Abstract. Background and Objectives. Previous studies have shown alterations in the cell cycle regulatory proteins in breast carcinomas. However, the results of these studies remain controversial. Cyclin D1 (CCND1) and p27KIP1 (CDKN1B) are two essential regulators of cell cycle progression. This study aimed to investigate the associations of CCND1 A870G and CDKN1B C79T polymorphisms with breast cancer risk.

Patients and Methods. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine the genotype and allelic frequencies of polymorphisms. Seventy-eight breast cancer patients and 84 age-matched healthy controls were included in the study.

Results. Frequencies of CT genotype and T allele of CDKN1B were found to be higher in breast cancer patients than in controls (p=0.013, OR: 1.514 95% CI: 1.086-2.114; p=0.007, OR=1.496; 95% CI: 1.111-2.014, respectively). The frequency of AA genotype of CCND1 was decreased in hormone receptor- (estrogen and progesterone receptors) negative patients with breast cancer (p<0.049, OR=0.286; 95% CI: 0.071-1.142).

Conclusions. Even though CDKN1B polymorphism appears to be an important predictive factor for breast cancer risk and CCND1 polymorphism may be a prognostic biomarker for breast cancer, further investigations with larger study groups are needed to fully elucidate the role of CCND1 and CDKN1B polymorphisms in the development and prognosis of breast cancer.
degradation, and which is hypothesized to be more stable compared to the product of the 870G allele. It has been shown that transcript-b leads to a longer half-life of CCND1, which may bypass the G1/S-checkpoint (7).

Several molecular epidemiological studies have been conducted to examine the association between CCND1 G870A polymorphism and breast cancer risk (11-17), but the results remain inconsistent.

Progression through the cell cycle is governed by the activation of cyclin-dependent kinases (CDKs), and sequential activation of CDK–cyclin complexes leads to the phosphorylation and inactivation of RB, allowing transcription of cell cycle genes by the E2F family of transcription factors (18). This process is kept in check by inhibitors (CKIs). These are referred to by various names, but are generally classified into two groups: inhibitors of kinase 4 (INK4) and CDK inhibitory protein/kinase inhibitor protein (CIP/KIP). The INK4 groups includes CDKN2A (INK4A/p16 and ARF/p14), CDKN2B (INK4B/p15), CDKN2C (INK4C/p18) and CDKN2D (INK4D/p19). These bind to both CDK4 and CDK6 to prevent their association with cyclin D. The CIP/KIP group comprises CDKN1A (WAF1/p21/CIP1) and CDKN1B (KIP1/p27), which form heterotrimeric complexes with the G1 to S transition CDKs. The CIP/KIP proteins do not affect cyclin binding. At low concentrations, they have been shown to improve complexing between CDK4/6 and cyclin D, but still inhibit CDK2–Cyclin-E (19). Loss of CDKN1B expression is also a common event in breast cancer, and has been strongly associated with high tumour grade and poor prognosis (20). The V109G polymorphism of the p27 gene, CDKN1B, examined by polymerase chain reaction analysis of tumour specimens, was associated with shortened disease-free survival in a subset of patients with infiltrating metastasis-free breast cancer (19). However, association of the CDKN1B C79T polymorphism and breast cancer risk has been analysed previously only in two studies (21, 22).

The aim of this study was to investigate the possible correlation between the polymorphisms of CCND1 G870A and CDKN1B C79T and development of breast cancer.

### Patients and Methods

#### Study population

The patient group consisted of 78 consecutive breast cancer patients with median age 52.5 (range 30-79) years, who were admitted to Istanbul University Cerrahpasa Medical Faculty, Department of General Surgery, Breast Services. The control group consisted of age- and sex-matched healthy subjects. The control subjects were randomly selected among volunteer blood donors. Subjects with a personal or family history of any cancer and chronic diseases such as cardiovascular or cerebrovascular disease,
diabetes mellitus, hypertension, or renal disease were excluded from the study. Control subjects were not taking any regular medication at time of the study. Eighty-four healthy women, presenting a median age 46 (range 30-81) years, were used as a control group. Informed consent was obtained from all participants. Breast cancer patients had previously undergone appropriate surgery. Questionnaires, medical records, and pathological reports were used to confirm the diagnosis and cancer status. This study protocol was approved by the Local Ethical Committee at Istanbul University Cerrahpasa Medical School.

Polymorphism Analysis. This study was carried out in the Department of Molecular Medicine, Istanbul University Experimental Medicine and Research Institute. Genomic DNA was extracted from peripheral whole blood containing EDTA according to the salting-out technique (23). Genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), the procedures of which are given in Table I (22, 23). The appropriate primers were used to amplify the corresponding gene of the subjects by PCR. The reaction products were digested by using the appropriate enzyme at 37°C. The digested products were analysed on 3% agarose gel stained with ethidium bromide and examined under transillumination. Each gel was read by two observers unaware of the subject’s status. If there was any conflict, samples were repeated. The expected results after restriction for each gene are also given in Table I.

Statistical analysis. Statistical analyses were conducted using the SPSS version 13 software package (SPSS Inc, Chicago, IL, USA). Distributions of genotypes and haplotypes were compared using the chi-square test. The distribution of genotypes in all groups was tested for deviation from Hardy-Weinberg equilibrium (HWE) using the goodness-of-fit test. Data are expressed as mean±standard deviation (SD). Univariate analysis was performed to compare the distribution of age and gender and the frequencies of alleles and genotypes. A multivariate logistic regression model was performed to investigate possible independent effects of the cell cycle genes genotypes to the patient’s characteristics and clinicopathological parameters after adjustment for age. P-values less than 0.05 were considered statistically significant.

Results

Characteristics of patients and primary tumours. Patient and control characteristics are given Table II. Sixty-four patients (82%) were diagnosed with stage I (n=33) or II (n=31) disease, whereas 14 patients (18%) were diagnosed with stage III (n=8) or IV (n=6) disease. In the pathological assessment of the tumours, the majority of the tumours (70%) were poorly (50%) or intermediate (20%) differentiated invasive ductal carcinomas. Three patients (4%) with stage II/III disease received preoperative chemotherapy, while 64 patients (82%) received adjuvant chemotherapy following surgery, and five patients (6%) with stage I estrogen receptor (ER)-positive disease received only adjuvant hormonal therapy. Six patients with stage IV disease (8%) received chemotherapy after the biopsies of the lungs or the bone metastases.

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<table>
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Association between the CCND1 and CDKN1B genes and patients’ characteristics. Table III summarizes the distributions of CCND1 and CDKN1B genotypes and alleles in patients with breast cancer and controls.

When the study groups were analysed for CCND1 A870G polymorphism, it was found that the genotype distributions and allelic frequencies were in agreement with HWE in controls (p=0.650) but not in patients (p=0.043). For CDKN1B, genotype distributions and allelic frequencies deviated from the HWE in patients (p=0.019), but not in the controls (p=0.646).

In the present study, frequencies of CT genotype and T allele of CDKN1B genetic variants were higher in the patients with breast cancer compared with the control group (57.7% vs. 38.1%, 35.3% vs. 23.8%; p=0.013, OR: 1.514; 95% CI 1.086-2.111; p=0.007, OR: 1.496; 95% CI: 1.111-2.014, respectively, Table III).

There was no significant difference in the distribution of the CCND1 A870G genotypes (p=0.05). The frequencies of the AA, AG and GG genotypes of CCND1 were 26.9%, 60.3%, and 12.8% in breast cancer patients and 22.6%, 52.4%, and 25% in healthy controls, respectively.)
In the subgroup analysis, the frequency of the AA genotype of \textit{CCND1} was lower in hormone receptor-negative patients with breast cancer compared with those whose tumours expressed hormone receptors ($p=0.041$, OR=$0.286$, 95% CI: 0.071-1.142).

Regarding the G870A polymorphism of \textit{CCND1} and C79T polymorphism of \textit{CDKN1B}, the frequencies of genotypes and alleles were not found to be associated with other clinicopathological parameters such as tumour size, grade and nodal involvement ($p>0.05$).

**Discussion**

Cyclin D1 and CDKIs are important cell cycle regulators. \textit{CCND1} is one of the most commonly overexpressed oncogenes in breast cancer, being overexpressed in 45-50% of primary ductal carcinomas (5). Several studies have investigated the potential link between \textit{CCND1} and \textit{CDKN1B} gene polymorphisms and breast cancer. This study is the first to examine the relationship between \textit{CCND1} and \textit{CDKN1B} variants of cell cycle genes and breast cancer in Turkish women.

In the present study, it was found that \textit{CCND1} G870A polymorphism was not significantly associated with the risk of breast cancer. The risk of breast cancer was increased in the presence of the A allele of \textit{CCND1} in patients with breast cancer (OR: 1.162, 95% CI: 1.001-1.350). It was also found that the frequency of AA genotype of \textit{CCND1} was decreased in hormone receptor negative breast cancer patients. In a previous report, the \textit{CCND1} AA genotype was found to be associated with increased breast cancer risk in both the Ontario (OR:1.3) and the Finland samples (OR:1.4), 95% CI (1.01-1.84) (17). The \textit{CCND1} AA genotype and A allele were also reported to be associated with increased breast cancer risk in Chinese samples (OR: 1.34 and 1.23 respectively) (14). In further support of the current study, recent large-scale case-control and case-cohort studies of breast cancer have reported that there was no association between \textit{CCND1} G870A polymorphism and breast cancer risk in other populations including Australian (11), Singapore Chinese (13), Finnish (15), Austrian (16), Malaysian (25) and German (26) populations. On the other hand, the GA genotype of \textit{CCND1} was found to be weakly associated with breast cancer risk (OR: 1.2), whereas the A allele and AA genotype had a similar distribution between the breast cancer cases and controls (OR: 1.1 and 1.2 respectively) among the Shanghai-Chinese population (12). Yu et al. (14) indicated that \textit{CCND1} G870A polymorphism was associated with breast cancer in young women in China. They have reported that the AG and AA genotypes were associated with increased risk of breast cancer. Recently, Lu et al. (10) conducted a meta-analysis on the association between G870A polymorphism and the risk of breast cancer and showed that there was an increased risk of breast cancer for carriers of variant 870A allele in Caucasians (OR: 1.14) but not in an Asian population (OR:1.1), whereas in another meta-analysis, A allele and AG genotype of \textit{CCND1} was found to be associated with breast cancer risk (OR:1.12) (27). Betticher et al. (7) reported that individuals with the AA genotype produce altered transcript (the transcript-b) that may have longer half-life. Therefore, cells with damaged DNA carrying allele A may bypass the G1/S phase checkpoint easily compared with those not carrying the polymorphism. Sawa et al. (8) demonstrated that high levels of normal transcript (transcript-a) inhibit entry into and completion of the S phase. These observations suggest that the genotyping difference of \textit{CCND1} polymorphism may influence the biological behavior of cells, thus altering the risk of developing of breast cancer by producing different transcripts of \textit{CCND1}.

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Regarding the G870A polymorphism of \textit{CCND1}, the frequency of AA genotype was significantly lower in breast cancer patients with ER and progesterone receptor (PR)
negative-compared with those that were positive. In support of the current study, it has been reported that single nucleotide polymorphism rs3212879 of CCND1 exhibited a similar distribution among ER-negative breast cancer patients even though genome-wide significance levels were not reached (28). Results from the current study suggest that associations of CCND1 genetic variants and breast cancer may be exerted in specific subtypes of breast cancer.

Although there is also great interest in understanding the role of CDKN1B amplification/expression in breast cancer risk and prognosis, surprisingly little on the role of polymorphisms is known. In this study, the frequencies of CT heterozygote genotypes and T allele of CDKN1B gene were significantly increased in breast cancer patients as compared with controls. CDKN1B belongs to the CIP/KIP family inhibitors. The main inhibitory effect of CDKN1B is the prevention of RB phosphorylation by CDK2-cyclin E (18). In concordance with the current findings, two studies among the Chinese population and the population of the SEARCH (breast) study group (22, 29) found an association of C79T polymorphism of CDKN1B gene with breast cancer risk. Biologically, it is possible that the CDKN1B C-79T polymorphism may be involved in the aetiology of breast cancer. Because p27KIP1 protein levels can be affected by the rates of gene transcription (30), P27KIP1 protein levels may be affected by sequence variants in the 5'-untranslated region of the CDKN1B transcript (31, 32).

In this study, in the single locus analyses, it was found that the CDKN1B C-79T heterozygote, but not the homozygote, had a significantly increased risk of breast cancer. Although the reason for a higher risk associated with the C-79T variant heterozygote remains unknown, it is possible that this heterozygote may result in impaired protein function leading to the potential imbalance of the protein structure. Another possible explanation is that these genes may be in linkage disequilibrium with another susceptibility locus.

In conclusion, CT genotype and T allele of CDKN1B appear to be associated with breast cancer risk in Turkish population. On the other hand, the CCND1 AA genotype is associated with hormone receptor-negative breast cancer, suggesting that subgroup analysis would elucidate the role of cell cycle gene polymorphisms in the pathogenesis and treatment of breast cancer.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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References


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