The Association of the CYP19 and CYP17 Polymorphic Markers with Sporadic Breast Cancer

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Abstract—Polymorphic alleles of CYP17 and CYP19, which are involved in estrogen biosynthesis, were tested for association with breast cancer (BC). Microsatellite (TTTA)6 and 3-bp deletion of CYP19 and single-nucleotide polymorphism T27C of CYP17 were analyzed in 123 BC patients and 119 healthy women. Of the six (TTTA)6 alleles observed, allele (TTTA)8 proved to be associated with BC (11.8% vs. 6.3%, P = 0.04). Genotype A2/A2 of CYP17 was also associated with BC (32.5% vs. 20.2%, P = 0.04). Risk of BC was especially high in the presence of both factors (7.3% vs. 0%, P < 0.01). Allele (TTTA)8 and genotype A2/A2 were assumed to be risk factors of BC.

Key words: breast cancer, CYP17, CYP19, DNA polymorphism

INTRODUCTION

Breast cancer (BC) is the most common malignancy in women. In Russia, BC accounts for 18.3% oncolgical diseases [1]. No more than 5% BC cases are associated with mutations of highly penetrant genes such as BRCA1, BRCA2, or P53 [2, 3]. In most cases, BC is multifactorial. The disease has not been associated with any particular gene so far; rather, numerous factors are thought to underlie it. As epidemiological studies have shown, risk of BC indeed depends on certain factors affecting people throughout their life. Some of these are related to the level of endogenous steroid hormones. These are early menarche, pregnancy at a relatively old age, delayed menopause, excessive body weight (obesity) in the postmenopausal period (since estrogens are synthesized in adipose tissue in obese women), etc. [4].

It is known that elevated estrogens and prolongation of their effect on the organism increase risk of BC [5, 6]. An increase in serum estrogens and especially in estradiol, which is the most biologically active estrogen, contributes substantially to the risk of BC [7]. A contribution is also made by the local effect of estrogens produced in the adipose tissue of the mammary gland [8–10]. The hypotheses ascribing a substantial role in carcinogenesis to steroid hormones proceed from the assumption that factors (hormones, interleukins) stimulating the mitotic activity of mammary-gland cells increase risk of BC [11].

We assumed that CYP17 and CYP19, which participate in estrogen biosynthesis, have a bearing on predisposition to BC. Possibly, some polymorphisms of these genes affect, if even slightly, the activity of relevant enzymic systems, while a combination of certain alleles substantially changes hormone production and thereby greatly increases individual risk of BC. Specific combinations of certain alleles may be associated with higher risk of BC, increasing estrogens in serum or in the adipose tissue of the mammary gland.

The product of CYP17 belongs to the P450 cytochrome family. This enzyme has a 17-hydrolase and 17,20-lyase activities, and participates in biosynthesis of human steroid hormones, including estradiol [12]. The 5'-untranslated region of CYP19 contains a single-nucleotide polymorphism, T27C, with alleles distinguishable by MspAI digestion. Serum estradiol is elevated in women carrying allele A2 [14–16]. This polymorphism has already been tested for association with BC, but the results are discrepant [15–20].

The product of CYP19, aromatase, is also involved in estrogen biosynthesis. This enzyme converts dehydroepiandrosterone (a testosterone form) into estron (estrogen) [21]. In the postmenopausal period, cells of the adipose tissue are the major source of estrogens, and expression of aromatase increases with age and body weight. Elevated local production of estrogens in the adipose tissue of the mammary gland increases risk of tumorigenesis [22, 23]. Intron 4 of CYP19 con-
tains polymorphic microsatellite (TTTA)ₗ. This polymorphism has been associated with BC, but there is no consensus as to which allele is predisposing. The candidates are (TTTA)₇ [24], (TTTA)₈ [25], (TTTA)₁₀ [25, 26], (TTTA)₁₂ [27], and alleles containing more than 10 repetitive units [28].

In addition to the short tandem repeat (STR), intron 4 has polymorphic deletion of three nucleotides (TCT) in the vicinity of (TTTA)₉. This polymorphism has not been associated with BC [24–26]; yet the presence of the deletion has been reported to increase risk of BC in premenopausal vs. postmenopausal women [30].

A combination of certain CYP17 and CYP19 alleles may increase the estrogen level in the organism so that the estrogen concentration in the mammary gland is higher than in serum. This effect is probably especially high in postmenopausal women. At high concentrations, estrogens may trigger cell malignant transformation.

In this work, we tested the above polymorphisms of CYP19 and CYP17 for association with BC in women from East European Russia.

**EXPERIMENTAL**

**Blood specimens** were obtained from blood of 123 BC patients (mean age 56.6 ± 11.0) who were treated in the Blockhin Cancer Research Center. The control sample included 119 blood specimens of women (mean age 40.3 ± 8.7) who had no BC. Control blood specimens were obtained from the blood collection unit of the Sechenov Medical Academy.

**Genomic DNA** was isolated by phenol–chloroform extraction with modification [31].

**Amplification.** Primer sequences and PCR conditions were as described earlier [18, 29]. To identify the CYP17 alleles, the amplification product was digested with MspAl (Fermentas, Lithuania) for 4 h. The products amplified from CYP17 and CYP19 were resolved by PAGE in 8% gel and silver stained [31]. To identify the (TTTA)ₙ alleles, DNA was sequenced with ABI Prism 310 Genetic Analyzer kits as recommended by Applied Biosystems.

**Statistical analysis.** The association of alleles (genotypes) with risk of BC was analyzed by Fisher’s exact test. Two-way significance (P) was estimated, and α = 0.05 used as its critical level. Allele and genotype frequency distributions were used to estimate odds ratio (OR) and the corresponding confidence intervals (CI). Samples with or without a given character were compared. The sample size was characterized by test potency β. Statistical analysis employed the GrafPad InStat program (ver. 3.05).

To characterize the diagnostic potential of predisposition factors identified, sensitivity and specificity were estimated respectively as the proportion of detectable cases and that of reliable detections.

**RESULTS**

Analysis of the STR polymorphism in CYP19 intron 4 revealed six (TTTA)ₙ alleles differing in electrophoretic mobility of the amplified fragment.

To establish the TTTA unit number, specimens of each group were sequenced. The alleles proved to contain 7–12 repeats; allele (TTTA)₉ was not found. In total, seven alleles with unit number ranging from 7 to 13 have been reported for this polymorphism [24–28]. In addition to seven TTTA units, the smallest allele contained trinucleotide deletion (TCT) 50 bp upstream of the repeat. The allele and genotype frequencies observed in patients and controls are shown in Table 1.

To check the other alleles for TCT deletion, DNA specimens were subjected to SSCP analysis. Each of the allele groups isolated by electrophoretic mobility proved to be homogeneous (data not shown), testifying to the absence of the deletion from all alleles but (TTTA)₇.

Analysis of the allele frequencies in the two samples identified allele (TTTA)₁₀ as predisposing to BC (11.8% vs. 6.3%, P = 0.04, OR = 1.99, 95% CI 1.04–3.81), which agreed with the published data [25]. No significant difference was observed for alleles (TTTA)₁₀ (2.4% vs. 4.6%, P = 0.22, OR = 0.52, CI 0.19–1.42) and (TTTA)₁₂ (3.7% vs. 3.8%, P = 0.99, OR = 0.97, CI 0.38–2.48), although these alleles have been associated with BC in West European, Scandinavian, and East Asian populations [22–25]. With our sample sizes and allele frequencies, sensitivity was estimated at 0.35 in the case of (TTTA)₁₀ and 0.50 in the case of (TTTA)₁₂ at P = 0.05. Analysis of the trinucleotide deletion frequencies did not reveal any significant difference between the two groups (32.9% vs. 37.4%, P = 0.34, OR = 0.82, CI 0.57–1.19); sensitivity was estimated at 0.48. This polymorphism has been associated with BC only in premenopausal women [30]. Since the relevant data were unavailable, such analysis was not carried out with our sample. Reliable comparison of the genotype frequency distribution for the two groups was impossible because of the low sample sizes. Yet risk of BC tended to be higher in women with genotype (TTTA)₉/(TTTA)₁₁ (9.8% vs. 3.4%, P = 0.07, OR = 3.12, CI 0.97–9.93).

The SNP polymorphism of CYP17 was studied with the same groups of patients and controls. The allele and genotype frequencies are shown in Table 2.

As analysis of the allele frequencies demonstrated, allele A2 is associated with higher risk of BC (59.3% vs. 48.3%, P = 0.02, OR = 1.56, CI 1.09–2.24). Yet no significant difference was observed between the