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Diet and lifestyle factors modify immune/inflammation response genes to alter breast cancer risk and prognosis: The Breast Cancer Health Disparities Study

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ABSTRACT

Tumor necrosis factor-α (TNF) and toll-like receptors (TLR) are important mediators of inflammation. We examined 10 of these genes with respect to breast cancer risk and mortality in a genetically admixed population of Hispanic/Native American (NA) (2111 cases, 2597 controls) and non-Hispanic white (NHW) (1481 cases, 1585 controls) women. Additionally, we explored if diet and lifestyle factors modified associations with these genes. Overall, these genes (collectively) were associated with breast cancer risk among women with >70% NA ancestry (P_{ARTP} = 0.0008), with *TLR1* rs7696175 being the primary risk contributor (OR 1.77, 95% CI 1.25, 2.51). Overall, *TLR1* rs7696175 (HR 1.40, 95% CI 1.03, 1.91; P_{adj} = 0.032), *TLR4* rs5030728 (HR 1.96, 95% CI 1.30, 2.95; P_{adj} = 0.014), and *TNFRSF1A* rs4149578 (HR 2.71, 95% CI 1.28, 5.76; P_{adj} = 0.029) were associated with increased breast cancer mortality. We observed several statistically significant interactions after adjustment for multiple comparisons, including interactions between our dietary oxidative balance score and *CD40LG* and *TNFSF1A*; between cigarette smoking and *TLR1*, *TLR4*, and *TNF*; between body mass index (BMI) among pre-menopausal women and *TRAF2*; and between regular use of aspirin/non-steroidal anti-inflammatory drugs and *TLR3* and *TRA2*. In conclusion, our findings support a contributing role of certain *TNF*-α and *TLR* genes in both breast cancer risk and survival, particularly among women with higher NA ancestry. Diet and lifestyle factors appear to be important mediators of the breast cancer risk associated with these genes.

1. Introduction

Tumor necrosis factor-α (TNF), a pro-inflammatory cytokine, stimulates cell proliferation and induces cell differentiation and is thought to be one of the most important promoters of inflammation. Additionally, TNF is a modulator of insulin resistance, especially among individuals who are obese or have chronic inflammation conditions; TNF has been reported to inhibit insulin-induced glucose uptake by targeting components of the insulin-signaling cascade [1–5]. TNF mediates cell survival and apoptosis through TNF receptors by activating at least two major signaling pathways, NFκB and the p38 mitogen-activated protein (MAP) kinase pathway. Tumor necrosis factor receptor superfamily member 1A (*TNFRSF1A* or *TNFR1*) is a major receptor for TNF-alpha that activates NFκB, mediates apoptosis, and functions as a regulator of inflammation. TNF receptor-associated factor 2 (TRAF2) is a member of the TRAF protein family that interacts with TNF receptors. TRAF2 is required for TNF activation of mitogen activated protein kinase 8 (MAPK8 alias JNK1) as well as NFκB and therefore is thought to influence the apoptotic effects of TNF. TNFSF10 (TRAIL) protein expression has been elevated in adriamycin-treated breast cells [6]. This protein preferentially

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induces apoptosis in transformed and tumor cells. CD40LG, also known as TNFSF5 and TRAP, is involved in TNF-signaling pathway and related cytokine activity. Toll-like receptors (TLR) also are mediators of inflammation and potentially important modulators of cancer risk through their involvement in the NFκB-signaling pathway [7,8]. TLR4 specifically has been linked to breast cancer [9] and to colon tumor progression and metastatic potential [10,11]. TRAIL has been designated CD253 (cluster of differentiation 253); TLR2 has been designated as CD282; and TLR3 has been designated as CD283.

In this study we examine genetic variation in *TLR* and *TNF*-related genes as they relate to breast cancer risk and survival. *TNF* rs1800629 has been associated with breast cancer risk in a small case–control study of Mexican women [12], suggesting that this gene and possibly its related pathway are important for breast cancer risk in Latina women. We evaluate associations by genetic ancestry since breast cancer incidence rates differ between non-Hispanic white (NHW), Hispanic, and Native American (NA) women living in the Southwestern United States [13]. We also evaluate associations by lifestyle factors that are associated with inflammation and insulin and could therefore modify risk associated with these genes and pathway. Factors we evaluate include dietary oxidative balance score (DOBS) [14], body mass index (BMI), regular cigarette smoking, use of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs), and having been diagnosed with diabetes. Given the association of these genes with apoptosis and metastatic potential, we evaluate their association with breast cancer mortality.

2. Methods

The Breast Cancer Health Disparities Study includes participants from three population-based case–control studies [13], the 4-Corners Breast Cancer Study (4-CBCS) [15], the Mexico Breast Cancer Study (MBCS) [16], and the San Francisco Bay Area Breast Cancer Study (SFBCS) [17,18], who completed an in-person interview and who had a blood or mouthwash sample available for DNA extraction. Information on exposures was collected up to the referent year, defined as the calendar year before diagnosis for cases or before selection into the study for controls. 4-CBCS participants were between 25 and 79 years; MBCS participants were between 28 and 74 years; and SFBCS participants were between 35 and 79 years. All participants signed informed written consent prior to participation and each study was approved by their Institutional Review Board for Human Subjects.

2.1. Data harmonization

Data were harmonized across all study centers and questionnaires as previously described [13]. Women were classified as either pre-menopausal or post-menopausal based on responses to questions on menstrual history. Pre-menopausal women were those who reported still having periods during the referent year (defined as the year before diagnosis for cases or before selection into the study for controls). Post-menopausal women were those who reported either a natural menopause or if they reported taking hormone therapy (HT) and were still having periods or were at or above the 95th percentile of age for those who reported having a natural menopause (i.e., ≥12 months since their last period). Women in 4-CBCS and SFBCS were asked to self-identify their race/ethnicity and were classified as non-Hispanic white (NHW), Hispanic, Native American (NA) or a combination of these groups. Women in MBCS were not asked their race or ethnicity.

Lifestyle variables included BMI calculated as self-reported weight (kg) during the referent year divided by measured height squared (m^2) and categorized as normal (<25 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30 kg/m^2). Cigarette smoking was evaluated as current, former, or never a regular smoker, where regular was defined as having smoked one or more cigarettes for six months or longer in 4-CBCS and SFBCS (data available for a subset of subjects only) or having smoked 100 or more cigarettes in MCBCS. A dietary oxidative balance score (DOBS) that included nutrients with anti- or pro-oxidative balance properties was developed as previously reported [14]. Dietary information was collected via a computerized validated diet history questionnaire in 4-CBCS [19,20], a 104-item semi-quantitative Food Frequency Questionnaire (FFQ) in MBCS [21], and a modified version of the Block Food Frequency Questionnaire in SFBCS [22]. Alcohol consumption was based on long-term use; consumption during the referent year was used for a subset of SFBCS women without information on long-term use. Regular use of aspirin or NSAIDS defined as three or more times a week for at least one month was available for the 4-CBCS only. A history of diabetes was defined as ever being told by a health care provider that you had diabetes or high blood sugar (available only for a subset of SFBCS participants).

2.2. Genetic data

DNA was extracted from either whole blood (n = 7287) or mouthwash (n = 634) samples. Whole Genome Amplification (WGA) was applied to the mouthwash-derived DNA samples prior to genotyping. A tagSNP approach was used to capture variation across the entire candidate genes. Genes were selected based on the literature available at the time the platform was developed that indicated a potential effect on inflammation. TagSNPs were selected using the following parameters: linkage disequilibrium (LD) blocks were defined using a Caucasian LD map in concordance with the custom-made GoldenGate chemistry array and an r^2 = 0.8; minor allele frequency (MAF) >0.1; range = −1500 bps from the initiation codon to +1500 bps from the termination codon; and 1 SNP/LD bin. Additionally, 104 Ancestry Informative Markers (AIMs) were used to distinguish European and NA ancestry [13]. All markers were genotyped using a multiplexed bead array assay format based on GoldenGate chemistry (Illumina, San Diego, California). A genotyping call rate of 99.93% was attained (99.65% for WGA samples). We included 132 blinded internal replicates representing 1.6% of the sample set. The duplicate concordance rate was 99.996% as determined by 193,297 matching genotypes among sample pairs. In the current analysis we evaluated tagSNPs for *CD40LG* alias *TNFSF5* and *TRAP* (three SNPs), *TLR1* (one SNP), *TLR2* (four SNPs), *TLR3* (four SNPs), *TLR4* (eight SNPs), *TNF* (two SNPs on Illumina and one taqman), *TNFRSF1A* (four SNPs), *TNFRSF11A* (25 SNPs), *TNFSF10* (12 SNPs), and *TRAF2* (four SNPs). Online Supplementary Table 1 provides a description of these genes and SNPs.

Supplementary table related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mrfmmm.2014.08.009.

2.3. Tumor characteristics and survival

Data on estrogen receptor (ER) and progesterone receptor (PR) tumor status and survival were available for cases from 4-CBCS and SFBCS only. Cancer registries in Utah, Colorado, Arizona, New Mexico, and California provided information on stage at diagnosis, months of survival after diagnosis, cause of death, and ER and PR status. Surveillance Epidemiology and End Results (SEER) disease stage was categorized as local, regional, or distant.