Breast Cancer Risk Is Modified by CYP19 Polymorphisms in Ashkenazi Jews

Leon Raskin, Flavio Lejbkowicz, Ofra Barnett-Griness, Sara Dishon, Ronit Almog, and Gad Rennert

Abstract

Exposure to sex hormones is a major risk factor for breast cancer and current treatments include hormone modifying drugs, among them aromatase inhibitors. We studied the association of CYP19 (Val80 and [TTTA]n) polymorphisms, the gene translated to aromatase, and the risk of breast cancer in BRCA carriers and noncarriers. The study consisted of 958 cancer cases and 931 healthy controls, including 474 carriers and 1,415 noncarriers. Cases and controls came from a population-based study of breast cancer in Israel, enriched with BRCA carriers from a clinical familial cancer service. Val80 G/G genotype was associated with significantly increased risk of breast cancer compared with the Val80 A/A genotype in BRCA1 carriers ages <50 years (odds ratio, 2.81; 95% confidence interval, 1.09-7.22; P = 0.032) but not in BRCA2 carriers or noncarriers of any age.

Introduction

Breast cancer is one of the leading causes of cancer morbidity and mortality worldwide (1). In the past decade, two major genes have been shown to be related to breast (and ovarian) cancer susceptibility, BRCA1 and BRCA2 (2). Three Ashkenazi Jewish founder mutations in these genes (BRCA1: 185delAG and 5382insC and BRCA2: 6174delT) have a combined frequency of 2.5% in the Ashkenazi population and appear in ~10% (3-5) of breast cancer cases in Ashkenazi Jewish women. Estimates of penetrance vary greatly across different studies, ranging from 37% to 90% (6-12). This heterogeneity in risk among women who carry BRCA1/2 mutations suggests the existence of modifying genetic and/or environmental factors. Polymorphisms in several genes have been suggested to modify breast and ovarian cancer risk in BRCA1 and BRCA2 carriers, although most have not been replicated (13-21). The only confirmed BRCA2 breast cancer risk modifier is RAD51 135G>C (22). No similar magnitude suggestive association, although nonstatistically significant, was found between Val80 polymorphism and estrogen receptor-negative status of the breast tumors. A common haplotype composed of the Val80 G allele and three haplotype-tagging single nucleotide polymorphisms (rs727479, rs10046, and rs4646) in the CYP19 coding region showed a trend to association with breast cancer risk in BRCA1 carriers ages <50 years. Published expression data show higher estrogen levels with higher repeats in [TTTA]n found in linkage disequilibrium with Val80. The present study suggests that the CYP19 Val80 polymorphism and a haplotype that includes this polymorphism are associated with increased breast cancer risk in young women with BRCA1 mutations. (Cancer Epidemiol Biomarkers Prev 2009;18(5):1617–23)
Table 1. Study population characteristics

<table>
<thead>
<tr>
<th>BRCA status*</th>
<th>Age category (y)</th>
<th>Breast cancer cases</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD) age</td>
<td>n</td>
<td>Mean (SD) age</td>
</tr>
<tr>
<td>BRCA1 carriers</td>
<td>&lt;50</td>
<td>83</td>
<td>97</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>&gt;50</td>
<td>62</td>
<td>54</td>
<td>116</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>48.9 (13.5)</td>
<td>151</td>
<td>296</td>
</tr>
<tr>
<td>BRCA2 carriers</td>
<td>&lt;50</td>
<td>41</td>
<td>57</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>&gt;50</td>
<td>37</td>
<td>43</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>41.1 (6.1)</td>
<td>100</td>
<td>178</td>
</tr>
<tr>
<td>Noncarriers</td>
<td>&lt;50</td>
<td>117</td>
<td>94</td>
<td>211</td>
</tr>
<tr>
<td></td>
<td>&gt;50</td>
<td>618</td>
<td>96</td>
<td>1,204</td>
</tr>
<tr>
<td>Total</td>
<td>735</td>
<td>63.5 (12.5)</td>
<td>680</td>
<td>1,415</td>
</tr>
</tbody>
</table>

*Ashkenazi founder mutations in BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT).

†Breast cancer combines breast cancer and breast/ovarian cancer cases.

rs727479(T), rs10046(T), and rs4646(G) were associated with higher levels of estrogens (27, 35-39). The Val80 (rs700518) G allele was found to be associated with elevated aromatase expression (40).

Estrogen receptor (ER) status is an important prognostic factor of breast tumors. A higher prevalence of ER-negative breast tumors in BRCA1 carriers was described (41). It has been shown that this association is neither a consequence of the young age at onset nor high grade but is an intrinsic property of BRCA1-related cancers (42). Recent studies found a significant association of the CYP19 [TTTA]7(delTCT) allele with ER-positive and CYP19 Trp59Arg (TC/CC) genotypes with ER-negative breast tumors (32, 43).

Previous genotyping efforts established the LD haplotype block structure of CYP19 (44). In block 4, which spans the entire coding region of CYP19, four common haplotypes cover 88% of haplotype diversity in Caucasians and can be distinguished by only three single nucleotide polymorphisms (SNP; rs727479, rs10046, and rs4646). None of these haplotypes was found to be significantly associated with breast cancer risk in a large multiethnic case-control study (44), but individual alleles rs727479(T), rs10046(T), and rs4646(G) were shown to be in association with elevated estrogen levels in postmenopausal women (39).

The present study investigates the causality of breast cancer in the unique population of Ashkenazi Jewish women, some of which carry the BRCA1 and BRCA2 founder mutations. Based on the previously published data concerning the role of CYP19 polymorphisms in breast cancer predisposition, it was hypothesized that the Val180 polymorphism, the [TTTA]n repeat polymorphism, or specific haplotypes in the CYP19 coding region can modify breast cancer risk. The aim of the study is to determine the clinical value of CYP19 polymorphisms in assessing breast cancer risk and their interaction with known genetic risk factors.

Materials and Methods

Study Population. The study population included 474 Ashkenazi Jewish women (223 breast cancer cases and 251 healthy controls) carrying Jewish founder mutations in the BRCA1 and BRCA2 genes and 1,415 Ashkenazi Jewish noncarrier women (733 cases and 680 controls; Table 1). Carriers were determined to have one of the three Jewish founder mutations in BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) and were cared for by the Clalit Health Services National Familial Cancer Consultation Service at the time of study. The series of 1,405 noncarriers derived from an ongoing population-based case-control study of the molecular and environmental etiology of breast cancer in Israel. In this study, which was initiated in 2000, all incident breast cancer cases in a distinct geographic region in Northern Israel are invited to participate after signing an informed consent approved by the Carmel Medical Center Institutional Review Board Committee. Controls are randomly sampled from the list of all women enrolled in the healthcare program provided by Clalit Health Services, the largest health services provider in Israel covering the majority of the Israeli population, and matched on age, residence, and Jewish/Arab status. Participants are interviewed by trained nurses to evaluate risk factors, including a detailed three-generation family history of cancer. Blood is drawn from each subject for DNA extraction and molecular analysis, including BRCA1 and BRCA2 founder mutation genotyping. DNA extracted from the blood is studied for a variety of molecular events, among them the existence of one or more of the Jewish founder mutations in the BRCA1 and BRCA2 genes. Participants who are found to be BRCA carriers are referred to the Clalit Health Services National Familial Cancer Consultation Service. This Service is a referral center, which can be approached by the population at large or by health professionals for advice. Most women who are evaluated by this service with regards to the breast/ovary syndromes either have a significant family history or have a personal history of breast cancer appearing at an early age, bilateral breast cancer, or breast cancer appearing in conjunction with ovarian cancer. Medical records were extracted, when available, for all study participants with breast cancer. Clinical data extracted from these records includes the ER and progesterone receptor status of the primary tumor when available.

The carriers group included 474 carriers of Jewish founder mutations: 296 of BRCA1 (218 of 185delAG and 78 of 5382insC) and 178 of BRCA2 (6174delT). Carriers with ovarian cancer only and two compound heterozygotes with mutations in both BRCA genes were not included into this study.
DNA Extraction and Genotyping. Genomic DNA was extracted from whole blood using a commercially available kit according to the manufacturer’s protocol (Puregene DNA extraction kit; Gentra Systems). Genetic testing for BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) was done using the Pronto BRCA kit (Pronto). Genomic DNA from breast cancer patients known to carry one of the three mutations (185delAG, 5382insC, and 6174delT) served as positive controls for each assay. All positive samples were confirmed by restriction enzyme digestion as described previously (4).

The genotyping of CYP19 rs727479 (C_4749_10), Val80 (rs700518; C_8794675_10), rs10046 (C_8234731_1_), and rs4646 (C_8234730_1_) was done by allelic discrimination using the 5′-nuclease Assay-on-Demand on 7900HT sequence detection system (Applied Biosystems). The assay was done in a 15 μL reaction volume containing 1× TaqMan PCR core reagents (Applied Biosystems), 5 mmol/L MgCl2, 200 nmol/L each PCR primer, 100 nmol/L MGB probes (Applied Biosystems), 0.5 units AmpliTaq Gold, 0.2 units AmpliTag Gold, 0.2 units AmpliTag Gold, and 40 ng genomic DNA. The Val80 5′-nuclease assay was validated by genotyping of 587 individuals using TaqMan and designed restriction fragment length polymorphism. No discrepancies were detected in the validation process.

The region of [TTTA]n repeats was amplified by PCR using the following primers: TTTA-F 5′-TACTTAGTTAGCTAC-3′ and TTTA-R 5′-TTACAGTGAGCCTGCT-3′. The PCR was carried out in a 25 μL containing 3 μL DNA template (~50 ng), 10 pmol of each primer, and 1 unit Taq polymerase (TaqKaRa). The reaction was incubated at 95°C for 5 min before 30 cycles of denaturation of 30 s at 95°C, annealing of 1 min at 55°C, and extension of 30 s at 72°C followed by a final extension of 10 min at 72°C. The amplified products were run on 2% agarose gels, excised, and purified by QiaGen MinElute Gel Extraction Kit (Qiagen). The cleaned PCR products were sequenced at Weizmann Institute DNA Sequencing Service.

Statistical Methods. Hardy-Weinberg equilibrium was tested in controls using the χ² goodness-of-fit test. CYP19 (Val80) genotype frequencies were compared between cases and controls using the Pearson χ² test and Armitage’s test for trend. Genotype odds ratios (OR) and their 95% confidence intervals (95% CI) were obtained using logistic regression, with Val80 A/A genotype as the reference category.

Multivariate logistic regression was used to adjust OR estimates for age and to test for gene-gene interaction. Genotype tests and trend tests were done using number of Val80 G alleles as categorical and continuous variable, respectively.

The carriers group included 474 women clustered in 341 families. To take into account the possible correlations within these families, we applied logistic regression models for correlated data (GEE model, SAS Genmod). As these additional analyses gave similar results to the ordinary logistic regression results, only the latter are presented.

The distribution of [TTTA] alleles was compared between cases and controls using Pearson χ² test and Armitage’s trend test. Exact test was employed when appropriate. Logistic regression based on allelic data was used to estimate ORs per 1 repeat increase. For this purpose, number of repeats was considered as a continuous variable and the associated P value was reported as P trend. When the global test for association was significant, allelic OR of a specific

### Table 2. CYP19 Val80 genotype and breast cancer risk in BRCA1/2 carriers and noncarriers

<table>
<thead>
<tr>
<th>BRCA1/2 status</th>
<th>Val80 genotype</th>
<th>Cases/controls</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1 carriers</td>
<td>A/A</td>
<td>15/24</td>
<td>Reference</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>39/54</td>
<td>1.41 (0.61-3.26)</td>
<td>0.418</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>27/19</td>
<td>2.81 (1.09-7.22)</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>81/97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA2 carriers</td>
<td>A/A</td>
<td>10/13</td>
<td>Reference</td>
<td>0.888</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>22/29</td>
<td>1.21 (0.42-3.47)</td>
<td>0.719</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>8/15</td>
<td>0.91 (0.26-3.20)</td>
<td>0.885</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>40/57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncarriers</td>
<td>A/A</td>
<td>28/26</td>
<td>Reference</td>
<td>0.628</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>67/51</td>
<td>1.22 (0.59-2.38)</td>
<td>0.549</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>22/17</td>
<td>1.20 (0.51-3.21)</td>
<td>0.664</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>117/94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1 carriers</td>
<td>A/A</td>
<td>15/12</td>
<td>Reference</td>
<td>0.321</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>33/25</td>
<td>1.05 (0.42-2.63)</td>
<td>0.922</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>13/17</td>
<td>0.6 (0.21-1.71)</td>
<td>0.336</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>61/54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA2 carriers</td>
<td>A/A</td>
<td>9/10</td>
<td>Reference</td>
<td>0.946</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>20/27</td>
<td>0.82 (0.28-2.39)</td>
<td>0.716</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>7/8</td>
<td>0.98 (0.25-3.82)</td>
<td>0.978</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>36/45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncarriers</td>
<td>A/A</td>
<td>153/146</td>
<td>Reference</td>
<td>0.346</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>299/303</td>
<td>0.94 (0.69-1.24)</td>
<td>0.677</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>166/137</td>
<td>1.16 (0.83-1.63)</td>
<td>0.366</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>618/586</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age.


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[TTTA]₇ repeat was estimated, where the group with [TTTA]₇ and [TTTA]₇delTCT alleles served as a reference category.

We used the haplo.stats package (45) for R for reconstruction of haplotypes and analysis of their potential association with breast cancer. Haplotype frequencies were estimated and compared using age-adjusted global and haplotype-specific score tests ("haplo.score" function). Rare haplotypes (combined frequency <10%) were pooled. In addition, based on inferred haplotype data, possible haplotype pairs and their posterior probability were calculated using the haplo.em function in R. A continuous variable counting the number of haplotype copies as well as indicator variables for one and two copies were created. Expectations of these variables were computed for each subject to account for the haplotype ambiguity. Using logistic regression, expected haplotype values were used to estimate age-adjusted ORs in both log-additive and dominant model. Pairwise LD (D and r²) between the haplotype-tagging SNPs was tested using the m⁺ statistic for LD in the "genetic" package for R.

In all analyses, P values < 0.05 were considered statistically significant. Unless otherwise specified, all results are age-adjusted. Analyses were done in SPSS (version 14) and SAS (version 9.1).

Results

The frequency of the CYP19 Val⁸⁰ G allele among controls was 50% in BRCA1 carriers, 50% in BRCA2 carriers, and 49% in noncarriers. CYP19 genotypes did not deviate from the Hardy-Weinberg equilibrium in controls.

In all BRCA1 mutation carriers, CYP19 Val⁸⁰ was not associated with breast cancer risk (OR per G allele increase, 1.17; 95% CI, 0.83-1.63; P = 0.370). However, it was found that, among the 180 BRCA1 carriers ages <50 years, the Val⁸⁰ G allele was associated with increased breast cancer risk (P trend = 0.028; G/G versus A/A; OR, 2.81; 95% CI, 1.09-7.22; P = 0.032; Table 2). No significant association between CYP19 Val⁸⁰ and breast cancer risk was found in either BRCA1 carriers of older age (age ≥50 years; P trend = 0.321) or BRCA2 carriers (P trend = 0.888 and 0.946 for ages <50 and ≥50 years, respectively; Table 2). There was no difference between cases and controls in Val⁸⁰ genotype distribution among the non-carriers of BRCA1/2 mutations (P trend = 0.300, 0.628, and 0.300).

Table 3. CYP19 Val⁸⁰ genotype and ER-negative status in BRCA1/2 carriers

<table>
<thead>
<tr>
<th>BRCA1/2 status</th>
<th>Val⁸⁰ genotype</th>
<th>ER(−)</th>
<th>ER(+)</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1 carriers</td>
<td>A/A</td>
<td>6</td>
<td>4</td>
<td>1.00</td>
<td>0.173</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>21</td>
<td>7</td>
<td>1.95 (0.49-9.44)</td>
<td>0.405</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>16</td>
<td>3</td>
<td>3.53 (0.58-21.51)</td>
<td>0.171</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>43</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1 carriers</td>
<td>A/A</td>
<td>7</td>
<td>5</td>
<td>1.00</td>
<td>0.747</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>14</td>
<td>11</td>
<td>0.75 (0.18-3.21)</td>
<td>0.697</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>7</td>
<td>5</td>
<td>0.76 (0.14-4.09)</td>
<td>0.746</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>28</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age.

Table 4. CYP19 coding region TGTG haplotype and breast cancer risk in BRCA1 carriers before and after age 50 y and BRCA2 carriers

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Frequency</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 &lt;50 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other/other</td>
<td>21.5</td>
<td>30.7</td>
<td>Reference</td>
<td>0.063</td>
</tr>
<tr>
<td>TGTG/other</td>
<td>50.8</td>
<td>50.7</td>
<td>1.63 (0.72-3.66)</td>
<td>0.241</td>
</tr>
<tr>
<td>TGTG/TGTG</td>
<td>27.7</td>
<td>18.5</td>
<td>2.42 (0.95-6.16)</td>
<td>0.063</td>
</tr>
<tr>
<td>BRCA1 &gt;50 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other/other</td>
<td>28.6</td>
<td>25.4</td>
<td>Reference</td>
<td>0.454</td>
</tr>
<tr>
<td>TGTG/other</td>
<td>53.7</td>
<td>50.6</td>
<td>0.94 (0.38-2.30)</td>
<td>0.887</td>
</tr>
<tr>
<td>TGTG/TGTG</td>
<td>17.7</td>
<td>24.0</td>
<td>0.65 (0.22-1.93)</td>
<td>0.440</td>
</tr>
<tr>
<td>BRCA2 &lt;50 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other/other</td>
<td>32.2</td>
<td>24.9</td>
<td>Reference</td>
<td>0.803</td>
</tr>
<tr>
<td>TGTG/other</td>
<td>48.3</td>
<td>50.6</td>
<td>0.99 (0.36-2.75)</td>
<td>0.983</td>
</tr>
<tr>
<td>TGTG/TGTG</td>
<td>19.4</td>
<td>24.5</td>
<td>0.85 (0.25-2.93)</td>
<td>0.793</td>
</tr>
<tr>
<td>BRCA2 &gt;50 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other/other</td>
<td>25.7</td>
<td>25.5</td>
<td>Reference</td>
<td>0.977</td>
</tr>
<tr>
<td>TGTG/other</td>
<td>60.8</td>
<td>61.4</td>
<td>0.98 (0.34-2.83)</td>
<td>0.973</td>
</tr>
<tr>
<td>TGTG/TGTG</td>
<td>13.5</td>
<td>13.1</td>
<td>1.03 (0.23-4.58)</td>
<td>0.965</td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age.

The haplotypes consist of haplotype-tagging SNPs tagging haplotype block 4 of CYP19 gene that cover the entire coding region of the gene: rs727479, rs700518 (Val⁸⁰), rs10046, and rs4646. Note that Val⁸⁰ (rs700518) was not included by Haiman et al. (44) to haplotype-tagging SNPs.
In addition, a recent study found the association of
CYP19

cancer risk among premenopausal women is reasonable.

A case-only analysis of
BRCA1

carriers ages <50 years showed a trend toward association between
Val80

G/G

and ER-negative tumors although not statistically significant (G/G versus A/A; OR, 3.53; 95% CI, 0.58-21.51; P = 0.171; \( P_{\text{trend}} = 0.173 \); Table 3).

We genotyped haplotype-tagging SNPs in haplotype block 4 covering the
CYP19

coding region [rs727479, rs700518 (Val80), rs10046, and rs4646] and reconstructed haplotypes. Haplotype analysis of the younger
BRCA1

carriers showed an increase trend in breast cancer risk associated with the common TGTG haplotype (frequency, 48.1%; OR per haplotype copy, 1.56; \( P_{\text{trend}} = 0.066 \); OR for TGTG haplotype homozygotes versus no copies of TGTG, 2.42; 95% CI, 0.95-6.16; P = 0.063; Table 4). This is consistent with the suggestive association between the rs10046 T allele and risk of breast cancer among younger
BRCA1

carriers (\( P_{\text{trend}} = 0.062 \); OR for T/T versus C/C, 2.46; 95% CI, 0.96-6.28; P = 0.060) and the OR of rs4646 G/G versus T/T was 2.69 (95% CI, 0.72-10.05; P = 0.140; data not shown).

The intro 4 [TTTA\(_r\)] polymorphism was genotyped in a subset of 104 noncarriers and 284
BRCA1

carriers and was found to be in complete LD with the
CYP19

Val80

A higher number of repeats (>7) was linked with the Val80 G allele (\( D' = 1; r^2 = 1 \); data not shown). The rare [TTTA\(_r\)] allele was not found in our sample. Among the younger
BRCA1

carriers, increasing number of repeats tended to be associated with increased breast cancer risk (OR per 1 repeat increase, 1.18; \( P = 0.053 \); data not shown).

Discussion

In the present study, we found an association between SNPs and haplotypes of the
CYP19

gene and breast cancer risk in
BRCA1

mutation carriers ages <50 years. Although many studies have been published supporting the role of
CYP19

polymorphisms in breast cancer (26, 27, 29, 31-33), the modifying effect of these polymorphisms on
BRCA1-related breast cancer risk had not been previously studied. Our finding may shed light on possible mechanisms by which the penetrance of BRCA mutations is altered. Such partial penetrance reflects the possible existence of modifying genes or lifestyle factors that have been formerly suggested (46).

The previously reported prevalence of ER-negative tumors in premenopausal
BRCA1

cancer (24) and relatively high frequency of breast cancer in the general population may bring to conclusion about higher frequency of phenocopies among women with postmenopausal breast cancer. From this point of view, the association shown in our study of
CYP19

polymorphisms with breast cancer risk among premenopausal women is reasonable. In addition, a recent study found the association of
CYP19

rs10046 T/T with elevated breast cancer risk in middle age group (ages 45-54 years; ref. 47).

The association of
CYP19

with breast cancer risk was found in
BRCA1

carriers ages <50 years but not in
BRCA2

carriers. This agrees with known biological and clinical differences between breast tumors in
BRCA1

and
BRCA2

carriers (2).

Several studies found an association between shorter [TTTA\(_r\)] alleles ([TTTA\(_r\)] and [TTTA\(_r\)]-9) and lower blood estrogen levels, whereas longer alleles ([TTTA\(_r\)] and [TTTA\(_r\)]-9) were found to be associated with higher blood estrogen levels (27, 35, 38). These observations support our finding that the Val80 G allele (and therefore [TTTA\(_r\)] alleles) increases the risk of breast cancer compared with shorter [TTTA\(_r\)] allele, which may be due to higher lifetime exposure to estrogens. Furthermore, our results show a suggestive positive association between the Val80 G/G genotype ([TTTA\(_r\)] homozygotes) and ER-negative tumors in young
BRCA1

carriers. Although this association did not reach statistical significance, this may be due to a lack of power from missing data on tumors collected at the beginning of the study. A potential association between
Val80

G/G

genotype and ER-negative tumors would have implications for our understanding of the factors that cause a breast cancer cell to develop into an ER-positive or ER-negative tumor. Higher circulating levels of estrogens associated with [TTTA\(_r\)] homozygotes (or
Val80

G/G

in ER-negative tumors can thus be a key element in the tumor microenvironment directing its receptor commitment.

Suitability for hormonal chemoprevention or treatment with selective ER modulators or aromatase inhibitors is another implication of the potential association between
Val80

and ER status. Whereas most studies show that ER-negative tumors do not respond to hormonal interventions, one case-control study (48) in
BRCA1

carriers suggested the opposite. The role of estrogen in
BRCA1-related breast cancer pathogenesis (and not in
BRCA2)

depends on the direct carcinogenic effect of estrogen metabolites and not on ER binding (49). The ER-negative breast stem cells are surrounded by ER-positive cells that can exert a paracrine effect on these stem cells (49, 50).

BRCA1

is potentially involved in the maturation of ER-negative stem cells into mature ER-positive cells (42). In
BRCA1

carriers, the differentiation is impaired (51) and the enlarged pool of stem cells has greater chance of malignant transformation. The higher estrogen levels potentially associated with the
Val80

G/G

genotype can promote the synthesis of growth factors (such as epidermal growth factors and insulin-like growth factors) by ER-positive mature breast cells surrounding ER-negative stem cells (52). Growth factors could further enhance stem cells proliferation, which in turn could increase the rate of stem cell transformation.

In spite of the demonstrated modifying effect of
Val80

and [TTTA\(_r\)] polymorphisms, it is likely that the biologically functional change in the aromatase protein is due to
an unknown causal allele in the coding region of CYP19, related to the risk haplotype TGTG. Breast cancer risk modification seems to be restricted to two SNPs of these haplotypes, Val80 (rs700518) and r10046€untranslatedregion). These SNPs are in very strong LD \( (D' = 0.93, r^2 = 0.88) \) and cover almost the entire coding region of CYP19 from exons 3 to 10. Nevertheless, our findings provide what seems to be a breast cancer risk modifier for younger women carrying BRCA1 gene mutations.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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