BRCA1 Breast Cancer Risk Is Modified by *CYP19* Polymorphisms in Ashkenazi Jews

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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* (Val^{80} 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. Val^{80} G/G genotype was associated with significantly increased risk of breast cancer compared with the Val^{80} 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. A similar magnitude suggestive association, although nonstatistically significant, was found between Val^{80} polymorphism and estrogen receptor-negative status of the breast tumors. A common haplotype composed of the Val^{80} 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 Val^{80}. The present study suggests that the *CYP19* Val^{80} 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)

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 studies have, as yet, evaluated the association between polymorphisms in the genes involved in estrogen metabolism and breast cancer risk in BRCA mutation carriers.

Because estrogens play an important role in carcinogenesis and progression of breast cancer (23, 24), genes encoding for enzymes involved in estrogen biosynthesis and metabolism are plausible candidates as breast cancer susceptibility genes. One such candidate is *CYP19* (p450arom), which encodes for aromatase, an enzyme that converts androgens to estrogens. The association of polymorphisms in the *CYP19* with breast cancer risk has previously been studied, with particular focus on the [TTTA]$_n$ repeats polymorphism (25-31). A single study observed a slightly higher [TTTA]$_7$ allele frequency among breast cancer cases in the Caucasian population (31). In this study, the allele A of the silent variant at exon 3 [rs700518 (Val^{80}Val)] is in complete linkage disequilibrium (LD) with [TTTA]$_7$. Subsequent studies of [TTTA]$_n$ repeats showed inconsistent results. The following alleles have been implicated as possible breast cancer susceptibility alleles: [TTTA]$_7$delTCT and [TTTA]$_{>10}$ (32, 33), [TTTA]$_7$ repeats (31), [TTTA]$_8$ and [TTTA]$_{10}$ (26, 27), [TTTA]$_{11}$ (34), and [TTTA]$_{12}$ (27, 29).

Several studies have observed an association between *CYP19* polymorphisms and concentrations of serum circulating estrogen-related metabolites. The alleles [TTTA]$_7$ and [TTTA]$_{<9}$ were associated with lower estrogen levels; conversely, the alleles [TTTA]$_8$, [TTTA]$_{>9}$,

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Table 1. Study population characteristics

BRCA status*	Age category (y)	Breast cancer† cases		Controls		Total
		n	Mean (SD) age	*n*	Mean (SD) age	
BRCA1 carriers	<50	83	39.7 (6.2)	97	32.9 (9.1)	180
	>50	62	62.8 (8.7)	54	60.8 (10.4)	116
	Total	145	48.9 (13.5)	151	42.9 (16.5)	296
BRCA2 carriers	<50	41	41.1 (6.1)	57	35.2 (9.5)	98
	>50	37	61.1 (8.8)	43	61.8 (11.4)	80
	Total	78	50.6 (12.5)	100	46.6 (16.8)	178
Noncarriers	<50	117	43.6 (5.0)	94	32.2 (5.0)	211
	>50	618	66.5 (10.3)	586	66.6 (10.3)	1,204
	Total	735	62.8 (12.8)	680	63.5 (12.5)	1,415

*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 Val^{80} (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* $Trp^{39}Arg$ (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 Val^{80} 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 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) or *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), Val^{80} (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 $MgCl_2$, 200 nmol/L each PCR primer, 100 nmol/L MGB probes (Applied Biosystems), 0.5 units AmpliTaq Gold, 0.2 units AmpErase UNG, and 40 ng genomic DNA. The Val^{80} 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′-GCAGGTACTTAGTTAGCTAC-3′ and TTTA-R 5′-TTACAGTGAGCCAAGGTCGT-3′. The PCR was carried out in a total volume of 25 μL containing 3 μL DNA template (~50 ng), 10 pmol of each primer, and 1 unit Taq polymerase (TaKaRa). 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 χ^2 goodness-of-fit test. *CYP19* (Val^{80}) genotype frequencies were compared between cases and controls using the Pearson χ^2 test and Armitage's test for trend. Genotype odds ratios (OR) and their 95% confidence intervals (95% CI) were obtained using logistic regression, with Val^{80} 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 Val^{80} 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 χ^2 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* Val^{80} genotype and breast cancer risk in *BRCA1/2* carriers and noncarriers

BRCA1/2 status	Val^{80} genotype	Cases/controls	OR (95% CI)	P	P_{trend}
<50 y					
BRCA1 carriers	A/A	15/24	Reference		0.028
	G/A	39/54	1.41 (0.61-3.26)	0.418	
	G/G	27/19	2.81 (1.09-7.22)	0.032	
	Total	81/97			
BRCA2 carriers	A/A	10/13	Reference		0.888
	G/A	22/29	1.21 (0.42-3.47)	0.719	
	G/G	8/15	0.91 (0.26-3.20)	0.885	
	Total	40/57			
Noncarriers	A/A	28/26	Reference		0.628
	G/A	67/51	1.22 (0.59-2.38)	0.549	
	G/G	22/17	1.20 (0.51-3.21)	0.664	
	Total	117/94			
≥50 y					
BRCA1 carriers	A/A	15/12	Reference		0.321
	G/A	33/25	1.05 (0.42-2.63)	0.922	
	G/G	13/17	0.6 (0.21-1.71)	0.336	
	Total	61/54			
BRCA2 carriers	A/A	9/10	Reference		0.946
	G/A	20/27	0.82 (0.28-2.39)	0.716	
	G/G	7/8	0.98 (0.25-3.82)	0.978	
	Total	36/45			
Noncarriers	A/A	153/146	Reference		0.346
	G/A	299/303	0.94 (0.69-1.24)	0.677	
	G/G	166/137	1.16 (0.83-1.63)	0.366	
	Total	618/586			

NOTE: Adjusted for age.

Table 3. *CYP19* Val^{80} genotype and ER-negative status in *BRCA1/2* carriers

BRCA1/2 status	Val^{80} genotype	ER(−)	ER(+)	OR (95% CI)	*P*	P_{trend}
<50 y						
BRCA1 carriers	A/A	6	4	1.00		0.173
	G/A	21	7	1.95 (0.40-9.44)	0.405	
	G/G	16	3	3.53 (0.58-21.51)	0.171	
	Total	43	14			
≥50 y						
BRCA1 carriers	A/A	7	5	1.00		0.747
	G/A	14	11	0.75 (0.18-3.21)	0.697	
	G/G	7	5	0.76 (0.14-4.09)	0.746	
	Total	28	21			

NOTE: Adjusted for age.

$[TTTA]_n$ repeat was estimated, where the group with $[TTTA]_7$ and $[TTTA]_7$delTCT 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 r^2) between the haplotype-tagging SNPs was tested using the χ^2 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* Val^{80} 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* Val^{80} 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 Val^{80} 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* Val^{80} 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 Val^{80} genotype distribution among the noncarriers of *BRCA1/2* mutations (P_{trend} = 0.300, 0.628, and

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

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

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 (Val^{80}), rs10046, and rs4646. Note that Val^{80} (rs700518) was not included by Haiman et al. (44) to haplotype-tagging SNPs.

0.346 for the overall and ages <50 and ≥50 years, respectively; Table 2).

Of the 956 breast cancer cases in the study, ER status was available for 772 (81%) cases (108 *BRCA1* carriers, 55 *BRCA2* carriers, and 609 noncarriers). There were 18 (2.9%) tumors diagnosed with ductal carcinoma *in situ*. Tumors were ER-negative in 67% of the *BRCA1* carriers (72 of 108), 29.1% of the *BRCA2* carriers, and 20.4% of the noncarriers. A case-only analysis of *BRCA1* carriers ages <50 years showed a trend toward association between Val^{80} G/G and ER-negative tumors although not statistically significant (G/G versus A/A; OR, 3.53; 95% CI, 0.58-21.51; $P = 0.171$; $P_{trend} = 0.173$; Table 3).

We genotyped haplotype-tagging SNPs in haplotype block 4 covering the *CYP19* coding region [rs727479, rs700518 (Val^{80}), 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; $P_{trend} = 0.066$; 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 ($P_{trend} = 0.062$; 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 intron 4 $[TTTA]_n$ polymorphism was genotyped in a subset of 104 noncarriers and 284 *BRCA1* carriers and was found to be in complete LD with *CYP19* Val^{80}. A higher number of repeats (>7) was linked with the Val^{80} G allele ($D' = 1$; $r^2 = 1$; data not shown). The rare $[TTTA]_{13}$ allele was not found in our sample. Among the younger *BRCA1* mutation 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* breast cancer (42) 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). *BRCA1*-related tumors are more commonly ER-negative, differently related to reproductive risk factors, less often detected by mammography, and more commonly involve ovarian cancers than are BRCA2-related tumors. These differences may be attributed to differences in estrogen metabolism or availability.

The complete LD between $[TTTA]_n$ repeats and Val^{80} observed in our study is in line with former reports (26, 27, 29, 31-34). Several studies found an association between shorter $[TTTA]_n$ alleles ($[TTTA]_7$ and [TTTA]<9) and lower blood estrogen levels, whereas longer alleles ($[TTTA]_8$ and $[TTTA]_{>9}$) were found to be associated with higher blood estrogen levels (27, 35, 38). These observations support our finding that the Val^{80} G allele (and therefore $[TTTA]_{>7}$ alleles) increases the risk of breast cancer compared with shorter $[TTTA]_7$ allele, which may be due to higher lifetime exposure to estrogens. Furthermore, our results show a suggestive positive association between the Val^{80} G/G genotype ($[TTTA]_{>7}$ 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 Val^{80} 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 $[TTTA]_{>7}$ homozygotes (or Val^{80} 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 Val^{80} and ER status. Whereas most studies show that ER-negative tumors do not respond to hormonal interventions, one case-control study (48) in BRCA carriers suggested the opposite. The role of estrogen in *BRCA1*-related breast cancer pathogenesis (and not in *BRCA2*) may depend 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* mutation 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 Val^{80} 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 Val^{80} and $[TTTA]_n$ 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, Val^{80} (rs700518) and rs10046 (3′-untranslated region). 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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