Abstract. Background: Breast cancer (BC), is more prevalent in subjects who have had prolonged exposure to heterocyclic amines, aromatic amines and high levels of oestradiol. Cytochrome P450 1B1 (CYP1B1) and N-acetyltransferase 2 (NAT2) have complementary role in metabolism of xenobiotics such as arylamines and heterocyclic amines. CYP1B1 also hyroxylates 17-β oestradiol. CYP1B1*3 polymorphism and seven missense and four silent polymorphisms of NAT2 were investigated. Patients and Methods: Sixty Turkish female BC patients and 103 healthy controls were phenotyped by polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP). Results and Conclusion: The distribution of NAT2 activity in the healthy control group was found to be correlated with that of healthy caucasians. Patients had slow acetylator phenotypes of NAT2, 1.8 times higher than controls but no statistical differences were found (p=0.07). In addition, the NAT2*5 allele was more statistically correlated with breast cancer patients rather than the controls (p=0.02). More, NAT2*5B was the most frequent haplotype of the NAT2*5 family (p=0.000). Breast cancer patients were detected to possess more CYP1B1*3 mutant alleles than the controls (p=0.043). The combined effect of CYP1B1*3 polymorphism and NAT2 slow acetylator genotype contributed to an increased risk for breast cancer in patients in this study (p=0.004).

Differences in the distribution of gene polymorphisms in key discriminant genes involved in oestrogen and xenobiotic metabolism is considered to contribute to variations in breast cancer susceptibility among different racial/ethnic populations (1). Biotransformation of xenobiotics in the body includes activating hydroxylation reactions by Cytochrome P450 enzymes and detoxification by conjugating enzymes such as catechol-O-methyltransferase (COMT), glutathione-S-transferases (GSTs) and N-acetyltransferases (NATs) (1). Polymorphisms in these enzymes may cause modifications in catalysing activity and stabilisation of the enzymes, thus increasing susceptibility to cancer due to prolonged exposure to oestradiol and xenobiotics (1). In this study, genetic polymorphisms of Cytochrome P450 1B1 (CYP1B1*3) and N-Acetyltransferase 2 (NAT2), and their contribution to breast cancer susceptibility was investigated among Turkish Women. Different populations have different polymorphism patterns, and the Turkish population is expected to display the genetic traits of the Caucasian population (1).

CYP1B1 is a haeme-thiolate monooxygenase that is involved in the NADPH-dependent monooxygenation of a considerable variety of carcinogens such as polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines (HAA), arylamines and nitrosamines formed in cooked food, tobacco products and found in industrial chemicals. CYP1B1 is located on chromosome 2p21 with three exons and two introns, encoding a 543 aminoacid length enzyme. CYP1B1 is mainly expressed in most cell types and secretory cells of breast tissue(2). CYP1B1 also mediates hydroxylation of 17-β oestradiol mainly to 4-hydroxyestradiol, the excess of which reacts further with DNA to form carcinogenic DNA adducts (3). Nearly, 80% of oestradiol is converted to 2-hydroxyestradiol (2-OHE2) by CYP1A1 in the liver and 20% of it to 4-hydroxyestradiol by CYP1B1 in breast tissue and uterus (3, 4). Various studies have established that increased blood oestrogen levels have the potential to induce breast and endometrial cancer, either by binding of oestrogen to its receptors, resulting in cell proliferation or, by conversion of cathechol form of oestrogens to reactive oxygen species.
(ROS) and related quinone and semiquinone products via redox cycling (5).

N-acetyltransferase-accompanied detoxification is a basic biotransformation pathway for the metabolism of drugs and environmental xenobiotics, specifically hydrazine drugs, homo- and hetero-cyclic amines and arylamines (6). The NAT2 detoxifying enzyme represents the N- and O-acetylation process in phase 2 metabolisation of biomolecules forming unstable N-acetylated compounds (7-8). If not sufficiently excreted from the organism, these N-acetoxy metabolites may be converted to aryl nitrenium ions that have the potential to react with DNA (6-9).

NAT2 is located at chromosome 8p21.3-23.1 and polymorphisms in this region cause individual and interethnic variation, thus affecting the acetylation capacity of the individual. Human populations can be divided into subgroups according to their acetylation capacities. To date 25 polymorphisms have been identified, of which 7 single nucleotide polymorphisms (SNPs) are the most abundant in various human populations, and are all found within the 870 bp coding region of the NAT2. The wild type allele is denoted as NAT2*4. NAT2*12 and NAT2*13 represent other fast phenotypes. NAT2*5, NAT2*6 and NAT2*7 haplotypes are documented as the slowest acetylating phenotypes (7).

Individuals possessing both slow haplotypes are classified as slow acetylators. In this regard, individuals possessing one fast and one slow allele are classified as intermediate acetylators (6-8).

This population based case-control study conducted among Turkish women, investigates the genetic frequencies of CYP1B1*3 and NAT2, and their possible individual or cooperative involvement in susceptibility to breast cancer.

**Patients and Methods**

**Patient selection.** A total of 163 unrelated individuals were included in this study: 60 female breast cancer patients, (median age: 53.7±11.31, range 31-72 years) and 103 healthy controls, (median age: 41.87±14.33, range 22-86 years). Surgical samples from the 60 breast cancer patients were obtained by the Department of Pathology, Istanbul University Cerrahpasa Medicine School, Istanbul. The healthy control group consisted of female healthy blood donors with no prior cancer history or any other vital disease history. Both postmenopausal and premenopausal women were included in the study. Twenty six (43.3%) patients and 70 (68.0%) controls were premenopausal and 34 (56.7%) patients and 33 (32.2%) controls were postmenopausal.

**DNA extraction.** Patient DNA was extracted from paraffin-embedded tissue using the method of Wright and Manos (10). Blood samples from the control group were collected in tubes containing EDTA, and DNA was prepared from leukocyte pellets by SDS lysis, ammonium acetate extraction and ethanol precipitation (11).

**Genotyping method for CYP1B1*3 and NAT2.** For genotyping, DNA was extracted from the blood of the controls and samples embedded in paraffin from non-tumoural neighbouring breast tissue. The CYP1B1*3 genotype was determined following Polymerase Chain Reaction (PCR) according to the method of Zheng et al. (12). Genotyping for NAT2 polymorphism, including 11 SNPs and 7 missense mutations, was conducted according to the method of Deitz et al. (13). The NAT2 haplotype nomenclature used in this study was based on the study of Sabbagh et al. (7, 8).

**Statistical analysis.** Statistical analyses were performed using SPSS version 7.5 (SPSS Inc. Chicago, USA) including the Chi-square (χ²) test, Fisher’s exact test and the Pearson correlation test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated.

**Results**

The allelic variant frequencies of CYP1B1*3 polymorphism are shown on Table I. 20.3% of the patients were homozygous for mutant allele (Val allele), while 40.7% of the patients were heterozygous mutant and 39% were homozygous for wild allele (Leu) (p=0.043, χ²: 3.911, OR: 2.58, 95% CI: 0.99-6.75).
The NAT2 gene was analysed for 11 polymorphisms: T111C, G191A, C282T, T341C, A434C, C481T, G590A, C759T, A803G, A845C, and G857A. None of the polymorphisms at nucleotides 111, 191, 434, 759 and 845 were observed in either control or patient samples. NAT2 allelic frequencies using the nomenclature of Sabbagh et al. (7, 8) are shown on Table II.

N-Acetyltransferase 2 genotyping in patients and controls was identified through the combinations of all polymorphisms. When these combined genotypes were evaluated with their phenotypic activities, fast acetylators and intermediate acetylators were found to be less frequent in the patient group (Figure 1). Slow acetylator phenotype in the patient group was observed to be 1.8 times higher than in the control group. Nevertheless, the results did not reach statistical significance ($p=0.07$, $\chi^2$: 3.24, OR: 1.82, 95% CI: 0.94-3.50).

NAT2*4/4 ‘Fast Acetylator’ phenotype, as the wild type, was observed in 3% of the controls, while the haplotype was not present in any of the patients. Total NAT2*5 haplotype frequency was statistically more abundant in patients than in controls. 56% of controls and 75% of patients carried NAT2*5 ($p=0.02$, $\chi^2$: 5.04, OR: 2.22, 95% CI: 1.10-4.50). Within these NAT2*5 haplotypes, NAT2*5B was the most frequent ($p=0.000$, $\chi^2$: 14.75, OR: 4.06, 95% CI: 1.94-8.51).

In a combined analysis of CYP1B1*3 and NAT2 phenotypes, the frequency of individuals carrying both the CYP1B1 Val/Val and NAT2 slow haplotypes was 6.2 times higher in the patient group than in the control group ($p=0.004$, $\chi^2$: 8.82, OR: 6.70, 95% CI: 1.63-23.55).

**Discussion**

This study investigated the risk of breast cancer in association with metabolic transformations of chemical carcinogens and analysed genetic modifications in order to determine risk assessment. Cytochrome P450 1B1 activation of chemicals and N-Acetyltransferase 2 detoxifying mechanisms may indicate either an increased or decreased cancer risk (12, 14).

N-Acetyltransferase 2 is one of the mostly differentiated gene family whose pharmacokinetic characteristics have already been studied (14, 15). Individual genetic variations lead to differences in NAT2 activation and susceptibility to diseases (14, 15). Smoking, consuming well-cooked meat and exposure to industrial chemicals are all risk inducing factors. The NAT2*5 family, including the T341C polymorphism, has been determined to be the most dominant subtype that causes declination in N-, O-acetylation functions. NAT2*14 including G191A, NAT2*6 including G590A and NAT2*7 including G857A are also the other slow acetylator subtypes in the order of increasing activity (7,8). Moreover, it has been demonstrated that T341C, A434C and G590A polymorphisms reduce protein expression and G191A, A845C and G857A cause decreases in protein stabilisation (7, 8). These studies show that a slow acetylator phenotype is not homogenous but is composed of phenotypes related on the heritage of certain SNPs and alleles.

The association between NAT2 acetylation and breast cancer has been evaluated by a number of studies. Among them are studies that obtained positive correlations while there has been research with no consistent associations (15). Alberg et al. established fast acetylation as a risk factor in women in all menopausal status (16). In contrast, Sillanpaa et al. and Huang et al. stated that slow acetylation increases cancer risk and the risk reaches the highest levels in postmenopausal women (17, 18).

In the present study, NAT2*4/4 homozygous wild type variant was present in 3% of the controls. NAT2*4 allele frequency was 18% in controls and 10% in patients.
Combinations of fast variants with other variant types were 8.9% of controls and 8.3% of patients. In conclusion, the frequency of fast variants in Turkish population seems to be in accordance with that of the Caucasian population and to be smaller than in Asian populations according to Sabbagh et al. (8).

Phenotypic schematisation of patients and controls confers 1.8 times elevated but not significant risks of breast cancer for slow acetylators. Statistically, NAT2*5 allele is harboured mostly by patients (p=0.02). Meanwhile, NAT2*5B allele is determined to be the most abundant phenotype among the NAT2 family with a frequency of 43% in patients and 15.8% in controls. In support of this observation, this risk associated phenotype (NAT2*5B) is also the most common phenotype among Turkish population according to Sabbagh et al. (8).

Studies combining acetylator phenotype and smoking status in breast cancer does not provide consistent associations. Huang et al., Hunter et al. and Millikan et al. have shown association with different acetylator haplotypes (17, 19, 20). Turkish women over fifty years old have low smoking rates and smoking rates of participants in our study were found to be quite low, only 9 of the patients and 23 of the cases were smoking 3.66±10.66 and 3.91±9.11 packs/year respectively. Therefore the smoking status was not included in the statistical analysis.

CYP1B1*3 induces risk with respect to activation of xenobiotics and oestrogen catabolism. Expression of CYP1B1 takes place mainly in the liver and is also detected in normal tissues (21). However, CYP1B1 expression level, determined in tumor tissues in immunohistochemical studies and enzyme activity experiments, reaches a negligible level in pretumoral progression and neoplastic tissues (21). Cancer risk due to conversion of 4-OH oestradiol to quinone and semiquinone metabolites and formation of DNA adducts has been investigated by Cavaliere et al. (22). Hanna et al. examined functional differences in oestrogen hydroxylating activities and concluded that CYP1B1*3 had a nearly 4-fold 4-hydroxylase function (23). CYP1B1*3 polymorphism localised in the haeme-binding region of the enzyme, leads to changes in functional activities with polymorphic modifications. As a consequence, excessive functioning of the enzyme may increase oestrogen catabolism and enzyme activity experiments, reaches a negligible level in pretumoral progression and neoplastic tissues (21). Cancer risk due to conversion of 4-OH oestradiol to quinone and semiquinone metabolites and formation of DNA adducts has been investigated by Cavaliere et al. (22). Hanna et al. examined functional differences in oestrogen hydroxylating activities and concluded that CYP1B1*3 had a nearly 4-fold 4-hydroxylase function (23). CYP1B1*3 polymorphism localised in the haeme-binding region of the enzyme, leads to changes in functional activities with polymorphic modifications. As a consequence, excessive functioning of the enzyme may increase oestrogen catabolism and enzyme activity experiments, reaches a negligible level in pretumoral progression and neoplastic tissues (21). Cancer risk due to conversion of 4-OH oestradiol to quinone and semiquinone metabolites and formation of DNA adducts has been investigated by Cavaliere et al. (22). Hanna et al. examined functional differences in oestrogen hydroxylating activities and concluded that CYP1B1*3 had a nearly 4-fold 4-hydroxylase function (23).

In line with previous studies, risk association seems more intense from the side of xenobiotic/oestrogen activation and acetylation mechanisms in the present study. To the authors' knowledge, only one previous study evaluated CYP1B1*3 and NAT2 slow acetylator phenotypes with significant results, however, only in light smokers (26). With respect to the present results, the coexistence of CYP1B1*3 and slow acetylator phenotype both demonstrate a positive relationship with breast cancer in patients and pose a statistically significant risk of breast cancer compared to women carrying the other variations (p=0.004, χ²: 8.82, OR: 6.70, 95% CI: 1.63-23.55). Due to the relatively small number of individuals included in this study, statistically significant findings were limited. It is therefore necessary to perform this study with larger groups and various populations. Furthermore both individual and cooperative effects of CYP1B1*3 and NAT2 polymorphisms should be investigated.

References


