T29C Polymorphism in the Transforming Growth Factor β1 Gene and Postmenopausal Breast Cancer Risk: The Multiethnic Cohort Study

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Abstract

Laboratory studies suggest a dual role for the transforming growth factor-β (TGF-β) signaling pathway in breast cancer. The normal antiproliferative activity of TGF-β in early breast tumor development is replaced by a promoting effect in later stages. A T29C transition polymorphism in the TGFBI gene has been associated with higher circulating TGF-β1 levels, and inconsistently with breast cancer risk in three recent studies. We tested the association of this variant with invasive breast cancer in a case-control study of 1123 cases and 2314 controls nested in the Multiethnic Cohort (MEC) Study. This study is a large prospective study being conducted in Hawaii and Los Angeles that includes Japanese, white, African American, Latino, and Native Hawaiian women who were predominantly postmenopausal at baseline. After adjustment for breast cancer risk factors, the odds ratio (OR) and 95% confidence interval (95% CI) for the TGFBI 29 CC genotype was 0.95 (95% confidence interval: 0.76–1.18), compared to the TT genotype. Analyses stratified by race/ethnicity, stage, or age category did not reveal any association of this variant with breast cancer. Given the strong biological rationale and the scarce and divergent epidemiologic data to date, additional investigations of the relationship between breast cancer and genetic variants in the TGF-β signaling pathway appear warranted.

Introduction

The transforming growth factor-β (TGF-β) family of cytokines is known to regulate many cellular processes involved in carcinogenesis, including cell proliferation, differentiation, motility, adhesion, and death (1). Each of the three TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) is encoded by a different gene and is expressed in a tissue-specific manner. TGF-β1 is expressed in endothelial, hematopoietic, and connective tissues (1). TGF-β demonstrates a growth-inhibiting effect on normal epithelial cells, including mammary epithelium (2). However, TGF-β has been suggested to play a dual role, acting as a tumor suppressor in early stages, through its antiproliferative activity, and as a tumor promoter in later stages, by enhancing tumor cell motility and invasiveness (3, 4). This duality of effects has been demonstrated for TGF-β1 in a number of genetically modified mouse models of breast cancer (5, 6).

Several polymorphisms have been described in the TGF-β1 gene, including a T-to-C transition at nt 29 in the region encoding the signal sequence (nt 869 relative to the transcription start site) which results in a Leu-Pro substitution at the 10th amino acid. This common variant has been associated with higher TGF-β1 serum levels (7, 8), an increased risk of osteoporosis (9), and a decreased risk of myocardial infarction in men but not in women (8). The investigation of this polymorphism with regard to breast cancer risk has recently begun and the data published to date have been inconsistent. The CC genotype has been associated with a 64% decreased breast cancer risk in a cohort study of 3075 white American women over age 65 at recruitment (10). In contrast, in a pooled analysis of three European case-control studies (3987 cases, 3867 controls), the CC genotype was associated with a 21% increased risk of breast cancer (11). Finally, in a hospital-based study of 232 cases and 172 controls conducted in Japan, no significant association was found between the CC genotype and breast cancer (12).

In an attempt to clarify the role of the TGF-β signaling pathway in breast carcinogenesis, we tested the association of this polymorphism with postmenopausal breast cancer in a large case-control study nested in the Multi-Ethnic Cohort (MEC) study.

Methods

The design and baseline characteristics of the MEC study have been described in detail elsewhere (13, 14). In short, participants are Hawaii or Los Angeles residents...
who entered the cohort from 1993 to 1996 by completing a 26-page mail questionnaire about demographic factors, lifestyle (including diet and smoking), medical history, medication use, family history of common cancers and, for women, reproductive history and hormone use. The cohort included 96,810 men and 118,441 women aged 45–75 at baseline. Among the women, 25% were Japanese American, 22% white, 21% Latino, 19% African American, 7% Hawaiian, and 6% other ethnic/racial origin. A nested case-control study was performed among women of the five main ethnic groups. Invasive breast cancer cases were identified through the Rapid Reporting System of the Hawaii Tumor Registry and through quarterly linkage to the Los Angeles County Cancer Surveillance Program, two cancer registries that are members of the Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute. This was complemented by annual linkages to the State of California’s cancer registry. A sample of cohort participants was randomly selected to serve as controls; the selection was stratified by sex and ethnicity. Incident breast cancer cases occurring since January 1995 and controls were contacted for donation of a blood sample. Samples were collected at the subjects’ homes, processed within 8 h and stored in liquid nitrogen freezers. The participation rate among cases was 74% and varied from 70% in African Americans to 81% in Latinos. The corresponding rate for controls was 66% and varied from 60% in African Americans to 71% in whites.

DNA was purified from buffy coats of peripheral blood and buccal cell samples using a PureGene Blood Kit (Gentra Systems, Minneapolis, MN) or a QIAamp 96 DNA Blood Kit (Qiagen, Valencia, CA). A genotyping method was developed for the TGFB1 T29C polymorphism using the fluorogenic 5'-nuclease assay (TaqMan Assay) (15). The assays were performed using a TaqMan PCR Core Reagent kit [Applied Biosystems (ABI), Foster City, CA] according to manufacturer’s instructions. The fluorogenic oligonucleotide primers for amplification of the polymorphic region of TGFB were GC088for (5'-CCACCCAC-CAGCCCCTTGC-3') and GC088rev (5'-CAGCTCCATGT-3') labeled with VIC to detect the T allele. PCR amplification using 10 ng of genomic DNA was performed in a thermal cycler (MWG Biotech, High Point, NC) with an initial step of 95°C for 10 min, followed by 50 cycles of 95°C for 25 s and 68°C for 1 min. The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System and the results analyzed with Sequence Detection Software (ABI). Experimental samples were compared to 12 controls to identify the three genotypes at each locus (CC, CT, TT). Any samples that were outside the parameters defined by the controls were identified as non-informative and were retested. Data for 166 samples that showed a weak signal and 332 samples for which the genotyping remained unsuccessful were excluded, leaving 3437 subjects for analysis. Results of all 204 blind duplicate pairs inserted in the sample plates for quality control were concordant.

The statistical analysis used unconditional logistic regression to compute odds ratios (ORs) and 95% confidence intervals (CIs) for the genotypes (16). All models were adjusted for ethnicity, age at blood draw, age at menarche, parity, and age at first birth. Genotype was modeled as indicator variables representing the genotypes at the candidate locus, or as a gene dosage effect variable assigned a value of 1, 2, or 3 according to the number of variant alleles at this locus (zero, one, and two variant alleles, respectively). The likelihood ratio test was used to determine interactions among certain variables with respect to breast cancer. The test compares a main effects, no interaction model with a fully parameterized model containing all possible interaction terms for the variables of interest. Deviation from the Hardy Weinberg equilibrium was tested with the χ² test.

Results

Table 1 shows the characteristics of the controls by genotype and known breast cancer risk factors, other variables of interest. Deviation from the Hardy Weinberg equilibrium was tested with the χ² test. No association was found between the genotypes and known breast cancer risk factors, other than race/ethnicity (Table 1).

Table 2 presents the ORs for the TGFB1 T29C polymorphism for all subjects combined and each ethnic group.

### Table 1. Characteristics of controls by TGFB1 T29C genotype

<table>
<thead>
<tr>
<th>TGFB1</th>
<th>TT (n = 690)</th>
<th>TC (n = 1103)</th>
<th>CC (n = 521)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American (%)</td>
<td>33.5</td>
<td>47.2</td>
<td>19.3</td>
</tr>
<tr>
<td>Latina (%)</td>
<td>27.7</td>
<td>47.6</td>
<td>27.7</td>
</tr>
<tr>
<td>White (%)</td>
<td>35.1</td>
<td>45.5</td>
<td>19.4</td>
</tr>
<tr>
<td>Japanese (%)</td>
<td>23.6</td>
<td>47.5</td>
<td>28.8</td>
</tr>
<tr>
<td>Hawaiian (%)</td>
<td>27.6</td>
<td>52.2</td>
<td>20.2</td>
</tr>
<tr>
<td>Age at draw (yrs)</td>
<td>63.3</td>
<td>62.9</td>
<td>63.0</td>
</tr>
<tr>
<td>Age at menarche (yrs)</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
</tr>
<tr>
<td>Age at first birth (yrs)</td>
<td>23.1</td>
<td>23.0</td>
<td>23.4</td>
</tr>
<tr>
<td>No. of births</td>
<td>2.9</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4</td>
<td>27.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Menopausal at baseline (%)</td>
<td>82.7</td>
<td>83.7</td>
<td>82.6</td>
</tr>
<tr>
<td>Ever used of HRT at baseline (%)</td>
<td>46.9</td>
<td>48.2</td>
<td>47.8</td>
</tr>
<tr>
<td>History of breast cancer in mother or sisters (%)</td>
<td>10.9</td>
<td>11.8</td>
<td>13.1</td>
</tr>
<tr>
<td>Ever smoked at baseline (%)</td>
<td>45.6</td>
<td>46.0</td>
<td>42.9</td>
</tr>
</tbody>
</table>

*Percentage (where indicated) or median.*
group separately. No association was found with the C allele overall and in each ethnic group. An analysis stratified by extent of disease at diagnosis revealed no difference in effect across stages, with an OR for the CC genotype versus the TT genotype of 1.01 (95% CI: 0.79–1.29) for localized breast cancer (814 cases, 2314 controls) and 0.84 (95% CI: 0.59–1.19) for tumors with regional or distant spread (301 cases, 2314 controls); 8 patients had unknown stage. A similar analysis stratified on age at blood draw also did not reveal any effect modification by age. The OR for the CC genotype versus the TT genotype was 0.85 (95% CI: 0.61–1.19) for women drawn at age 63 or earlier (452 cases, 1214 controls) and 1.02 (95% CI: 0.76–1.37) for women drawn after age 63 (671 cases, 1100 controls). Interactions were also tested between TGFB1 and age at menarche, age at first birth, parity, and BMI. No interactions were suggested.

**Discussion**

In this case-control study nested in a multiethnic cohort of predominantly postmenopausal women, we failed to find evidence for an association between breast cancer and the TGFB1 T29C polymorphism which has been both positively and negatively associated with this disease in recent studies. There is a strong rationale for investigating the role of this genetic variant as a possible contributor to cancer susceptibility. Laboratory studies have shown that TGFB-β is an important regulator of various cellular processes in the normal and malignant mammary gland (1). Acting through its downstream elements, the SMAD proteins and its receptors, TGFB-β inhibits cell cycle progression in lobular and ductal epithelial cells and, thus, acts as a tumor suppressor in early stages of breast tumor development (2–4). In later stages, as a result of changes in tumor cell responsiveness, TGFB-β acts as a promoter by enhancing tumor cell motility and invasiveness (17). This dual role has been clearly demonstrated in several transgenic mouse models (18), some highly relevant to human breast carcinogenesis (6). Also, suggestive of a role for the TGFB-β signaling pathway in the progression of human breast cancers are the findings of somatic mutations in type I and II TGFB-β receptor genes (TBR1 and TBR2) in recurrent or metastatic breast tumors (19, 20). Furthermore, TGFB-RII expression has been shown to inversely correlate with breast tumor aggressiveness, as assessed by tumor grade, mitotic count, and clinical stage (21).

On the basis of these laboratory data, it is hypothesized that sequence variants which modify the function of genes in the TGFB-β signaling pathway may affect breast cancer risk. The common T29C transition in TGFB1, resulting in a Leu10Pro substitution in the signal peptide sequence, is a good candidate locus because it has been associated with higher circulating TGFB-1 levels (7, 8). Three published studies have examined its association with breast cancer. In a cohort study of 3075 US white women recruited at age 65 or older, the CC genotype was associated with a markedly lower risk of breast cancer [hazard ratio of 0.36 (95% CI: 0.17–0.75)] and a delayed age at diagnosis (10). Although some in situ cases were included, 86% of the tumors were invasive in this study (10). In a pooled analysis of three European, population-based case-control studies (3987 cases with invasive tumors, 3867 controls), the TGFB1 29 CC genotype was found to be weakly associated with an increased breast cancer risk [OR = 1.21 (95% CI: 1.05–1.31)] (11). No data were presented stratified on stage or age at diagnosis, although it was reported that there was “no significant differences in the magnitude of the ORs...by patient age, tumor stage, or grade at diagnosis” (11). In vitro transfection experiments by the same group showed that the signal peptide with Pro at residue 10 caused a 2.8-fold increase in secretion compared with the Leu form (11). Finally, in a hospital-based case-control study of 232 prevalent breast cancer cases and 172 controls in Japan, Hishida et al. (12) reported ORs of 0.81 (95% CI: 0.50–1.34) and 0.77 (95% CI: 0.45–1.34) for the TC and CC genotypes, respectively, compared to the TT genotype. The OR for the CC genotype among premenopausal women was 0.45 (95% CI: 0.20–0.98), whereas it was 1.40 (95% CI: 0.64–3.08) for postmenopausal women, suggesting a modifying effect of menopausal status. Consistent with the lack of agreement in the published data (which are not easily explainable by the minor differences in design), our results did not suggest any clear association between the TGFB1 29 CC genotype and breast cancer. However, due to the composition of our sample, our findings only pertain to invasive postmenopausal breast cancer.

The strengths of the present study warrant consideration. Information on potential confounders was obtained before diagnosis and the samples were analyzed without knowledge of the case-control status of the subjects, eliminating the possibility of differential misclassification. The findings were reproduced across several ethnic groups, arguing against residual

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**Table 2. Breast cancer odds ratios** (95% CI) for **TGFB1 T29C genotype**

<table>
<thead>
<tr>
<th></th>
<th>TT</th>
<th>TC</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>n b</td>
<td>338/690</td>
<td>550/1103</td>
<td>235/521</td>
</tr>
<tr>
<td>OR</td>
<td>1.00</td>
<td>1.07 (0.89–1.28)</td>
<td>0.95 (0.76–1.18)</td>
</tr>
<tr>
<td>P for gene dosage</td>
<td>0.71</td>
<td>0.52</td>
<td>0.39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>African-American</th>
<th>Latina</th>
<th>White</th>
<th>Japanese</th>
<th>Hawaiian</th>
</tr>
</thead>
<tbody>
<tr>
<td>n b</td>
<td>76/205</td>
<td>67/179</td>
<td>114/141</td>
<td>62/91</td>
<td>19/74</td>
</tr>
<tr>
<td>OR</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.11 (0.77–1.61)</td>
<td>1.04 (0.72–1.52)</td>
<td>1.00 (0.70–1.42)</td>
<td>1.25 (0.83–1.88)</td>
<td>0.88 (0.44–1.76)</td>
</tr>
<tr>
<td>P for gene dosage</td>
<td>0.52</td>
<td>0.39</td>
<td>0.46</td>
<td>0.74</td>
<td>0.39</td>
</tr>
</tbody>
</table>

aAdjusted for age of blood draw, race (where indicated), age at menarche, parity, and age at first birth.

bNumber of cases/number of controls.

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confounding by ethnicity. The allele frequency for the variant allele was similar to that available in the literature for Caucasians and Japanese and the genotype distributions were in Hardy Weinberg equilibrium, arguing against selection bias. Finally, the study was sufficiently powered, because it had an 80% power to detect an OR of 1.21, as reported in the study by Dunning et al. (11).

In summary, the present study suggests that the TGFBI 29C variant is not associated with postmenopausal breast cancer. Given the strong biological rationale and the paucity and inconsistency of the epidemiological data on this relationship to date, additional investigations appear warranted. However, future studies should also consider other variants in the TGF-signaling pathway (22).

References
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