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Functional Polymorphism of Thymidylate Synthase, But Not of the *COMT* and *IL-1B* Genes, Is Associated With Breast Cancer

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Polymorphic variations may affect the rate of gene transcription, the stability of the mRNA, or the quantity and activity of the resulting protein. In this study we evaluated the association between the interleukin-1B C-31T, catechol-*O*-methyltransferase Val158Met, and thymidylate synthase (TS) 1494del6 polymorphisms and breast cancer. Each genetic polymorphism was investigated by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis. No significant difference in either the genotype distribution or the allelic frequencies of the IL-1B and COMT gene polymorphisms was observed between the patient and control groups. For the TS 1494del6 polymorphism a significant difference was observed for both the genotypes ($P = 0.01$) and the allele frequencies ($P = 0.0097$), indicating a decreased risk associated with the variant allele. Our data do not provide evidence for an association between the polymorphic variants of the IL-1B and COMT genes and breast cancer risk. On the other hand, the TS 1494del6 polymorphism is associated with a significantly lower risk of breast cancer and may be a potential genetic marker. J. Clin. Lab. Anal. 21:97–102, 2007. © 2007 Wiley-Liss, Inc.

Key words: IL-1B; COMT; TS; polymorphism; breast cancer

INTRODUCTION

Breast carcinogenesis is a complex multistep process that is influenced by several factors. Polymorphisms in specific genes may affect the rate of gene transcription, the stability of the mRNA, or the quantity and activity of the resulting protein (1). Proinflammatory cytokines produced by inflammatory cells have been associated with inflammatory diseases and cancer (2). The interleukin (IL) family includes three members: IL-1A, IL-1B, and IL-1Ra. IL-1A and IL-1B are potent proinflammatory cytokines, whereas IL-1Ra is an anti-inflammatory cytokine that competes with IL-1A and IL-1B for binding to IL-1 receptors without an intrinsic effect (1). Two potentially functional SNPs are observed in the *IL-1B* promoter. The C-31T variant is located in a promoter TATA-box that markedly affects DNA–protein interactions in vitro and has been associated with fivefold elevated binding activity to initiation factors (3). Associations with *IL-1B* polymorphisms have been reported for stomach cancer (3), *Helicobacter pylori* infection (4), periodontitis (5), and inflammatory bowel diseases (6). In breast cancer cells, IL-1B combines with the estrogen receptor-α, resulting in transcriptional activation (7). Higher IL-1B levels than IL-1A are observed in invasive breast cancer tissue (8). These findings suggest that interindividual variations in IL-1 production may play a role in the development of breast cancer.

COMT (catechol-*O*-methyltransferase) is involved in the activation and detoxification of catecholestrogens, which can cause oxidative damage (9,10). O-methylation increases the concentrations of 4-methoxyestradiol (4-MEO-E2) and 2-methoxyestradiol (2-MEO-E2). 2-MEO-E2 possesses antiproliferative, cytotoxic, and apoptotic activity, and thus decreases the potential for DNA damage (11). In the past several years, research interest in the catechol-metabolizing system has been revived because of its potential pathophysiological and pathogenic significance in estrogen-induced hormonal cancers (12). Two distinct variants of COMT are encoded by a single gene located at chromosome 22q11: a soluble protein with 221 residues, and a membrane-bound protein with an additional 50

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residues at the N-terminus (13). A single base polymorphism (G→A) at codon 108 of the soluble *COMT* and codon 158 of the membrane-bound form results in an amino acid change (Val→Met) leading to the synthesis of a variant, thermolabile enzyme (14). Homozygote individuals for the Met allele (*COMT*-L) display three- to fourfold decreased enzyme activity resulting in increased levels of circulating catechol estrogens and decreased formation of the antitumorigenic 2-methoxyestradiol (14,15). So far, there are only limited data on the effect of *COMT* polymorphisms in carcinogenesis (16,17). However, epidemiological studies indicate that the low-activity *COMT* genotype may be associated with an increased breast cancer risk (18–20).

Thymidylate synthase (TS) is a key enzyme that catalyzes methylation of deoxyuridine-5′-monophosphate (dUMP) to deoxythymidine-5′-monophosphate (dTMP) with 5,10-methylene tetrahydrofolate (CH2-THF) as the cofactor. This reaction is the source of de novo cellular thymidylate production (21). TS is an essential enzyme in proliferating cells, and is also an important target for a variety of chemotherapeutic drugs, including 5-FU, capecitabine, and pemetrexed (21,22). Resistance to fluoropyrimidines can occur through a variety of mechanisms, including elevated TS protein expression as a result of higher transcription (23) and translation rate (24). A polymorphism within the 3′-untranslated region of the *TS* gene, consisting of a 6-bp deletion at nucleotide 1494 of the TS mRNA, has been associated with decreased mRNA stability and an enhanced rate of mRNA decay (21,22). This common polymorphism is also transcribed into the 3′-untranslated region (3′-UTR) of the primary *TS* transcript. 3′-UTRs are implicated in the modulation of gene regulation at the post-transcriptional level in many mammalian systems. Thus, functional genetic variants of the *TS* gene may represent risk factors for breast cancer because of their central role in cellular folate metabolism.

In this study we investigated the association of the *IL-1B* C-31T, *COMT* Val158Met, and *TS* 1494del6 gene polymorphisms with the risk of breast cancer in the Turkish population.

MATERIALS AND METHODS

We evaluated the IL-1B C-31T polymorphism in 126 breast cancer patients (mean age = 51.1 ± 11.3 years), the *COMT* Val158Met polymorphism in 114 breast cancer patients (mean age = 51.1 ± 13.1 years), and the TS 1494del6 polymorphism in 150 breast cancer patients (mean age = 49.6 ± 9.8 years). We also evaluated these polymorphisms in 110 (mean age = 40.6 ± 11.6 years), 108 (mean age = 41.2 ± 11.4 years), and 141 (mean age = 40.1 ± 11.0 years) control subjects, respectively. Blood samples were collected and DNA was extracted by standard (phenol-chloroform) methods. The polymorphisms were analyzed by means of polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

TABLE 1. The primer pairs used in the study

IL-1B-F	5′ - AGA AGC TTC CAC CAA TAC T -3′
IL-1B-R	5′- TAG CAC CTA GTT GTA AGG A -3′
COMT-F	5′ - TAC TGT GGC TAC TCA GCT GTG C -3′
COMT-R	5′ - GTG AAC GTG GTG TGA ACA CC -3′
TS-F	5′ - CAA ATC TGA GGG AGC TGA GT -3′
TS-R	5′ - CAG ATA AGT GGC AGT ACA GA -3′

The primer sequences used in the study for the *IL-1B*, *COMT*, and *TS* genes are shown in Table 1 (22,25,26).

To investigate the *IL-1B* C-31T and *COMT* Val158-Met polymorphisms, we performed PCR amplification in a volume of 25 μL 1X PCR buffer containing 0.5 ng/μL of genomic DNA, 30 nM of specific primer pair, 10 mM of each dNTP, and 1.5 mM $MgCl_2$ and 1 U of Taq-Polymerase (Fermantas, Vilnius, Lithuania). For the *IL-1B* C-31T polymorphism, the amplification conditions were as follows: 5 min of initial denaturation at 94 °C, followed by 25 cycles of 1 min at 94 °C, 1 min at 54 °C, and 1 min at 72 °C, and a 5-min final extension at 72 °C (25). Then 8 μL of the PCR product was digested with 4 U of AluI (Fermantas) at 37 °C. The cycling conditions for the *COMT* codon 158 polymorphism consisted of 35 cycles of 94 °C for 30 sec, 56 °C for 30 sec, and 72 °C for 30 sec. The PCR product was digested with Hsp92II (Promega, Madison, WI) by overnight incubation at 37 °C.

To analyze the TS 1494del6 polymorphism, we performed amplification with 1 μg/μL of genomic DNA in a total volume of 25 μL containing 1X PCR buffer, 1.5 mM of $MgCl_2$, 10 mM of each dNTP, 30 nM of primers, and 1 U of Taq-Polymerase (Fermantas). The amplification conditions were as follows: denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 30 sec, 58 °C for 45 sec, and 72 °C for 45 sec, and a final extension at 72 °C for 5 min. The amplified fragments were digested with 4 U of DraI (Fermantas) by overnight incubation at 37 °C.

The restriction products were separated on 15% polyacrylamide gels containing ethidium bromide (10 mg/μL) and evaluated using a video documentation system (Vilber-Lourmat, Marne-de Vallée, France). The chi-squared test was used to compare the allele and genotype frequencies.

RESULTS

Restriction analyses of the *IL-1B* C-31T, *COMT* codon 158 and TS1494del6 polymorphisms are shown in

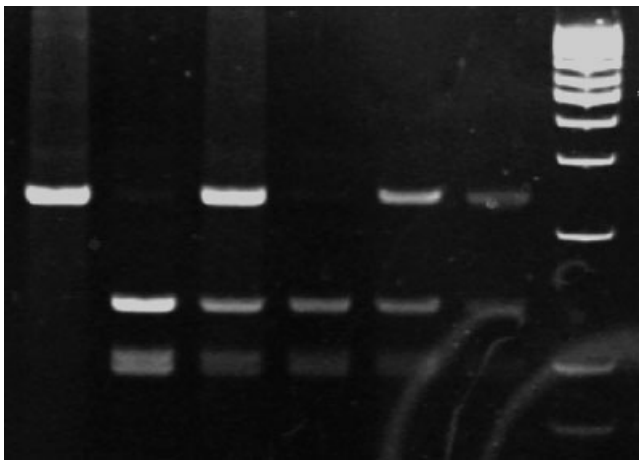

Fig. 1. *IL-1B* genotypes. Lane 1: Wild-type. Lanes 2 and 4: Homozygote variants. Lanes 3, 5, and 6: Heterozygote samples.

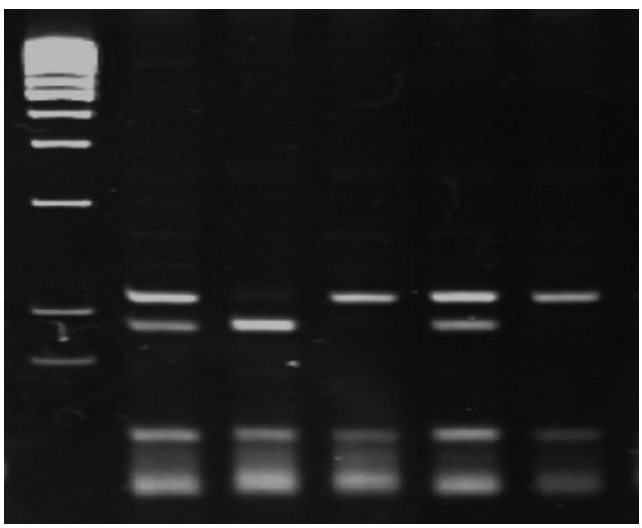

Fig. 2. Analysis of the *COMT* codon 158 polymorphism: Lanes 1 and 4: Heterozygote samples, Lane 2: Homozygote variant. Lanes 3 and 5: Wild-type homozygote samples.

Figs. 1, 2 and 3. The sizes of the restriction fragments of the *IL-1B* gene amplification product were 240 bp for the wild-type (CC), 138 and 103 bp for the homozygote variants (TT), and 240, 138, and 103 bp for the heterozygotes (CT) (Fig. 1). The frequencies of the variant (T) and wild-type alleles (C) were 39.3% vs. 60.7%, and 44.5% vs. 55.4% in the patient and control groups, respectively.

Restriction analysis of the *COMT* gene revealed a single fragment of 114 bp for the homozygote wild-type (GG), two fragments of 114 and 96 bp for heterozygotes (AG), and a single band of 96 bp for the homozygote variant (AA) types (Fig. 2). The distribution of the genotypes was compared between breast cancer patients and healthy individuals to determine whether the variant alleles were associated with breast cancer risk.

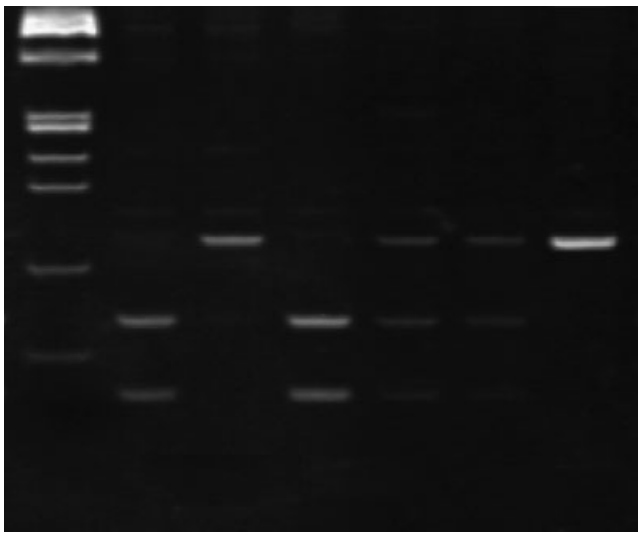

Fig. 3. Analysis of the TS1494 polymorphism. Lanes 1 and 3: Wild-type homozygote samples. Lanes 2 and 6: Homozygote variants. Lanes 4 and 5: Heterozygote samples.

The allele and genotype frequencies of each polymorphism in the patient and control groups are depicted in Table 2.

No association was observed between the genotypes and breast cancer risk for the *IL-1B* (C-31T) and *COMT* Val158Met polymorphisms ($x^2 = 1.393$, $P = 0.237$, and $x^2 = 0.929$, $P = 0.594$, respectively). The frequencies of the variant allele were similar in the patient and control groups, and the differences were not statistically significant ($x^2 = 1.336$, $P = 0.274$, and $x^2 = 0.986$, $P = 0.320$, respectively).

The *TS* gene restriction fragment sizes were 70 bp and 88 bp for the wild-type (+6 bp/+6 bp) allele. The absence of the polymorphic 6 bp demolishes the DraI restriction site. Thus, a 152 bp band is observed for the variant (−6 bp/−6 bp) allele. Heterozygote individuals (+6 bp/−6 bp) display all three fragments.

A significant difference was observed between the genotype ($x^2 = 6.587$, $P = 0.01$) as well as the allele frequencies ($x^2 = 6.68$, $P = 0.009$) of the TS 1494del6 polymorphism in the patient and control groups.

DISCUSSION

Different cytokines play a role in the growth and differentiation of mammary epithelial tissue (3). Peritumoral lymphocytes have been shown to secrete potent cytokines in breast tumors (27). Therefore, polymorphisms leading to low cytokine levels could influence the risk of cancer by decreasing the immuno-

TABLE 2. Genotype and allele frequencies in the patients and control groups

	IL-1B (C-31T)	*COMT* (Val158Met)	*TS* 1494del6
Patients (n)	126	114	150
Homozygote (wild type)	18 (%14.2) (CC)	29 (%25.4) (GG)	53 (%35.3) (+6 bp/+6 bp)
Heterozygote	63 (%50) (CT)	59 (%51.7) (AG)	77 (%51.33) (+6 bp/−6 bp)
Homozygote (variant)	45 (%35.7) (TT)	26 (%22.8) (AA)	20 (%13.33) (−6 bp/−6 bp)
Controls (n)	110	108	141
Homozygote (wild type)	21 (%19.09) (CC)	34 (%31.48) (GG)	29 (%20.56) (+6 bp/+6 bp)
Heterozygote	56 (%50.9) (CT)	53 (%49.07) (AG)	84 (%59.57) (+6 bp/−6 bp)
Homozygote (variant)	33 (%30) (TT)	21 (%19.4) (AA)	28 (%19.85) (−6 bp/−6 bp)
Allele frequencies			
Patients	C, 99 (%39.28)	G, 117 (%51.3)	+6 bp, 183 (%61)
	T, 153 (%60.71)	A, 111 (%48.6)	−6 bp, 117 (%39)
Controls	C, 98 (%44.54)	G, 121 (%56.01)	+6 bp, 142 (%50.3)
	T, 122 (%55.45)	A, 95 (%43.98)	−6 bp, 140 (%49.64)

logical response. The *IL-1B* C-31T polymorphism is located in a promoter region with a TATA-box that markedly affects DNA–protein interactions in vitro. The–31T allele is associated with a fivefold-elevated binding activity to the transcription factors (3). Previous studies have shown an association between the *IL-1B* C-31T polymorphism and risk of gastric adenocarcinoma development in different ethnic groups (3,28,29). A higher risk in individuals infected with *Helicobacter pylori* who harbor the variant allele has been explained by the induction of inflammatory products such as IL-1B by *H. pylori* (30). However, other studies in the literature report a lack of association between the *IL-1B* polymorphism and gastric cancer (31). A recent report showed an association between 1L1B gene polymorphisms and lung cancer risk (2), while another study suggested that the *IL-1B*-31T/T genotype and the *IL-1B*-511/-31 haplotype C-T are associated with hepatocellular carcinoma in Japanese patients with chronic hepatitis C virus infection (32).

In addition to these inconsistent findings, a previous study suggested that women with the CT/TT genotype of *IL-1B* C-31T have a reduced risk of breast cancer (33). In contrast to that report, we did not observe a significant difference in the frequency of the variant allele or the genotype distribution between the patient and control groups. There was no association between the *IL-1B* C-31T polymorphism and clinical features (stage, histology, nuclear and histologic grade, estrogen, and progesterone receptor).

Since estrogens play an important role in the genesis of breast cancer, considerable attention has been given to the polymorphisms of the estrogen biosynthesizing and metabolizing genes (34). *COMT* participates in estrogen metabolism after hydroxylation of estrogens to catecholestrogens by forming O-methylated derivatives. Hydroxylated estrogens can be oxidized to semiquinones and quinones with carcinogenic properties (35,36). The *COMT* Val158Met polymorphism has been analyzed in different ethnic groups (19,20,37,38). It has been hypothesized that the low-activity *COMT*-L allele increases breast cancer risk (10), but the results have been conflicting. A significantly increased risk of breast cancer has been reported in Korean *COMT*-L allele carriers (20) and postmenopausal Taiwanese women (39). In a study of Caucasian women from the United States, the *COMT*-L allele was associated with an increased risk of breast cancer in premenopausal women, and a decreased risk in postmenopausal women (40). An association with disease progression and lymph node metastasis has also been reported (41).

On the other hand, a population-based case-control study failed to observe an association between the *COMT* alleles and disease risk (42). Our results are in concordance with these data, since we did not observe significant differences in the genotype and allele distribution of the *COMT* codon 158 polymorphism. There was also no association between the *COMT* polymorphism and clinical parameters such as stage, histology, nuclear or histologic grade, and hormone receptor status. These findings are in agreement with a previous study on Turkish woman (19), as well as with reports on different ethnic populations, such as Chinese, African-American, and Caucasian women (37,38). The association between the *COMT*-L allele and breast cancer risk seems to be modified by several other risk factors, such as menopausal status, body mass index (BMI), and hormone replacement therapy (18,43).

Previous reports suggested that the *TS*1494del6 polymorphism is associated with decreased TS mRNA levels (21,22). This led to the hypothesis that TS protein levels may be similarly affected. However, more recent studies have failed to reveal a significant relationship between TS activity and the 3′ TS genotype (44).

In colon cancer patients homozygous for the variant allele, 4.2-fold lower *TS* mRNA levels compared to

homozygote wild-type allele carriers have been reported (22). It has been suggested that individuals carrying the variant alleles have a 40% increased risk of colon cancer compared to wild-type homozygotes. However, subsequent case-control studies have failed to confirm an association between the *TS*1494del6 polymorphism and colorectal carcinoma (45,46).

There are no reports in the literature on the role of the *TS*1494del6 polymorphism in breast cancer. Since the polymorphism has been linked to a reduced production of TS, we hypothesized that this genetic variant may be associated with a higher risk of breast cancer. We observed a significant difference for both genotypes and the allele frequencies of the *TS*1494del6 polymorphism between the patient and control groups. Interestingly, the homozygote variant genotype was found more frequently in the healthy controls, indicating a lower risk associated with the variant allele. Our study was sufficiently large to have statistical power. No significant difference was found between the distribution of genotypes and clinical features.

In conclusion, our data do not provide evidence for an association between the polymorphic variants of the *IL-1B* and *COMT* genes and breast cancer risk. In contrast to previous reports on colon cancer, we observed a lower risk in breast cancer patients carrying the variant *TS* allele. Large-scale, population-based studies are needed to investigate the range of risks associated with the *TS* alleles and breast cancer.

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