Abstract
There is compelling evidence to suggest that catecholestrogens may play a role in the development of breast cancer. Particularly, inactivation of catecholestrogens may prevent the genesis and arrest the development of breast cancer. Catechol-O-methyltransferase (COMT) is polymorphic and responsible for the detoxification of catecholestrogens. In the present study, we examined what role COMT gene polymorphisms may play in the development of breast cancer in a case–control study of 130 sporadic unrelated premenopausal Turkish breast cancer patients with 233 unrelated healthy controls. The frequency of COMT-L allele was more significantly represented in the breast cancer cases (48.08%) than in the controls (38.20%). The genotype frequencies of COMT-HH, HL and LL were 25.4, 53.1 and 21.5% in the breast cancer subjects and 26.6, 62.7 and 10.7% in the controls respectively. In conclusion, the COMT-L allele and COMT-LL genotype are genetic risk factors for sporadic breast cancer in premenopausal Turkish women.

Keywords: COMT; Polymorphism; Breast cancer; Premenopausal women; Turkey

1. Introduction
Catechol-O-methyltransferase (COMT, E.C. 2.1.1.6) catalyzes the transfer of a methyl group from the donor S-adenosylmethionine (SAM) to catecholamines (Axelrod and Tomchick, 1958). COMT is widely distributed in various mammalian tissues, mainly in brain, liver, kidney, endometrium and breast (Weisz et al., 1998), and whose physiological substrates include a wide range of chemical compounds such as catecholamine neurotransmitters and endogenous and exogenous catechol estrogens (Weisz et al., 1998; Karhunen et al., 1994; Raitiojans et al., 2000). In blood COMT is found mainly in erythrocytes;
in leukocytes it exhibits low activity (OMIM: http://www.ncbi.nlm.nih.gov/entrez). It has been a long-supported view that the main physiological function of the COMT is basically for the inactivation of biologically active and toxic endogenous and/or exogenous catechols (Axelrod and Tomchick, 1958). COMT is considered to play a critical role in the regulation of levels of endogenous catechols (catecholamines and CEs). Moreover, COMT provides a protective role by blocking oxidative metabolism of endogenous and exogenous catechols and, therefore, the generation of potentially mutagenic electrophiles (Raffogianis et al., 2000). COMT is produced as both a soluble protein with 221 residues (S-COMT, 25 kDa) and a membrane-bound protein with an additional 50 residues at the N-terminus (MB-COMT, 30 kDa) (Huh and Friedhoff, 1979). A single gene on chromosome 22q11.2 encodes both proteins, but separate promoters initiate their expression. A common single-nucleotide polymorphism (SNP) in the coding region of the human COMT gene results in substitution of methionine for valine at position 158 of MB-COMT and position 108 of S-COMT (Weinshilboum and Raymond, 1977; Lachman et al., 1996). Recent, several but not all, epidemiological studies have reported that the low-activity COMT genotype is associated with an increased breast cancer risk (Huang et al., 1999; Lavigne et al., 1997; Thompson et al., 1998; Millikan et al., 1998; Kocabas et al., 2002; Mitrunen et al., 2002; Matsui et al., 1997; Yim et al., 2001). The Val 108/158 Met polymorphism encodes a thermolabile variant of the enzyme that in homozygotes (COMT-LL) confers an about 2- to 4-fold lower catalytic activity (Dawling et al., 2001; Syvanen et al., 1997). Heterozygous individuals (COMT-HL) have intermediate and homozygous wild-type (COMT-HH) have high enzyme activity (Weden et al., 2003). A significant positive association between breast cancer risk and low-activity COMT genotype was identified. Nevertheless, the findings differ with respect to the age group in which this association is found (i.e., whether in pre or postmenopausal women or both).

In the present study, we studied the allele and genotype frequencies of COMT Val 108/158 Met polymorphisms in sporadic breast cancer and controls. We also determined which allele or genotype might cause susceptibility to sporadic breast cancer in premenopausal women in Turkey.

2. Materials and Methods

2.1. Subjects

Breast cancer patients were assessed on the basis of clinical examinations as well as mammographic and histopathological examinations. Breast cancer patients and controls were from the same geographical origin. A total of 130 breast cancer patients was enrolled in the study during the period of May 1999 to April 2003. The breast cancer patients studied here had not been previously exposed to either chemotherapy and/or radiotherapy. Control subjects, without history of breast cancer, were recruited to the study.

2.2. Genotype analysis

DNA was isolated from peripheral venous blood with a procedure previously published (Miller et al., 1988). In the COMT genotype analysis, a 217 bp fragment was first amplified using a forward primer 5′-TCG TGG ACG CCG TGA TTC AGG-3′, and a reverse primer 5′-AGG TCT GAC AAC GGG TCA GGC-3′ as described by Yim et al. (2001) with some modifications. Subsequently, a 10 ul of the 217 bp PCR amplified fragment was further digested by a 5 U of the Hsp 92 II restriction endonuclease (Promega, Madison, WI). The electrophoresis of the digested fragment was carried out at 20 W for 35 min on 10% polyacrylamide gels followed by silver staining. The gels were scanned using a scanner. Restriction fragments of 114, 83 and 20 bp revealed the COMT-H allele, while the 114 bp fragment was cut into 96 and 18 bp fragments in the COMT-L allele.

2.3. Statistical analysis

Odds ratios (OR), 95% confidence intervals (CI) and chi-squared analysis for a matched analysis were computed using conditional logistic regression. Genotype and allele frequencies for the COMT Val 108/158 Met polymorphism were estimated from observed genotype counts. The expected genotype and allele proportions according to the Hardy-Weinberg law were calculated and compared to observed genotypes and alleles. Genotype and allele frequencies were assessed for association with breast cancer using standard contingency table analysis incorporating the chi-squared test of independence. This analysis produces a chi-squared
Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Allele frequency:</th>
<th>OR, 95% CI, chi-square, d.f., P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 130</td>
<td>N = 233</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>COMT 100.0 (%) 100.0 (%)</td>
<td>48.08, 38.20</td>
<td>chi-square = 8.019, d.f. = 2, P = 0.018</td>
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<td></td>
</tr>
<tr>
<td>HH 33 (25.4) 62 (26.6)</td>
<td>0.938 (0.575–1.532)</td>
<td>chi-square = 0.065, d.f. = 1, P = 0.799</td>
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<tr>
<td>HL 69 (53.1) 146 (62.7)</td>
<td>0.674 (0.436–1.041)</td>
<td>chi-square = 3.174, d.f. = 1, P = 0.075</td>
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<tr>
<td>LL 28 (21.5) 16 (10.7)</td>
<td>2.284 (1.267–4.116)</td>
<td>chi-square = 7.819, d.f. = 1, P = 0.005</td>
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The P-value of 0.05 was specified as the significance threshold. All the statistical analyses were performed using the SPSS (v11).

3. Results

3.1. Subjects

The study consisted of 130 sporadic and unrelated breast cancer women, and 233 age frequency matched women controls. The age range of the Turkish women included in the breast cancer study was 20–42 with a mean age of 36.58 ± 9.02 years. The 233 healthy controls were from the same population from which the breast cancer patients arose. The age range of the controls was 19–43 with a mean age of 36.11 ± 10.14 years. In the control population, individuals were selected whether the family in which they were brought up had breast cancer in the past. Those who had not had, were included in the study and also they were examined by two of us for the suitability for control. The cohort breast cancer patients were diagnosed in the breast clinic of the Department of General Surgery of the University of Kocaeli, Turkey, based on mammographic, histopathological findings and clinical examination. Informed consent was obtained from all women who participated in the study. The study was also evaluated by the review board of the University of Kocaeli.

3.2. Genotype analyses in sporadic breast cancer patients and controls

We provide evidence for the involvement of COMT polymorphism in sporadic breast cancer in Turkish women. In this case–control study, 130 sporadic unrelated breast cancer women and 233 unrelated healthy women controls were studied for the possible effect of the COMT polymorphisms on breast cancer predisposition in Turkish women. The expected COMT-L allele frequency was 44.23% in the breast cancer cases, and 44.21% in the controls. Actually the observed L allele frequency was 48.08% in the breast cancer cases, and 38.20% in the controls. Frequencies of COMT-HH, HL and LL genotypes were 25.4, 53.1, and 21.5% in the breast cancer cases and 26.6, 62.7, and 10.7% in the controls, respectively. The COMT-LL genotype was overrepresented in the breast cancer cases (21.5%) compared with controls (10.7%) (OR = 2.284; 95% CI = 1.267–4.116; chi-square = 7.819, d.f. = 1, P = 0.005) (Table 1).

4. Discussion

Although the molecular mechanisms underlying the development of breast cancer are not known, it is generally believed that the initiation of breast cancer is a consequence of cumulative genetic damages leading to genetic alterations. Most of the risk factors for breast cancer relate to the increased or prolonged exposure to oestrogen. However, metabolic by-products of oestrogen in the body may also act as initiators of cellular alterations (Yager, 2000).

The COMT proteins are expressed constitutively but in slightly different proportions in most of the tissues analysed (Gulberg and Marsden, 1975; Lundström et al., 1995). Changes in the expression of COMT activity have been detected during mammalian development, ageing, and oestrous cycle (Inoue and Creveling, 1991). Elevated COMT activity has also been observed in some solid tumors; such as in melanomas (Smit et al., 1994) insulinomas (Feldman et al., 1979), and breast...
cancer (Assicot et al., 1977; Hoffman et al., 1979; Amin et al., 1983). Elevated COMT expression has also been reported in an MCF7 human breast adenocarcinoma cell line (Hoffman et al., 1979; Lavigne et al., 2001). Breast cancer has been shown to be connected with exposure to exogenous or endogenous steroid hormones and their metabolites. Estrogen has been reported to be one important risk factor for breast cancer in human (Stack et al., 1998; Yager and Liehr, 1996; Liehr, 1997; Service, 1998). Catecholestrogens may be metabolised to quinones or semiquinones, which have carcinogenic properties (Yager and Liehr, 1996; Ball and Knuppen, 1990). COMT enzyme is able to methylate catecholestrogens to produce 2- and 4-methoxyestradiols, which are noncarcinogenic metabolites. In fact, 2-methoxyestradiol seems to have potent tumor suppressor activity in vitro (Fotsis et al., 1994). Tenhunene et al. (1999) have reported that elevated S-COMT and MB-COMT proteins were present in neoplastic mammary gland samples of patients with breast cancer by a magnitude of 2.7- and 4.8-fold respectively. Polymorphisms associated with breast cancer risk have been identified in the genes involved in a wide variety of functions including steroid hormone metabolism, detoxification of environmental carcinogens, DNA damage repair genes, and tumor suppressor genes (Dunning et al., 1999). Genetic polymorphism existing in the coding region and/or promoter region is expected to be associated with a small to moderate increase in breast cancer risk (Weber and Nathanson, 2000).

Since, estrogens play an important role in the genesis of breast cancer, considerable attentions have been given to the polymorphisms in the estrogens biosynthesizing and metabolizing genes (Kristensen and Borresen-Dale, 2000). Such polymorphisms are expected to affect the synthesis or degradation of estrogens and, consequently, the risk of breast cancer. COMT is a phase II enzyme implicated in the inactivation of catechol estrogens by transfer of a methyl group. Methylated catechol estrogens have been demonstrated to lack estrogenic activity (Zhu and Conney, 1998). Since COMT 108/158 Met has been associated with three to four-fold decreased activity of the COMT compared with COMT 108/158 Val, these two forms are designated as COMT-L allele and COMT-H allele respectively. Associations between COMT-L allele and breast cancer have been investigated (Huang et al., 1999; Lavigne et al., 1997; Thompson et al., 1998; Millikan et al., 1998; Kocabas et al., 2002; Mitrunen et al., 2002; Matsui et al., 2000; Yim et al., 2001; Hamajima et al., 2001; Bergman-Junghoestrom and Wingren, 2001). In the study of US Caucasian women, Thompson et al. (1998) have reported that the COMT-L allele is associated with an increased risk for breast cancer in pre-menopausal women (OR = 2.4, 95% CI = 1.4–4.3), while a decreased risk in postmenopausal women (OR = 0.5, 95% CI = 0.3–0.9). Huang et al. (1999) have demonstrated that COMT-L allele carriers showed a significantly increased risk for breast cancer in postmenopausal Taiwanese women (OR = 3.55, 95% CI = 1.15–13.37). Yim et al. (2001) have reported that COMT-L allele showed an increased risk for both pre and postmenopausal breast cancer Korean women (OR = 1.7, 95% CI = 1.04–2.78). Lavigne et al. (1997) found significantly increased risk only among obese postmenopausal women carrying the COMT-LL genotype. Matsui et al. (2000) recently reported that the COMT-L allele was associated with progression and lymph node metastasis of breast cancer in Japanese women. In a recent study by Mitrunen et al. (2002), the COMT-LL genotype was found to be significantly associated with breast cancer in postmenopausal women with long-term use of estrogen in Finnish women (OR = 4.02, 95% CI = 1.13–14.3). On the contrary, Millikan et al. (1998) found no significant association in a large population-based case-control study. In the study of Turkish women, Kocabas et al. (2002) suggested that there was no association between COMT-L allele and breast cancer (OR = 0.86, 95% CI = 0.46–1.60). The COMT LL genotype was 13% in the controls and 17% in the breast cancer population. The COMT-L allele was 39% in the controls and 42% in the breast cancer subjects. The region we did the work is the most industrialised area in Turkey. Kocabas et al. (2002) did the work in Ankara where there is no industrial pollution. The study population size of them was 84 breast cancer patients and 103 controls, whereas our population included 130 breast cancer patients and 233 controls. In the present study, The COMT-L allele frequency was 38.20% in the controls and 48.08% in the breast cancer patients. The COMT-LL genotype frequency was 10.7% in the controls and 21.5% in the breast cancer patients (Table 1).

The variant L allele frequencies in Caucasian women, ranging from 49 to 63% are higher than those
in Asian women, ranging from 24 to 33% (Miayoshi and Noguchi, 2003). The pooled data of the L allele frequency of all Caucasian and Asian women was 53 and 28% respectively. The frequency in women of the COMT-L allele was 25% in Caucasian (Lavigne et al., 2001), 5% in Taiwanese (Huang et al., 1999), 10% in Korean (Yim et al., 2001), 13% in African-American (Milikan et al., 1998), 14% in Japanese (Hamajima et al., 2001), 50% in Finnish (Mitrnrun et al., 2002), 37% in Swedish (Bergman-Jungestrom and Wingren, 2001). As it appears from the presented data that the frequencies of COMT-L allele and COMT-LL genotype vary drastically worldwide with regard to the ethnic groups.

In conclusion, the COMT-L allele and COMT-LL genotype are associated with a significantly increased risk for breast cancer in pre-menopausal Turkish women with sporadic breast cancer.

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References


