clustered with HapMap 3 Europeans, Mexicans, or Asians. With these exclusions, all odds ratios changed by ≤3%. Therefore, results of the original analysis on the full sample remained the focus for interpretation.

Discussion

Our analyses found eight genes that may be involved in the etiology of breast cancer in African American women: *CETP* for overall breast cancer; *CALM2* for overall and ER+ breast cancer; *NR0B1, IGF2R,* and *CYP1B1* for ER+ disease; and *PGR, MAPK3,* and *MAP3K1* for ER- disease.

The most significant of these were *CALM2* (overall breast cancer), *NR0B1* (ER+), and *PGR* (ER-). The most significant SNP in *CALM2,* intronic SNP rs13032512, is less common in 1000 Genomes European ancestry samples (2.2%) than in African samples (5.9%). The other gene-wide significant SNP in *CALM2,* intronic SNP rs114416221, is rare in 1000 Genomes Europeans (0.1%). *CALM2* maps to chromosome 2p21 and is one of three genes that encode the protein calmodulin (CaM). *CALM2* was included in the present study because of CaM's involvement in gonadotropin-releasing hormone (GnRH) signaling: GnRH induces calcium influx, which activates CaM leading to a variety of downstream effects that result in gonadotropin gene expression [65]. Thus, *CALM2* may impact breast cancer susceptibility through its effects on hormone synthesis.

The most significant SNP in the *NR0B1* region, rs138860909, is located about 2kb upstream of the gene. This SNP is rare in 1000 Genomes European ancestry samples (0.3%). *NR0B1* maps to chromosome Xp21.2 and encodes the orphan nuclear receptor DAX1, for which high expression has been associated with excellent survival in node-negative breast cancer [66]. DAX-1 is expressed in tissues involved in steroid hormone function and acts as an anti-steroidogenic factor by serving as a corepressor for the expression of enzymes such as aromatase [67]. The ability of DAX-1 to inhibit aromatase expression suggests a possible role for *NR0B1* variants in the etiology of ER+ breast cancer, consistent with our results showing that associations with the top *NR0B1* SNPs were restricted to ER+ disease.

The most significant SNP in the *PGR* gene, intronic SNP rs11571215, is monomorphic in 1000 Genomes European ancestry samples. *PGR* is the progesterone receptor gene, located on chromosome 11q22.1. Multiple studies have reported breast cancer associations with *PGR* SNP rs1042838 [68–71], but a later, larger meta-analysis showed no association [72]. Breast cancer associations have also been reported in a small AA sample for rs590688 and rs10895054 [73]. These three SNPs were not associated with breast cancer in the present study. Several studies have reported evidence that *PGR* variants modify the effect of hormone replacement therapy on breast cancer risk [74–76], and a few studies have reported *PGR* associations with mammographic density [77, 78]. Still, the role of *PGR* variants in breast cancer development remains uncertain. Nevertheless, the associated variants in this AA study are of interest given that they are absent from or rare in European ancestry populations, the source of subjects for most prior studies.

Associations observed for *CYP1B1* are also of interest. This gene contains two common missense SNPs that were gene-wide significant for ER+ breast cancer: rs10012 (Arg48Gly) and rs1056827 (Ala119Ser). These two SNPs have previously been associated with other cancers (prostate [79–81], lung [82], endometrial [81, 83]) in mostly European samples. Modest associations have also been reported for rs1056827 with breast cancer in recent meta-analyses [80, 81], although another meta-analysis reported no association [84]. These two SNPs were correlated at $r^2 = 0.87$ in AMBER, and only rs10012 remained after pruning.

CYP1B1 maps to chromosome 2p22.2 and encodes the cytochrome P-450 1B1 enzyme, which is expressed in breast tumors [85–87]. CYP1B1 is capable of activating a variety of carcinogens and is responsible for the 4-hydroxylation of estradiol [85, 88], leading to the formation of carcinogenic semiquinones and quinones [82]. Our study suggested a protective

effect for the 48Gly allele. It is unclear what mechanism would explain such an effect given that this allele has been shown to cause enhanced activity of the CYP1B1 enzyme [89], which one might assume would lead to increased risk. However, the all cases odds ratio of 0.92 (95% CI 0.86, 0.99) in the current study is consistent with the odds ratio of 0.93 (95% CI 0.81, 1.08) reported for carriers of one Gly allele in the meta-analysis by Economopoulos and Sergentanis [84]. Another meta-analysis reported an odds ratio of 0.93 (95% CI 0.79, 1.10) for the 48Gly variant based on a recessive model [81]. While these modest associations were not significant in either meta-analysis, these studies did not conduct analyses by ER subtype, whereby we discovered a stronger ER+ association. Further evidence of subtype-specific effects comes from Wen and colleagues who reported that patients carrying the 48Gly allele were less likely to have ER+ disease [90]. Also of note, prior studies have included mostly Caucasian subjects, and the 48Gly allele, with a frequency of 29.0% in 1000 Genomes Europeans, is the major allele in African populations (57.4%).

One of the implicated genes from our study, *MAP3K1*, is a breast cancer GWAS locus [36]. Following its discovery, replication and fine mapping studies in Europeans, Asians, and AAs confirmed and identified a number of associated SNPs in this region [37, 39, 41, 42, 91– 96]. Two of these SNPs, rs16886397 and rs832539, were analyzed in the present study. Results for rs832539 were null, while rs16886397, previously shown to affect *MAP3K1* transcription [96], was nominally associated with ER+ breast cancer (OR = 1.22 for the G allele, p = 0.044). Of note, although *MAP3K1* was one of the top ER- genes in our analysis, it was also nominally associated with ER+ disease (p = 0.023). None of the gene-wide significant *MAP3K1* SNPs from our analysis were in high LD with the top *MAP3K1* SNPs from the literature, based on 1000 Genomes African ancestry samples.

Most of the SNPs of interest from the current study are of lower frequency in European vs. African populations. This may explain why aside from *MAP3K1*, statistically significant associations have not been found for these same genes/variants in the numerous European breast cancer GWAS. Reported gene associations may represent the causal effects of one or multiple variants. Therefore, although these analyses identify specific SNPs for follow-up, future work should consider the entire gene.

Despite having over 3,500 cases and 4,500 controls, the present study had limited power to detect individual SNP associations of small magnitude as well as stronger associations for rare SNPs, especially for subtype analyses. Nevertheless, it is the largest study to date on the genetics of breast cancer in African Americans. Results from sensitivity analyses minimized concerns about potential bias from PCA outliers or inflation of test statistics by relatives in the study. Most of the SNPs of interest presented here were imputed. Although this is a potential limitation, we focused on common SNPs with high imputation INFO scores (Table 2).

In summary, gene-based and single SNP analyses suggested that *CALM2*, *CETP*, *NR0B1, IGF2R, CYP1B1*, *PGR, MAPK3,* and *MAP3K1* may be involved in the etiology of breast cancer in African American women. The most significantly associated genes containing common SNPs of interest were *CALM2* for overall breast cancer, *NR0B1* for ER+ breast

cancer, and *PGR* for ER- disease. Several of the top SNPs identified here are rare or absent in European populations, possibly explaining their lack of discovery to this point.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Associations of genes from steroid hormone pathways with overall, ER+, and ER- breast cancer risk in the AMBER Consortium

Gene	Number of SNPs	Number of SNPs retained after pruning	All cases p-value	ER+ p-value	ER- p-value
GHRH	166	101	**1.0×10^{-3}**	0.16	0.41
CALM2	158	78	**5.0×10^{-3}**	**0.013**	0.48
CETP	312	176	**9.0×10^{-3}**	0.31	0.30
AKR1C1	484	143	**0.010**	0.15	0.061
CGA	101	53	**0.013**	0.074	0.74
CAMK2A	555	331	**0.016**	0.13	0.41
HSD17B3	509	191	**0.018**	**0.022**	0.27
CAMK2G	224	69	**0.018**	0.20	0.14
SULT1E1	206	89	**0.028**	0.32	0.12
PRKCD	298	158	**0.034**	0.096	0.30
ITPR2	3130	1251	**0.041**	0.36	0.61
GSTP1	170	75	**0.049**	0.41	0.14
NR0B1	79	68	0.067	**1.0×10^{-3}**	0.88
IGF2R	883	401	0.052	**7.0×10^{-3}**	0.59
CYP1B1	139	75	0.51	**0.013**	0.20
GRB2	607	129	0.33	**0.019**	0.14
SLC10A2	329	153	0.15	**0.025**	0.73
DIO3	53	30	0.59	**0.027**	0.75
CYP2R1	63	43	0.076	**0.028**	0.42
ADCY9	1414	644	0.38	**0.031**	0.85
PGR	711	202	0.47	0.61	**3.0×10^{-3}**
MAPK3	24	19	0.75	0.95	**0.010**
MAP3K1	544	141	0.13	**0.023**	**0.015**
LHCGR	426	224	0.84	0.81	**0.020**
SULT2B1	604	290	0.27	0.59	**0.026**
LSS	284	115	0.75	0.85	**0.026**
PLCB3	83	54	0.060	0.62	**0.029**
HSD17B2	677	296	0.12	0.18	**0.037**
DPE P2	128	49	0.78	0.69	**0.038**
MAP2K7	78	45	0.53	0.77	**0.039**
FABP6	513	216	0.72	0.15	**0.040**
PRKACA	99	51	0.71	0.64	**0.044**
HSD11B2	45	24	0.82	0.54	**0.048**
ACOT8	103	56	0.10	0.79	**0.049**

ER+ estrogen receptor positive, *ER-* estrogen receptor negative

Nominally significant results in bold font

Table 2

Relation of SNPs selected from gene-based analyses to risk of overall, ER+, and ER- breast cancer in AMBER

Gene	P-value threshold for gene-wide significance	SNP	Reference/effect allele	Effect allele frequency (%): AMBER controls, 1000G AFR, 1000G EUR	PLINK INFO score: All AMBER, BWHS/WCHS/ CBCS, MEC	3663 cases, 4687 controls		1983 ER+ cases, 4687 controls		1098 ER- cases, 4687 controls	
						OR	p-value	OR	p-value	OR	p-value
SNPs Gene-Wide Significant for All Breast Cancer											
CALM2	6.4×10^{-4}	rs13032512	G/A	5.5, 5.9, 2.2	0.92, 0.98, 0.77	**1.33**	1.3×10^{-4}	1.30	4.1×10^{-3}	1.35	8.2×10^{-3}
		rs114416221	T/G	2.6, 4.5, 0.1	0.97, 0.97, 0.97	**1.43**	3.6×10^{-4}	**1.58**	8.9×10^{-5}	1.40	0.026
CETP	2.8×10^{-4}	rs28888131	G/A	19.9, 19.4, 18.5	0.97, 0.98, 0.95	**0.84**	8.9×10^{-5}	0.87	8.0×10^{-3}	0.82	3.5×10^{-3}
SNPs Gene-Wide Significant for ER+ Breast Cancer											
NR0B1	7.4×10^{-4}	rs138860909	G/A	16.6, 22.8, 0.3	0.87, 0.87, 0.91	0.88	0.012	**0.80**	3.4×10^{-4}	1.00	0.97
		rs5927492	G/A	40.2, 34.7, 82.1	gtyped, gtyped, gtyped	1.06	0.095	**1.15**	6.8×10^{-4}	0.94	0.21
IGF2R	1.2×10^{-4}	rs76778371	G/A	7.2, 4.8, 7.7	0.94, 0.93, 0.97	0.77	2.1×10^{-4}	**0.68**	2.0×10^{-5}	0.92	0.41
CYP1B1	6.7×10^{-4}	rs10012	G/C	50.8, 57.4, 29.0	0.99, 0.99, 0.98	0.92	0.023	**0.85**	1.1×10^{-4}	1.01	0.81
SNPs Gene-Wide Significant for ER- Breast Cancer											
PGR	2.5×10^{-4}	rs11571215	T/C	9.2, 12.0, 0.0	0.97, 0.98, 0.93	0.90	0.083	1.02	0.75	**0.64**	1.0×10^{-5}
		rs11571247	T/C	11.7, 14.4, 0.0	0.99, 1.00, 0.94	0.94	0.29	1.08	0.24	**0.69**	4.8×10^{-5}
		rs2124761	C/A	17.5, 21.9, 0.7	0.99, 1.00, 0.94	0.90	0.020	0.96	0.48	**0.76**	1.6×10^{-4}
MAPK 3	2.6×10^{-3}	rs78564187	G/A	18.0, 21.0, 9.4	1.03, gtyped, 0.99	1.07	0.13	1.03	0.58	**1.26**	3.7×10^{-4}
MAP3K1	3.5×10^{-4}	rs10075381	A/C	8.0, 3.6, 35.6	1.00, gtyped, 0.97	0.94	0.35	1.05	0.55	**0.66**	1.5×10^{-4}

1000G 1000 Genomes Project Phase 3, *AFR* African, *EUR* European, *OR* odds ratio (adjusted for study, age, region, DNA source, and genotype principal components), *gtyped* genotyped

Odds ratios in bold font indicate the phenotype (overall, ER+, or ER- breast cancer) for which the gene was a top hit and the SNP was gene-wide significant. *CALM2* was a top hit for both overall and ER+ breast cancer, and the *CALM2* SNP rs114416221 was gene-wide significant for both of these outcomes.