HHS Public Access

Author manuscript

Breast Cancer Res Treat. Author manuscript; available in PMC 2014 September 14.

Published in final edited form as:

Breast Cancer Res Treat. 2013 September ; 141(2): 287–297. doi:10.1007/s10549-013-2690-z.

Associations between genetic variants in the TGF-β signaling pathway and breast cancer risk among Hispanic and non-Hispanic white women

Stephanie D. Boone,
Department of Epidemiology and Population Health, School of Public Health and Information Sciences, James Graham Brown Cancer Center, University of Louisville, 485 E. Gray St. Room, Louisville, KY 40202, USA

Kathy B. Baumgartner,
Department of Epidemiology and Population Health, School of Public Health and Information Sciences, James Graham Brown Cancer Center, University of Louisville, 485 E. Gray St. Room, Louisville, KY 40202, USA

Richard N. Baumgartner,
Department of Epidemiology and Population Health, School of Public Health and Information Sciences, James Graham Brown Cancer Center, University of Louisville, 485 E. Gray St. Room, Louisville, KY 40202, USA

Avonne E. Connor,
Department of Epidemiology and Population Health, School of Public Health and Information Sciences, James Graham Brown Cancer Center, University of Louisville, 485 E. Gray St. Room, Louisville, KY 40202, USA

Christina M. Pinkston,
Department of Epidemiology and Population Health, School of Public Health and Information Sciences, James Graham Brown Cancer Center, University of Louisville, 485 E. Gray St. Room, Louisville, KY 40202, USA

Esther M. John,
Cancer Prevention Institute of California, Fremont, CA 94538, USA

Division of Epidemiology, Department of Health Research and Policy, and Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA 94305, USA

Lisa M. Hines,
Department of Biology, University of Colorado, Colorado Springs, CO 80045, USA

Mariana C. Stern,
Norris Comprehensive Cancer Center, Keck School of Medicine of USC, Department of Preventive Medicine, University of Southern California, Los Angeles, CA 90089, USA

Correspondence to: Stephanie D. Boone, srdenk01@louisville.edu.

Electronic supplementary material The online version of this article (doi:10.1007/s10549-013-2690-z) contains supplementary material, which is available to authorized users.

Anna R. Giuliano,
H. Lee Moffit Cancer Center & Research Institute, Tampa, FL 33612, USA

Gabriela Torres-Mejia,
Centro de Investigacion En Salud Poblacional, Instituto Nacional de Salud Publica, Av. Universidad No. 655, Col. Sta. Ma. Ahuacatitlan, CP 62100 Cuernavaco, Morelos, Mexico

Guy N. Brock,
Department of Bioinformatics and Biostatistics, School of Public Health & Information Sciences, James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA

Frank D. Groves,
Department of Epidemiology and Population Health, School of Public Health and Information Sciences, James Graham Brown Cancer Center, University of Louisville, 485 E. Gray St. Room, Louisville, KY 40202, USA

Richard A. Kerber,
Department of Epidemiology and Population Health, School of Public Health and Information Sciences, James Graham Brown Cancer Center, University of Louisville, 485 E. Gray St. Room, Louisville, KY 40202, USA

Roger K. Wolff, and
Department of Internal Medicine, University of Utah, Salt Lake City, UT 84132, USA

Martha L. Slattery
Department of Internal Medicine, University of Utah, Salt Lake City, UT 84132, USA

Stephanie D. Boone: srdenk01@louisville.edu

Abstract

The *TGF-β* signaling pathway has a significant role in breast cancer initiation and promotion by regulating various cellular processes. We evaluated whether genetic variation in eight genes (*TGF-β1*, *TGF-β2*, *TGF-βR1*, *TGF-βR2*, *TGF-βR3*, *RUNX1*, *RUNX2*, and *RUNX3*) is associated with breast cancer risk in women from the Breast Cancer Health Disparities Study. A total of 3,524 cases (1,431 non-Hispanic whites (NHW); 2,093 Hispanics/Native Americans(NA)) and 4,209 population-based controls (1,599 NHWs; 2,610 Hispanics/NAs) were included in analyses. Genotypes for 47 single nucleotide polymorphisms (SNPs) were determined. Additionally, 104 ancestral informative markers estimated proportion of NA ancestry. Associations with breast cancer risk overall, by menopausal status, NA ancestry, and estrogen receptor (ER)/progesterone receptor tumor phenotype were evaluated. After adjustment for multiple comparisons, two SNPs were significantly associated with breast cancer risk: *RUNX3* (rs906296 $OR_{CG/GG}$ = 1.15 95 % CI 1.04–1.26) and *TGF-β1* (rs4803455 $OR_{CA/AA}$ = 0.89 95 % CI 0.81–0.98). *RUNX3* (rs906296) and *TGF-βR2* (rs3773644) were associated with risk in pre-menopausal women (p_{adj} = 0.002 and 0.02, respectively) and in those with intermediate to high NA ancestry (p_{adj} = 0.04 and 0.01, respectively). Self-reported race was strongly correlated with NA ancestry (r = 0.86). There was a significant interaction between NA ancestry and *RUNX1* (rs7279383, p_{adj} = 0.04). Four *RUNX* SNPs were associated with increased risk of ER-tumors. Results provide evidence that genetic variation in *TGF-β* and *RUNX* genes are associated with breast cancer risk. This is the first report

of significant associations between genetic variants in *TGF-β* and *RUNX* genes and breast cancer risk among women of NA ancestry.

Keywords

reast cancer risk; Breast Cancer Health Disparities Study; TGF-β; signaling pathway; Native American ancestry; Hispanic; Non-Hispanic white

Introduction

The *TGF-β* signaling pathway includes several multifunctional cytokines and cell receptors that control the activity of particular genes to regulate cellular processes via a SMAD-mediated signaling cascade [1, 2]. Inhibition of *TGF-β* signaling occurs in several cancers, including breast cancer [3–5]. Genes in this pathway may play a dual role in breast cancer: as early tumor suppressors with growth-inhibitory effects, and as late promoters of invasiveness and angiogenesis [6]. Genes in the TGF-β-signaling pathway also have been linked to expression of estrogen receptors (ER) [7–10]. One study reported that absence of the *TGF-β* response was more likely in ER+ tumors and associated with a poor prognosis [10].

There is evidence that Runt-related transcription factors (*RUNX)* interact with receptor regulated *SMADs (R-SMADs)* and are down-stream effectors of the *TGF-β* signaling pathway [11]. Three *RUNX* family members play an important tissue-specific role in determining the fate of cells during differentiation and growth, and there is increasing evidence that loss of function is involved in carcinogenesis [12–14]. In most tumor cells, genetic variation in key members of the pathway causes resistance to the growth-inhibitory effects of *TGF-β* signaling [15, 16].

Despite evidence for a role of *TGF-β* signaling genes in breast cancer, there are sparse data for the association of variants in these genes with breast cancer risk. Studies of the associations of genetic variants in *TGF-β1*, *TGF-β2*, and *TGF-β* receptors (*TGF-βR1, TGF-βR2, TGF-βR3)* with breast cancer risk are inconsistent [5, 17–25], limited by small sample sizes [26–31], and data on non-white groups are lacking [29]. Meta-analyses, based primarily on non-Hispanic white (NHW) women, have not confirmed associations with the most commonly studied single nucleotide polymorphisms (SNPs) [*TGF-β1* (rs1800469 and rs1982073) and *TGF-βR1* (*6A/rs11466445)] with breast cancer risk [5, 18, 23–25], although mouse models have demonstrated that these SNPs increase (*TGF-β1*) and decrease (*TGF-βR1*) gene expression [28, 32–34]. To date, there are few, if any, published studies on the association of *RUNX* genes with breast cancer risk.

We investigated associations of 47 SNPs in eight genes (*TGF-β1, TGF-β2, TGF-βR1, TGF-βR2, TGF-βR3, RUNX1, RUNX2, and RUNX3*) with breast cancer risk in the Breast Cancer Health Disparities Study (BCHD), a population-based, collaborative study of Hispanic/Native American (NA) and NHW women living in the United States or Mexico [35]. We evaluated whether associations differ by European or NA ancestry, menopausal status, and

ER and progesterone receptor (PR) tumor phenotype. Additionally, SNP–SNP interactions were assessed.

Methods

Study population

The BCHD study is a consortium of three population-based case–control studies in the United States and Mexico: the 4-Corner's Breast Cancer Study (4-CBCS), the San Francisco Bay Area Breast Cancer Study (SFBCS), and the Mexico Breast Cancer Study (MBCS). Participants completed an in-person interview and provided a blood or saliva sample for DNA extraction [35]. The 4-CBCS participants were between 25 and 79 years of age. Eligible cases were diagnosed with a first primary breast cancer (in situ or invasive) between 10/1999 and 05/2004 in Arizona, Colorado, New Mexico, and Utah. Controls were randomly selected and frequency-matched to cases on 5 year age distributions and ethnicity. A total of 1,850 cases (1,244 NHW; 606 Hispanic/NA) and 2,057 controls (1,329 NHW; 728 Hispanic/NA) participated. SFBCS participants were between the ages of 35 and 79 years and lived in the San Francisco Bay Area. Eligible cases were diagnosed with a first primary invasive breast cancer between 04/1995 and 04/2002 (Hispanics) or between 04/1995 and 04/1999 (NHWs); controls were selected using random-digit dialing and frequency-matched to cases on 5 year age distributions and ethnicity. A total of 1,105 cases (312 NHW; 793 Hispanic) and 1,318 controls (320 NHW; 998 Hispanic) participated. Participants from the MBCS included women between 28 and 74 years of age. Eligible cases were diagnosed with a first primary breast cancer (in situ or invasive) between 01/2004 and 12/2007 across 12 participating hospitals in three main healthcare systems. Controls were selected from the participating hospitals' geostatistic catchment area using a probabilistic multistage design and matched to cases on 5 year age distributions, healthcare institute membership, and residency. A total of 1,881 women (850 cases, 1,031 controls) participated. All participants signed informed written consent prior to participation and the Institutional Review Board for Human Subjects at each institution approved the studies.

Data harmonization

Questionnaire data were harmonized across the three studies as previously described [35]. Women were classified as pre-menopausal or post-menopausal based on menstrual history and menopausal hormone therapy use. Women who reported still having periods were classified as pre-menopausal. Women were classified as post-menopausal if they reported natural menopause (≥12 months since last period), using hormone replacement therapy, or were at or above the 95th percentile of age for race/ethnicity of those reporting natural menopause within their study center. Mean ages for natural menopause were 58 and 56 years for 4-CBCS NHW and Hispanic/NA women, 55 and 56 years for SFBCS NHW and Hispanic women, and 54 years for MBCS Hispanic women.

Genetic data

DNA was extracted from either whole blood (n = 7,287) or saliva (n = 634) samples. Whole genome amplification (WGA) was conducted on saliva-derived DNA samples prior to genotyping. A tag-SNP approach was utilized to capture variation across candidate genes.

SNPs were selected using five parameters: (1) linkage disequilibrium (LD) blocks defined using a Caucasian LD map and an $r^2 = 0.8$; (2) minor allele frequency (MAF) > 0.1; (3) range = ±1500 base pairs (bps) from the initiation codon to the termination codon; (4) 1 SNP/LD bin; or (5) evidence of functionality. A multiplexed bead array assay based on GoldenGate chemistry (Illumina, San Diego, CA) was used for genotyping with a call rate of 99.93 % (99.65 % for saliva samples). There were 132 internal blinded replicates (1.6 % of the sample set). The duplicate concordance rate was 99.996 % as determined by 193,297 matching genotypes among sample pairs.

We investigated 47 SNPs in eight genes: *TGF-β1* (2 SNPs), *TGF-β2* (1 SNP), *TGF-βR1* (5 SNPs), *TGF-βR2* (1 SNP), *TGF-βR3* (5 SNPs), *RUNX1* (8 SNPs), *RUNX2* (17 SNPs), and *RUNX3* (8 SNPs). Supplemental Table 1 provides identification (rs) numbers and descriptions of selected SNPs. Minor allele frequencies (MAF) and Hardy-Weinberg Equilibrium (HWE) were calculated based on the frequencies of alleles and genotypes in the control population. We genotyped 104 Ancestral Informative Markers (AIMs) to characterize proportion of NA ancestry. AIMs were selected based on established differences in specific alleles between NA and European populations [36, 37].

Tumor characteristics

Statewide cancer registries in Utah, Colorado, Arizona, New Mexico, and California provided information on ER and PR tumor phenotype for a subset of 995 (69 %) NHW cases and 968 (75 %) Hispanic/NA cases. ER and PR were not available from the MBCS.

Statistical analysis

STRUCTURE 2.0 was utilized to calculate the proportion of genetic admixture based on European and NA ancestry [35, 37]. Percent ancestry was categorized, based on the distribution of NA ancestry in the control population, allowing sufficient power to assess associations of ancestral groups with breast cancer risk (≤28 %, 29–70 %,>70 %) [35]. Differences in covariates between self-reported ethnicity and case–control status were tested using the Mantel–Haenszel Chi square (χ^2). Logistic regression was used to estimate odds ratios (OR) for associations of genotypes with breast cancer risk adjusting for age (<40, 40–49, 50–59, 60–69, 70+), study center (4-CBCS, SFBCS, MBCS) and percent NA ancestry. Associations for each SNP were initially assessed assuming a co-dominant mode of inheritance. Dominant and/or recessive models were considered when the trend in ORs suggested a different mode of inheritance than co-dominant and increased statistical power could be gained by collapsing genotypes. Confounding by categories of age, study center, NA ancestry, body mass index (BMI), first-degree family history of breast cancer, age at menarche, age at menopause, menopausal status, parity, age at first birth, education, oral contraceptive use, hormone therapy use, alcohol consumption, and smoking status, and for continuous measures of physical activity and NA ancestry. Covariates were considered confounders if the univariate p value was ≤0.20 and adjustment produced a change of ≥10 % in the effect estimate for the overall association of the genotype with breast cancer risk [38]. We did not observe confounding by any factor; nonetheless all models were adjusted for age, study center and percentage of NA ancestry.

Statistical interactions of SNPs with NA ancestry, self-reported ethnicity, and menopausal status were assessed using the difference in maximum likelihood estimates for logistic regression models with and without an interaction term, using a χ^2 test with 2 degrees of freedom (2-df) for a co-dominant model, and 1 degree of freedom (1-df) for a dominant or recessive model. Risk of each breast cancer subtype (ER/PR tumor phenotype) was evaluated using multinomial logistic regression [39, 40]. SNP–SNP interaction models were assessed for those SNPs significantly associated with breast cancer risk and considering their biological function and potential interactions within the *TGF-β* signaling pathway.

P values, based on a 1-df Wald χ^2 test statistic, were adjusted for multiple comparisons taking into account tag-SNPs within each gene using a step-down Bonferroni correction method [41]. This method is based on the effective number of independent SNPs determined using the SNP spectral decomposition method based on the eigenvalues of the correlation matrix among the SNPs for each gene as proposed by Nyholt [42] and modified by Li and Ji [43]. This method is conservative, especially when evaluating correlated variables such as SNPs within a gene. An adjusted $P \leq 0.05$ for main effects and interactions was considered statistically significant. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Results

The analyses included 7,733 participants (3,524 cases, 4,209 controls) with complete data for the SNPs of interest. Approximately 60 % of participants were over 50 years of age, with a higher proportion of pre-menopausal women among Hispanic/NA cases (41 vs. 34 %) and Hispanic/NA controls (41 vs. 32 %) than NHW ($p < 0.0001$) (Table 1). Hispanic/NA cases tended to less educated ($p < 0.0001$) and have more ER−/PR− tumors than NHW (23 vs. 18 %) ($p = 0.001$). Self-reported race/ethnicity was strongly correlated with NA ancestry (*Cramer's* V, r = 0.86). Nearly all women (99.5 %) from the U.S. who self-reported being NHW were in the low NA ancestry group, whereas those who self-reported being Hispanic/NA were distributed across the intermediate (75.9 %), low (16.7 %), and high (7.4 %) NA ancestry groups. The majority of Mexico Hispanics (controls) had high (51.6 %) or intermediate (47.3 %) NA ancestry (data not shown).

Of the 47 SNPs evaluated, 10 [*RUNX1* (*rs7279383* and *rs8127225*); *RUNX2* (rs10948238 and rs13201287); *RUNX3* (rs906296); *TGF-β1* (rs4803455); *TGF-β2* (rs6604609); *TGF-βR1* (rs6478974); *TGF-βR2* (rs3773644); and *TGF-βR3* (rs6678564)] were significantly associated with breast cancer risk after adjustment for age, study, and NA ancestry (Table 2). After adjustment for multiple comparisons, a significant increase in breast cancer risk was observed for *RUNX3* (rs906296 $OR_{CG/GG} = 1.15$; 95 % CI1.04–1.26; $p_{adj} = 0.03$) and an inverse association was observed for *TGF-β1* (rs4803455, $OR_{CC/AA} = 0.89$; 95 % CI 0.81–0.98; $p_{adj} = 0.04$). *TGF-β2* (rs6604609) was significantly associated with reduced risk of breast cancer ($OR_{TA/AA} = 0.80$, 95 % CI 0.71–0.91, p = 0.0002), whereas *TGF-βR2* (rs3773644) was associated with increased risk of breast cancer ($OR_{TT} = 1.21$, 95 % CI 1.05–1.40, p = 0.004).

RUNX3 (rs906296) had a significant interaction with menopausal status before multiple comparison adjustment (p_{adj} = 0.08), and was significantly associated with increased breast cancer risk in pre-menopausal women (OR_{AA} = 1.33; 95 % CI 1.14–1.55 p_{adj} = 0.002) (Table 3). Although *RUNX1* (rs2268288, OR_{CC} = 1.47; 95 % CI 1.03–2.09, p_{adj} = 0.26) and *RUNX3* (rs4478762, OR_{AA} = 1.71; 95 % CI 1.04–2.82, p_{adj} = 0.21) were associated with a modest increase in post-menopausal breast cancer risk, these were not significant with adjustment for multiple comparisons. The SNPs *TGF-βR2* (rs3773644, OR_{TT} = 1.32, 95 % CI 1.04–1.68, p = 0.02) and *TGF-β2* (rs6604609) ($OR_{TA/AA}$ = 0.77, 95 % CI 0.66–0.91, p = 0.002) were significantly associated with breast cancer risk among pre-menopausal and post-menopausal women, respectively (data not shown).

There was significant interaction between NA ancestry and RUNX1 (rs7279383, p_{adj} = 0.04) (Table 4). Women with the highest NA ancestry had a significant increase in risk ($OR_{CG/GG}$ = 1.75 95 % CI 1.17–2.63, p_{adj} = 0.05), whereas those with low and high NA ancestry had nonsignificant reduced risks ($OR_{CG/GG}$ = 0.87 95 % CI 0.76–1.00, p_{adj} = 0.41 and $OR_{CG/GG}$ = 0.82 95 % CI 0.69–0.97, p_{adj} = 0.14, respectively). Divergent results were also observed by NA ancestry for *TGF-β1* (rs1800469); women with intermediate NA ancestry had a significant increased risk (OR_{TT} = 1.29, 95 % CI 1.04–1.58, p_{adj} = 0.04), whereas there was a null association in those with low and high NA ancestry (OR_{TT} = 0.96, 95 % CI 0.77–1.20, p_{adj} = 1.00, and OR_{TT} = 0.92, 95 % CI 0.65–1.30, p_{adj} = 0.94, respectively). Several SNPs were significantly associated with increased risk in women with intermediate NA ancestry, although results were not significantly divergent across NA ancestry: *RUNX3* (rs906296, $OR_{CG/GG}$ = 1.23, 95 % CI 1.06–1.43, p_{adj} = 0.04); and *TGF-βR2* (rs3773644, OR_{TT} = 1.44, 95 % CI 1.11–1.88, p = 0.01) (data not shown). One SNP was inversely associated with risk of women with intermediate NA ancestry: *TGF-β2* (rs6604609, $OR_{TA/AA}$ = 0.76, 95 % CI 0.63–0.91, p = 0.003, data not shown). Further evaluation of self-reported ethnicity yielded similar results, although no significant interactions were observed.

Table 5 shows associations between *TGF-β* signaling SNPs and breast cancer risk stratified by ER/PR tumor phenotype. Only one SNP was significantly different across ER/PR phenotypes after adjustment for multiple comparisons (*RUNX3*, rs7517302, p_{adj} = 0.04). However, five SNPs were significantly associated with risk within ER/PR phenotype strata that remained significant after adjustment for multiple comparisons. *RUNX1* (rs7279123) was associated with reduced breast cancer risk for ER+/PR− (p_{adj} = 0.03). There was increased breast cancer risk for ER−/PR+ (*RUNX3*, rs2236850, p_{adj} = 0.03); and increased risk for ER−/PR− for *RUNX2* rs9463090 (p_{adj} = 0.009), *RUNX2* rs12333172 (p_{adj} = 0.007) and *RUNX3* rs7517302 (p_{adj} = 0.03). There were no associations between SNPs and breast cancer risk defined by ER+/PR+ phenotype. We also evaluated interactions between individual SNPs and menopausal status within ER phenotypes: no significant interactions were observed.

Lastly, SNP–SNP interactions were examined for SNPs with statistically significant main effects for breast cancer risk. Only two were statistically significant *[RUNX3* (rs7517302 and *rs906296)*TGF-βR1* (rs6748974)]. The combined effect for rs 7517302*rs6748974 (p =

0.003) and rs906296*rs6748974 (p = 0.02) resulted in a slight increase in risk (OR = 1.20, data not shown).

Discussion

The present analyses suggest that genetic variation in the *TGF-β* signaling pathway *[TGF-β1, TGF-β2, TGF-βR1, TGF-βR2, TGF-βR3, RUNX1, RUNX2*, and *RUNX3]* influences breast cancer risk in Hispanic/NA and NHW women. Four SNPs were associated with breast cancer risk *[RUNX3* (rs906296); *TGF-β1* (rs4803455); *TGF-β2* (rs6604609); and *TGF-βR2* (rs3773644)]. Additionally, associations differed by ER/PR tumor phenotype, menopausal status, and percentage NA ancestry. In particular, two SNPs, *RUNX3* (rs906296) and *TGF-βR2* (rs3773644) were significantly associated with increased risk in pre-menopausal women and women of intermediate NA ancestry. Additionally one SNP, *TGF-β2* (rs6604609), was associated with decreased risk in these groups. This provides further evidence that *RUNX3* interacts with the *TGF-β* signaling pathway.

Genes from the *TGF-β* superfamily of cytokines and *RUNX* family have important roles regulating cellular processes and are part of one of the most commonly altered cellular signaling pathways in cancer, making them attractive candidates for cancer-related etiology [44, 45]. A general biological mechanism for the relationship between *TGF-β* signaling and breast cancer may be a SMAD-mediated pathway which facilitates the transduction of signals for target genes involved in cellular processes [1]. In normal mammary cells, *TGF-β1* is reported to have an anti-proliferative effect on epithelial and endothelial mammary cells by acting as a tumor suppressor down-regulating cellular growth, differentiation and apoptosis [3, 5]. Mouse models also offer evidence that increased levels of *TGF-β1* in serum strengthen tumor suppressor activity, reducing risk of breast cancer [4]. Immune cells, including B-cell, T-cell, and macrophages, secrete *TGF-β1*, which negatively regulates their proliferation, differentiation, and activation by other cytokines. This process makes *TGF-β1* an effective immunosuppressor, and disruption of the signaling pathway is linked to autoimmunity, inflammation, and cancer [46].

In most tumor cells, genetic variants can cause resistance to the inhibitory effects of *TGF-β* signaling [15, 16]. Exact mechanisms for resistance remain unknown, although evidence for other cancers suggests there may be decreased expression of *TGF-β* receptors [47, 48], oncoproteins such as *p*53 [49], other tumor suppressors that regulate the pathway such as *RUNX3* [50], or increased expression of inhibitory *SMADs* (*I-SMAD6* or *I-SMAD-7*) in the extracellular matrix [3]. There is also evidence that when *TGF-β1* and *TGF-βR1* are overexpressed following tumor initiation, they promote angiogenesis via cell migration and adhesion factors, resulting in metastasis [3]. *RUNX* genes have the ability to enhance growth regulation by making target cells sensitive to the effects of *TGF-β* family members. In turn, *TGF-β* genes can activate *RUNX* genes at the transcription and post-transcriptional levels [51, 52]. Most of our findings indicate that genetic variation is associated with increased breast cancer risk, suggesting that these genes are no longer acting as tumor suppressors via normal signaling.

Several epidemiological studies have investigated associations between select SNPs in *TGF-β1*, *TGF-β2*, and *TGF-β* receptors and breast cancer [15, 17, 18, 21, 28–32]. With the exception of one [17], most studies are underpowered and results are inconsistent. Only one study to date included Hispanic women, Le Marchand et al. [29] reported an inverse association for the (CC vs. TT) genotype of *TGF-β1* (rs1982073) (OR_{CC} = 0.81 95 % CI 0.52–1.27) in post-menopausal Hispanic women from the Multiethnic Cohort study; however, the number of Hispanic women was small (67 cases, 179 controls) and the analysis was underpowered.

Three meta-analyses were conducted for *TGF-β1* (rs1800469) and breast cancer risk: no association was observed for the recessive model (TT vs. CC/CT) [18, 23, 25]. Although we did not find an overall association for the recessive model of rs1800469 also, the co-dominant model suggested increased risk for women with intermediate NA ancestry. Moreover, present study suggests there is moderate LD (r^2 = 0.67) between *TGF-β1* (rs1800469) and *TGF-β1* (rs4803455) in both Hispanic/NA and NHW women. In our study, there was a significant inverse association of *TGF-β1* (rs4803455) with breast cancer risk.

The Shanghai Breast Cancer Study [21] conducted a multistage pathway analysis evaluating 11 genes (341 SNPs) in the *TGF-β* signaling pathway. Results for two SNPs can be compared to those from the present study. Statistically significant results were reported for the additive model of *TGF-βR1* (rs10733710, OR = 1.11, p = 0.02) and the recessive model of *TGF-bR3* (rs284185, OR_{AA} = 1.74, p = 0.004), which contrasts with our non-significant findings. The nominal p values reported by Ma et al. [21] suggest that their results may not be significant if adjusted for multiple comparisons.

Genetic variation among *RUNX* genes may be important in colorectal cancer [53], but there are no epidemiological data for breast cancer. We found several associations between *RUNX* SNPs and breast cancer risk (*RUNX3* rs9062696) in women of intermediate to high NA ancestry (RUNX3 rs906296 and *RUNX1* rs7279383), as well as an interaction with NA ancestry (*RUNX1* rs7279383). These findings support the hypothesis that genetic variation can influence breast cancer differently in Hispanic/NA than NHW women, possibly due to unmeasured biological functional variants that influence susceptibility.

TGF-β signaling may alter expression of the estrogen receptor (*ER*α) and estrogen may reciprocally interfere with this signaling [7–9]. *ER*α activation has been reported to inhibit *TGF-β1* transcription activity by up to 60 % [54, 55] and *RUNX1* has been considered an “accessibility factor” for *ER*α binding sites [9]. *RUNX2* decreases *ER*α mRNA and protein levels in breast cancer cells; and *RUNX3* may function as a tumor suppressor by destabilizing the *ER*α gene and inhibiting its expression [7, 56]. Our study is the first to evaluate associations between *TGF-β* and *RUNX* genes with risk of breast cancer stratified by ER/PR tumor phenotypes. Four *RUNX* SNPs were significantly associated with an increased risk of ER−/PR− (*RUNX2* rs9463090 and rs12333172, *RUNX3* rs2236850 and rs7517302) and ER−/PR+ (*RUNX1* rs7279123) tumors. These novel findings suggest that variation in *RUNX* genes may increase proliferation in ER− breast cells. Although the present analysis included 1,963 cases with available data on ER/PR phenotypes, tumor phenotype data for cases in MBCS were unavailable, limiting statistical power when

evaluating rarer phenotypes (ER−/PR+). Nonetheless, our results support the role of these genes in influencing estrogen-related associations with breast cancer.

There are several strengths and limitations for this study. The sample size provided sufficient power to evaluate different modes of inheritance and stratified analyses. Genetic ancestry, measured by 104 AIMs markers, was used determine biological ancestry in addition to self-reported ethnicity, allowing us to focus on the biological basis of associations while evaluating lifestyle factors that differ between groups. Because of sparse data in the literature for these genes, comparisons to other studies for specific SNPs and the interpretation of our findings were guided mostly by in vivo studies. However, we used a tag-SNP approach to cover variation across the entire gene, as the selected SNPs are in linkage disequilibrium with those not reported. Finally, adjustment for multiple comparisons is conservative and may result in false negative associations.

Results from the present study may have implications for the etiology of breast cancer phenotypes and disparities between race/ethnic groups for risk. The present study is the first to report associations between variants in these genes and ER/PR tumor phenotype, menopausal status, and NA ancestry. Our results suggest that variation in these genes may explain the previously reported results that Hispanic women have increased risk of pre-menopausal, ER− breast cancer compared to NHW. Biologic significance of the genes is strongly suggested, although specific SNPs that were evaluated may or may not be functional. A better understanding of how *TGF-β* and *RUNX* genes can switch their role from tumor suppressor to promoter in breast carcinogenesis is needed, as well as how these genes may influence ER expression. Studies evaluating a larger representation of SNPs in this complex signaling pathway will aid in validating our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was funded in part through a Susan G. Komen training grant: Breast Cancer Disparities Epidemiology Research Training Program (KG090926) to Dr. K. Baumgartner and Dr. R. Baumgartner at the University of Louisville. The Breast Cancer Health Disparities Study was funded by Grant CA14002 from the National Cancer Institute to Dr. Slattery. The San Francisco Bay Area Breast Cancer Study was supported by Grants CA63446 and CA77305 from the National Cancer Institute, Grant DAMD17-96-1-6071 from the U.S. Department of Defense and Grant 7 PB-0068 from the California Breast Cancer Research Program. The collection of cancer incidence data used in this study was supported by the California Department of Public Health as part of the statewide cancer reporting program mandated by California Health and Safety Code Section 103885; the National Cancer Institute's Surveillance, Epidemiology and End Results Program under contract HHSN261201000036C awarded to the Cancer Prevention Institute of California; and the Centers for Disease Control and Prevention's National Program of Cancer Registries, under agreement #1U58 DP000807-01 awarded to the Public Health Institute. The 4-Corner's Breast Cancer Study was funded by Grants CA078682, CA078762, CA078552, and CA078802 from the National Cancer Institute. The research also was supported by the Utah Cancer Registry, which is funded by contract N01-PC-67000 from the National Cancer Institute, with additional support from the State of Utah Department of Health, the New Mexico Tumor Registry, and the Arizona and Colorado cancer registries, funded by the Centers for Disease Control and Prevention National Program of Cancer Registries and additional state support. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official view of the National Cancer Institute or endorsement by the State of California Department of Public Health, the National Cancer Institute, and the Centers for Disease Control and Prevention or their Contractors and Subcontractors. The Mexico Breast Cancer Study was funded by Consejo Nacional de Ciencia y Tecnología (CONACyT) (SALUD-2002-C01-7462). We would also like to acknowledge the contributions of the following individuals to the

study: Sandra Edwards for data harmonization oversight; Erica Wolff and Michael Hoffman for laboratory support; Carolyn Ortega for her assistance with data management for the Mexico Breast Cancer Study, Jocelyn Koo for data management for the San Francisco Bay Area Breast Cancer Study, Dr. Tim Byers for his contribution to the 4-Corner's Breast Cancer Study, and Dr. Josh Galanter for assistance in selection of AIMS markers for the study.

References

1. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003; 113(6):685–700. [PubMed: 12809600]
2. Massague J. The transforming growth factor-beta family. Annu Rev Cell Biol. 1990; 6:597–641. [PubMed: 2177343]
3. Elliott RL, Blobe GC. Role of transforming growth factor beta in human cancer. J Clin Oncol. 2005; 23(9):2078–2093. [PubMed: 15774796]
4. Pierce DF Jr, et al. Mammary tumor suppression by transforming growth factor beta 1 transgene expression. Proc Natl Acad Sci USA. 1995; 92(10):4254–4258. [PubMed: 7753792]
5. Scollen S, et al. TGF-beta signaling pathway and breast cancer susceptibility. Cancer Epidemiol Biomarkers Prev. 2011; 20(6):1112–1119. [PubMed: 21527583]
6. Zu X, et al. Transforming growth factor-beta signaling in tumor initiation, progression and therapy in breast cancer: an update. Cell Tissue Res. 2012; 347(1):73–84. [PubMed: 21845401]
7. Huang, B., et al. RUNX3 acts as a tumor suppressor in breast cancer by targeting estrogen receptor alpha. New England: Oncogene; 2011.
8. Band AM, Laiho M. Crosstalk of TGF-beta and estrogen receptor signaling in breast cancer. J Mammary Gland Biol Neoplasia. 2011; 16(2):109–115. [PubMed: 21390570]
9. Chimge, NO.; Frenkel, B. The RUNX family in breast cancer: relationships with estrogen signaling. New England: Oncogene; 2012.
10. Bierie B, et al. Abrogation of TGF-beta signaling enhances chemokine production and correlates with prognosis in human breast cancer. J Clin Invest. 2009; 119(6):1571–1582. [PubMed: 19451693]
11. Miyazono K, Maeda S, Imamura T. Coordinate regulation of cell growth and differentiation by TGF-beta superfamily and Runx proteins. Oncogene. 2004; 23(24):4232–4237. [PubMed: 15156178]
12. Cameron ER, Neil JC. The Runx genes: lineage-specific oncogenes and tumor suppressors. Oncogene. 2004; 23(24):4308–4314. [PubMed: 15156187]
13. Ito Y. Oncogenic potential of the RUNX gene family: 'overview'. Oncogene. 2004; 23(24):4198–4208. [PubMed: 15156173]
14. Lund AH, van Lohuizen M. RUNX: a trilogy of cancer genes. Cancer Cell. 2002; 1(3):213–215. [PubMed: 12086855]
15. Levy L, Hill CS. Alterations in components of the TGF-beta superfamily signaling pathways in human cancer. Cytokine Growth Factor Rev. 2006; 17(1–2):41–58. [PubMed: 16310402]
16. Serra R, Crowley MR. Mouse models of transforming growth factor beta impact in breast development and cancer. Endocr Relat Cancer. 2005; 12(4):749–760. [PubMed: 16322320]
17. Cox A, et al. A common coding variant in CASP8 is associated with breast cancer risk. Nat Genet. 2007; 39(3):352–358. [PubMed: 17293864]
18. Qi X, et al. Transforming growth factor-beta1 polymorphisms and breast cancer risk: a meta-analysis based on 27 case-control studies. Breast Cancer Res Treat. 2010; 122(1):273–279. [PubMed: 20309626]
19. Liao RY, et al. TGFBR1×6A/9A polymorphism and cancer risk: a meta-analysis of 13,662 cases and 14,147 controls. Mol Biol Rep. 2010; 37(7):3227–3232. [PubMed: 19882361]
20. Colleran G, et al. The TGFBR1×6A/9A polymorphism is not associated with differential risk of breast cancer. Breast Cancer Res Treat. 2010; 119(2):437–442. [PubMed: 19390964]
21. Ma X, et al. Pathway analyses identify TGFBR2 as potential breast cancer susceptibility gene: results from a consortium study among Asians. Cancer Epidemiol Biomarkers Prev. 2012; 21(7): 1176–1184. [PubMed: 22539603]

22. Pasche B, et al. TGFBR1×6A and cancer: a meta-analysis of 12 case-control studies. J Clin Oncol. 2004; 22(4):756–758. [PubMed: 14966109]
23. Niu W, et al. Association of TGFB1 −509 C > T polymorphism with breast cancer: evidence from a meta-analysis involving 23,579 subjects. Breast Cancer Res Treat. 2010; 124(1):243–249. [PubMed: 20232138]
24. Wang YQ, et al. Association between TGFBR1 polymorphisms and cancer risk: a meta-analysis of 35 case-control studies. PLoS One. 2012; 7(8):e42899. [PubMed: 22905183]
25. Woo SU, et al. Association of a TGF-beta1 gene −509 C/T polymorphism with breast cancer risk: a meta-analysis. Breast Cancer Res Treat. 2010; 124(2):481–485. [PubMed: 20361250]
26. Baxter SW, et al. Transforming growth factor beta receptor 1 polyalanine polymorphism and exon 5 mutation analysis in breast and ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2002; 11(2): 211–214. [PubMed: 11867510]
27. Jin Q, et al. Polymorphisms and haplotype structures in genes for transforming growth factor beta1 and its receptors in familial and unselected breast cancers. Int J Cancer. 2004; 112(1):94–99. [PubMed: 15305380]
28. Kaklamani VG, et al. Combined genetic assessment of transforming growth factor-beta signaling pathway variants may predict breast cancer risk. Cancer Res. 2005; 65(8):3454–3461. [PubMed: 15833881]
29. Le Marchand L, et al. T29C polymorphism in the transforming growth factor beta1 gene and postmenopausal breast cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev. 2004; 13(3):412–415. [PubMed: 15006917]
30. Cox DG, et al. TGFB1 and TGFBR1 polymorphisms and breast cancer risk in the Nurses' Health Study. BMC Cancer. 2007; 7:175. [PubMed: 17848193]
31. Hishida A, et al. Transforming growth factor B1 T29C polymorphism and breast cancer risk in Japanese women. Breast Cancer. 2003; 10(1):63–69. [PubMed: 12525765]
32. Dunning AM, et al. A transforming growth factor beta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer Res. 2003; 63(10):2610–2615. [PubMed: 12750287]
33. Pasche B, et al. Constitutively decreased TGFBR1 allelic expression is a common finding in colorectal cancer and is associated with three TGFBR1 SNPs. J Exp Clin Cancer Res. 2010; 29:57. [PubMed: 20500843]
34. Wallace TA, Martin DN, Ambs S. Interactions among genes, tumor biology and the environment in cancer health disparities: examining the evidence on a national and global scale. Carcinogenesis. 2011; 32(8):1107–1121. [PubMed: 21464040]
35. Slattery, M., et al. Genetic variation in genes involved in hormones, inflammation, and energetic factors and breast cancer risk in an admixed population. Austin: Carcinogenesis; 2012.
36. Collins-Schramm HE, et al. Mexican American ancestry-informative markers: examination of population structure and marker characteristics in European Americans, Mexican Americans Amerindians and Asians. Hum Genet. 2004; 114(3):263–271. [PubMed: 14628215]
37. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155(2):945–959. [PubMed: 10835412]
38. Bursac Z, et al. Purposeful selection of variables in logistic regression. Source Code Biol Med. 2008; 3:17. [PubMed: 19087314]
39. Dubin N, Pasternack BS. Risk assessment for case-control subgroups by polychotomous logistic regression. Am J Epidemiol. 1986; 123(6):1101–1117. [PubMed: 3706280]
40. Hosmer, D., Jr; Lemeshow, S. Applied logistic regression. In: Sons, JWA., editor. Probability and mathematical statistics. New York: Wiley; 1989.
41. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat. 1979; 6:65–70.
42. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet. 2004; 74(4):765–769. [PubMed: 14997420]
43. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity (Edinb). 2005; 95(3):221–227. [PubMed: 16077740]

44. Akhurst RJ. TGF beta signaling in health and disease. Nat Genet. 2004; 36(8):790–792. [PubMed: 15284845]
45. Massague J. TGFbeta in Cancer. Cell. 2008; 134(2):215–230. [PubMed: 18662538]
46. Moustakas A, et al. Mechanisms of TGF-beta signaling in regulation of cell growth and differentiation. Immunol Lett. 2002; 82(1–2):85–91. [PubMed: 12008039]
47. Lee JD, et al. The type III TGF-beta receptor suppresses breast cancer progression through GIPC-mediated inhibition of TGF-beta signaling. Carcinogenesis. 2010; 31(2):175–183. [PubMed: 19955393]
48. Gobbi H, et al. Transforming growth factor-beta and breast cancer risk in women with mammary epithelial hyperplasia. J Natl Cancer Inst. 1999; 91(24):2096–2101. [PubMed: 10601380]
49. Ewen ME, et al. p53-dependent repression of CDK4 translation in TGF-beta-induced G1 cell-cycle arrest. Genes Dev. 1995; 9(2):204–217. [PubMed: 7851794]
50. Li QL, et al. Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell. 2002; 109(1):113–124. [PubMed: 11955451]
51. Blyth K, Cameron ER, Neil JC. The RUNX genes: gain or loss of function in cancer. Nat Rev Cancer. 2005; 5(5):376–387. [PubMed: 15864279]
52. Ito Y, Miyazono K. RUNX transcription factors as key targets of TGF-beta superfamily signaling. Curr Opin Genet Dev. 2003; 13(1):43–47. [PubMed: 12573434]
53. Slattery ML, et al. Associations between genetic variation in RUNX1, RUNX2, RUNX3, MAPK1 and eIF4E and risk of colon and rectal cancer: additional support for a TGF-beta-signaling pathway. Carcinogenesis. 2011; 32(3):318–326. [PubMed: 21088106]
54. Ito I, et al. Estrogen inhibits transforming growth factor beta signaling by promoting Smad2/3 degradation. J Biol Chem. 2010; 285(19):14747–14755. [PubMed: 20207742]
55. Matsuda T, et al. Cross-talk between transforming growth factor-beta and estrogen receptor signaling through Smad3. J Biol Chem. 2001; 276(46):42908–42914. [PubMed: 11555647]
56. Lau QC, et al. RUNX3 is frequently inactivated by dual mechanisms of protein mislocalization and promoter hypermethylation in breast cancer. Cancer Res. 2006; 66(13):6512–6520. [PubMed: 16818622]

Breast Cancer Res Treat. Author manuscript; available in PMC 2014 September 14.

Table 1

Descriptive covariates of study population, stratified by ethnicity and case–control status, Breast Cancer Health Disparities Study (n = 7,733)

Characteristic	Study population[a] (n = 7,733)									p^c
	Non-hispanic white(n = 3,030)					Hispanic/Native American (n= 4,703)				
	Controls (n = 1,599)		Cases (n = 1,431)		p^b	Controls (n = 2,610)		Cases (n = 2,093)		p^b	
	N	(%)	*N*	(%)		*N*	(%)	*N*	(%)		
Age (years)					0.04					0.22	
<40	87	6.1	117	7.3		313	12.0	198	9.5		<0.0001
40–49	401	28.0	409	25.6		834	31.9	708	33.8		
50–59	403	28.2	410	25.6		758	29.0	614	29.3		
60–69	340	23.8	356	22.3		530	20.3	425	20.3		
70+	200	13.9	307	19.2		175	6.7	148	7.1		
Study					0.37					0.94	
4-CBCS	1,335	83.5	1,177	82.3		736	28.2	579	27.7		<0.0001
Mexico	–	–	–	–		994	38.1	816	39.0		
San Francisco	264	16.5	254	17.8		880	33.7	698	33.4		
% Native american ancestry					0.21					0.0012	
≤0.28	1,591	99.5	1,420	99.2		280	10.7	276	13.2		<0.0001
0.28–0.70	7	0.44	7	0.5		1,697	65.0	1,373	65.6		
>0.70	1	0.1	4	0.3		633	24.3	444	21.2		
Menopausal status					0.13					0.71	
pre-/peri-	494	31.5	475	34.1		1,027	40.7	831	41.2		<0.0001
post-	1,075	68.5	919	65.9		1,499	59.3	1,186	58.8		
Education					0.57					\0.0001	
<High school	79	5.0	71	5.0		1538	60.2	1088	52.8		<0.0001
High school grad/GED	338	21.3	284	20.1		419	16.4	377	18.3		
Post-high school	1,168	73.7	1,059	74.9		597	23.4	594	28.9		
ER/PR status											
ER+/PR+	–	–	681	68.4	–	–	–	598	61.8	–	0.001
ER+/PR−	–	–	116	11.7		–	–	117	12.1		
ER−/PR+	–	–	17	1.7		–	–	28	2.9		

Characteristic	Study population[a] (n = 7,733)					p[c]				
	Non-hispanic white(n = 3,030)					Hispanic/Native American (n= 4,703)				
	Controls (n = 1,599)		Cases (n = 1,431)		p[b]	Controls (n = 2,610)		Cases (n = 2,093)		p[b]
	N	(%)	*N*	(%)		*N*	(%)	*N*	(%)	
ER−/PR−	–	–	181	18.2		–	–	225	23.2	

[a] Column percentages may not add up to 100 % due to rounding. Column totals (*n*) may not add up to total for each column due to missing observations: education (*n* = 121), menopausal status (*n* = 227)

[b] Case–control status comparisons within each ethnic group, Mantel–Haenszel chi square *p* values reported

[c] Ethnic group comparisons regardless of case–control status, Mantel–Haenszel chi square *p* values reported

Breast Cancer Res Treat. Author manuscript; available in PMC 2014 September 14.

Table 2

TGF-β signaling pathway genes: overall associations with breast cancer risk, the breast cancer health disparities study

Gene (SNP)	Genotype	Controls (*n*= 4,209)		Cases (*n*= 3,524)		OR[a]	(95 % CI)	*p*[b]
		N	(%)	*N*	(%)			
RUNX1 (rs7279383)	CC	3,098	73.6	2,647	75.1	1.00		0.032 (0.23)
	CG/GG	1,110	26.4	876	24.9	0.89	(0.80, 0.99)	
RUNX1 (rs8127225)	TT	2,591	61.6	2,117	60.1	1.00		0.029 (0.23)
	TC/CC	1,612	38.4	1,407	39.9	1.11	(1.01, 1.22)	
RUNX2 (rs10948238)	CC/CT	3,602	85.6	2,950	83.8	1.00		0.028 (0.42)
	TT	604	14.4	571	16.2	1.15	(1.01, 1.30)	
RUNX2 (rs13201287)	GG/GA	3,899	92.6	3,226	91.5	1.00		0.050 (0.69)
	AA	310	7.4	298	8.5	1.18	(1.00, 1.39)	
RUNX3 (rs906296)	CC	2,701	64.2	2,143	60.8	1.00		**0.004 (0.03)**
	CG/GG	1,505	35.8	1,379	39.2	**1.15**	**(1.04, 1.26)**	
TGF-β1 (rs4803455)	CC	1,400	35.3	1,193	37.0	1.00		**0.023 (0.04)**
	CA/AA	2,561	64.7	2,032	63.0	**0.89**	**(0.81, 0.98)**	
TGF-β2 (rs6604609)[c]	TT	3,455	82.1	3,009	85.4	1.00		**0.0002**
	TA/AA	753	17.9	515	14.6	**0.80**	**(0.71, 0.91)**	
TGF-βR1 (rs6478974)	TT/TA	3,531	83.9	2,884	81.9	1.00		0.045 (0.19)
	AA	677	16.1	639	18.1	1.13	(1.00, 1.28)	
TGF-βR2 (rs3773644)[c]	CC/CT	3,767	89.5	3,072	87.2	1.00		**0.004**
	TT	441	10.5	452	12.8	**1.21**	**(1.05, 1.40)**	
TGF-βR3 (rs6678564)	GG	3,523	83.7	3,004	85.2	1.00		0.03 (0.11)
	GC/CC	685	16.3	520	14.8	0.87	(0.77, 0.99)	

Bold text designates significant associations at the 0.05 level after adjustment for multiple comparisons

[a] Adjusted for age, study center, and genetic admixture

[b] Wald *p*-value for 1 df test; *p*-value adjusted for multiple comparisons (Bonferroni–Holm) in parentheses

[c] Only one SNP evaluated for each gene, multiple comparisons not applicable

Table 3

The Association of TGF-β signaling pathway genes and breast cancer risk stratified by menopausal status

Gene (SNP)	Genotype	Pre/peri-menopause (*n*= 2,827)						Post-menopause (*n* =4,679)						*p*-int[b]
		Controls (*n* = 1,306)		Cases (*n* = 1,521)		OR[a]	(95 % CI)	Contros (*n* = 21,105)		Cases (*n* = 2,574)		OR[a]	(95 % CI)	
		N	(%)	*N*	(%)			*N*	(%)	*N*	(%)			
RUNX1	TT/TC	1,480	97.3	1,273	97.5	1.00		2,515	97.7	2,033	96.6	1.00		0.10 (0.77)
(rs2268288)	CC	41	2.7	33	2.5	0.88	(0.55, 1.41)	59	2.3	71	3.4	1.47	(1.03, 2.09)	
RUNX3	CC	1,009	66.3	774	59.3	1.00		1,619	63.0	1,301	61.9	1.00		0.01 (0.08)
(rs906296)	CG/GG	512	33.7	532	40.7	**1.33**	**(1.14, 1.55)**	952	37.0	802	38.1	1.05	(0.93, 1.18)	
RUNX3	GG/GA	1,504	98.9	1,293	99.0	1.00		2,546	99.0	2,068	98.2	1.00		0.21 (1.00)
(rs4478762)	AA	16	1.1	13	1.0	0.98	(0.47, 2.05)	27	1.0	37	1.8	1.71	(1.04, 2.82)	

Bold text designates significant associations at the 0.05 level (within strata) after adjustment for multiple comparisons

[a] Odds Ratios adjusted for age, study, and genetic admixture (*n* = 7,506; missing observations: menopausal status (*n* = 227)

[b] Interaction *p*-value (SNP × menopause); Bonferroni *p*-value for adjustment for multiple comparisons shown in parentheses

Table 4

The Association of TGF-β signaling pathway genes and breast cancer risk stratified by proportion of Native American ancestry

Gene (SNP)	Genotype	≤0–28 %			29–70 %			71–100 %			*p*-int[b]
		Case/ control	OR[a]	(95 % CI)	Case/ Control	OR [a]	(95 % CI)	Case/ control	OR[a]	(95 % CI)	
RUNX1	CC	1,165/1,229	1.00		1,092/1,285	1.00		390/584	1.00		**0.004 (0.04)**
(rs7279383)	CG/GG	530/641	0.87	(0.76, 1.00)	288/419	0.82	(0.69, 0.97)	58/50	**1.75**	**(1.17, 2.63)**	
TGF-β1	CC	772/866	1.00		379/516	1.00		102/141	1.00		0.42 (0.84)
(rs1800469)	CT	692/759	1.01	(0.88, 1.17)	692/854	**1.11**	**(0.94, 1.31)**	206/299	0.95	(0.69, 1.30)	
	TT	1,90/216	0.96	(0.77, 1.20)	295/313	**1.29**	**(1.04, 1.58)**	133/193	0.92	(0.65, 1.30)	

[a] Odds Ratios adjusted for age and study

[b] Interaction *p*-value (SNP*admixture); Bonferroni–Holm *p*-value for multiple comparisons shown in parenthesis; bold text indicates significance after multiple comparisons. Wald *p*-value within strata adjusted for multiple comparisons by NA ancestry strata (Bonferroni–Holm step-down method), *bold* text indicates significance ($p \leq 0.05$) after multiple comparisons

Table 5

The association of TGF-P signaling pathway genes and breast cancer risk as defined by ER/PR Phenotypes

Gene (SNP)	Genotype	Controls[a]	ER+/PR+			ER+/PR−			ER−/PR+			ER−/PR−			p^c
		N	*N*	OR[b]	(95 % CI)	*N*	OR[b]	(95 % CI)	*N*	OR[b]	(95 % CI)	*N*	OR[b]	(95 % CI)	
RUNX1 (rs7279123)	CC	1,935	730	1.00		160	1.00		31	1.00		249	1.00		0.01 (0.06)
	CT	1,083	466	1.12	(0.97, 1.29)	65	**0.72**	**(0.53, 0.97)**	14	0.83	(0.44, 1.57)	135	0.98	(0.78, 1.22)	
	TT	184	79	1.10	(0.83, 1.46)	7	**0.44**	**(0.21, 0.96)**	0	0.00	(0.00, 0.00)	20	0.88	(0.55, 1.43)	
RUNX2 (rs9463090)	GG	2,051	818	1.00		155	1.00		25	1.00		238	1.00		0.01 (0.09)
	GA	1,016	410	1.02	(0.88, 1.17)	70	0.93	(0.70, 1.25)	17	1.34	(0.72, 2.50)	130	**1.10**	**(0.87, 1.38)**	
	AA	144	49	0.83	(0.59, 1.16)	8	0.72	(0.35, 1.50)	3	1.81	(0.54, 6.10)	38	**2.31**	**(1.57, 3.39)**	
RUNX2 (rs12333172)	CC/CT	3,109	1238	1.00		222	1.00		43	1.00		378	1.00		0.01 (0.10)
	TT	106	40	0.92	(0.63, 1.33)	11	1.44	(0.76,	2	1.36	(0.32, 5.72)	28	**2.12**	**(1.37, 3.27)**	
RUNX3 (rs2236850)	TT	1,073	401	1.00		77	1.00	2.73)	11	1.00		105	1.00		0.03 (0.17)
	TC	1,526	620	1.08	(0.93, 1.26)	112	1.02	(0.76, 1.38)	15	**0.96**	**(0.44, 2.10)**	216	1.44	(1.13, 1.85)	
	CC	609	257	1.12	(0.93, 1.35)	44	1.00	(0.68, 1.46)	18	**2.88**	**(1.35, 6.16)**	83	1.39	(1.02, 1.88)	
RUNX3 (rs7517302)	TT	2,664	1035	1.00		191	1.00		32	1.00		324	1.00		**0.005 (0.03)**
	TC/CC	548	242	1.13	(0.96, 1.34)	41	1.05	(0.74, 1.50)	13	2.02	(1.05, 3.88)	82	**1.25**	**(0.96) 1.62)**	

[a] ER/PR data were compared with 3,214 controls from sites where cases have ER/PR data Mexico data is excluded because they do not have data for ER/PR status (n = 1,810)

[b] Odds ratios (OR) and 95 % confidence intervals (CI) adjusted for age, study, and genetic admixture; Bonferroni-Holms p-value adjustment for multiple comparisons shown in parentheses

[c] Wald-p for overall model, Bonferroni-Holm p-value adjustment for MC shown in parentheses. Wald p-value within strata adjusted for multiple comparisons by admixture strata (Bonferroni-Holm step-down method), *bold* text indicates significance ($p \leq 0.05$) after multiple comparisons