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The Influence of *HER2* Genotypes as Molecular Markers on Breast Cancer Outcome

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Alterations of the human epidermal growth factor receptor 2 (*HER2*) protooncogene have been implicated in the carcinogenesis and prognosis of breast cancer. A polymorphism has been identified at codon 655 (ATC/isoleucine to GTC/valine [I655V]) in the transmembrane domain-coding region of this gene, which may be associated with the risk of breast cancer. In this study we aimed to determine whether the risk of breast cancer is associated with the I655V polymorphism of *HER2* transmembrane domain-coding region at codon 655. The genomic DNA from breast cancer patients and control subjects underwent analysis by the polymerase chain reaction–fragment length polymorphism. We observed no overall association between *HER2* genotype and breast cancer ($p = 0.53$). However, an elevated positive association was observed for Ile/Val+Val/Val versus Ile/Ile genotypes in women >age 60 years ($p = 0.02$). Further, other risk factors—namely, the body mass index and family history—were found to be risk factors for developing breast cancer ($p = 0.006$ and $p = 0.00$, respectively). In conclusion, results of this study suggest that polymorphisms of the *HER2* gene may be important susceptibility biomarkers for breast cancer risk, particularly among older women.

Introduction

Breast cancer is the most common invasive malignancy affecting women worldwide. The incidence and the mortality rates vary between different ethnically and geographically distinct populations (Kalemi *et al.*, 2005). Along with environmental factors, genetic factors have an important place in the etiology of breast cancer. Also, advanced age, age at menarche, age at menopause, nutritional habits, body mass index (BMI), and family history of breast cancer are among the other factors that are thought to play a role in breast cancer (Boring *et al.*, 1994). Breast cancer is associated with different types of somatic genetic alterations such as mutations in oncogenes and tumor suppressor genes (BRCA1 and BRCA2) (Kalemi *et al.*, 2005). Therefore, other genes are likely to modify the risk of breast cancer (Rebbeck, 1999).

One of the most important genes studied is the human epidermal growth factor receptor 2 (*HER2*), which has been classified as a potential molecular prognostic indicator (Kim *et al.*, 1998; Rèvillion *et al.*, 1998; Gullick, 2001; Olayioye, 2001; Rogers *et al.*, 2002). *HER2*, which is known as c-erbB-2 or HER-2/neu, is a member of the tyrosine kinase erb-B receptor family, which has four members, from *HER1* to *HER4* (Pinkas-Kramarski *et al.*, 1997). The members of this family possess intrinsic tyrosine kinase activity (Hynes, 2000; Stern, 2000), which allows them to play an important role in signal transduction pathways regulating many cellular functions, such as cell differentiation and proliferation (Goel *et al.*, 2002). *HER2* is the second member of this family. The *HER2* protooncogene (neu/ERBB2) is located at chromosome 17 (Coussens *et al.*, 1985) and codifies a 185 kDa transmembrane glycoprotein (Akiyama *et al.*, 1986; Tomassi *et al.*, 2004) involved in the regulation of cell growth, differentiation, and survival (Yarden, 2001). Alterations in this gene, such as gene amplification (Tsuda *et al.*, 2001) and point mutations (Bargmann *et al.*, 1986), are capable of activating the receptor, resulting in enhanced activation of intracellular signaling pathways such as the MAPK and PI3K/AKT, which can lead to uncontrolled cell proliferation (Yarden and Sliwkowski, 2001). It has been observed that there are overexpression *HER2* receptors in 20–30% of breast cancer cases (Cooke *et al.*, 2001). In the transmembrane coding part of human *HER2*, an Ile-to-Val single-nucleotide polymorphism was found at codon 655, resulting in the substitution of isoleucine (Ile:ATC) with valine (Val:GTC) (Papewalis *et al.*, 1991). But it is not known how this variant contributes to the risk of developing sporadic breast cancer (Puputti *et al.*, 2006).

Controversial studies have been published regarding the role of this polymorphism in the development of breast

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cancer, in different populations (Ameyaw *et al.*, 2002; Xie *et al.*, 2000; Baxter and Campbell, 2001; Keshava *et al.*, 2001; Wang-Gohrke and Claude, 2001; Pinto *et al.*, 2004, An *et al.*, 2005).

Within this scope, the primary aim of the current study was to determine whether there were associations between *HER2* I655V polymorphism and risk of breast cancer for the Turkish population in the Mersin sample. The secondary aim of the study was to evaluate the potential interaction between genotype and other risk factors for breast cancer, the age at menarche, age at menopause, smoking, BMI, and family history of breast cancer.

Materials and Methods

Study population

The control group comprised 208 individuals, and the mean age was 49.01 ± 9.26. The control group was randomly selected from healthy women at ages similar to case group.

The case group consisted of 166 women with breast cancer with a mean age of 51.97 ± 11.48. The case group comprised women who had been diagnosed with breast cancer at the Mersin University Medical Faculty Oncology Clinic during the years 2004–2006. When blood samples were obtained from the individuals and information and permissions were received, questionnaire was applied to all individuals. The following information was obtained from this questionnaire: age, height, weight, cigarette smoking status, age at menarche, age at menopause, and family history of breast cancer. Menopausal status assessed as follows: premenopause, occurrence of regular menstrual period within the previous 3 months; postmenopause, amenorrhea for at least 12 months or a bilateral oophorectomy performed ≥6 months previously. The patients were informed that the DNA samples acquired from the obtained samples and the information in the registration form would be used in a scientific study, and then their consents were obtained. More than 95% of the samples were successfully genotyped on the first attempt, and samples failing genotyping were not used in further analyses. A total of 166 cases and 208 controls were successfully genotyped and were included in the analyses.

Genotyping

Blood samples were collected, and DNA was extracted using the standard (phenol-chloroform) methods. Polymorphisms were analyzed using polymerase chain reaction–restriction fragment length polymorphism (PCR and RFLP). For the *HER2* I655V polymorphism, genomic DNA was amplified in a 25 μL reaction mixture containing 0.5 ng/μL DNA, 0.6 nM of the primers (*HER2*-F: 5′-AGA GAG CCA GCC CTC TGA CGT CCA T-3′; *HER2*-R: 5′-TCC GTT TCC TGC AGC AGT CTC CGC A-3′), 0.2 mM of each of dNTP, 1× PCR buffer, 1.75 mM $MgCl_2$, and 1 U Taq polymerase. The samples were denatured for 5 min at 94°C. This was followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 56°C, and extension for 1 min at 72°C, with a final extension for 7 min at 72°C (Akısık and Dalay, 2004). Amplicons (148 bp) were digested with 4 U *Bsm*AI (MBI Fermentas, Vilnius, Lithuania) by overnight incubation at 37°C. Restriction fragments were separated on 3% agarose gels. The gel was stained with ethidium bromide (10 mg/μL) and evaluated using a gel documentation system (Vilber Lourmat, Marne La Vallée, France). Digestion of each PCR product with *Bsm*AI gave 116- and 32-bp fragments for the Val allele and a single 148 bp fragment for the Ile allele.

Statistical analysis

To assess whether the *HER2* genotypes were in Hardy–Weinberg equilibrium and to determine *p*-values for differences in genotype frequencies between cases and controls, χ^2 tests were used. Unconditional logistic regression was performed to calculate the odds ratios (ORs), and 95% confidence intervals (CIs) to assess the risk of breast cancer. In addition to the corresponding factors, analyses were per-

Table 1. Characteristics of Study Subjects

Variable	*Cases*	*Controls*	p-*value*
Subjects (*n*)	166	208	
First-degree family history of breast cancer, *n* (%)			
Yes	36 (21.7)	16 (7.7)	$\chi^2 = 15.10$; OR, 3.3; 95% CI, 1.771–6.237; $p = 0.00$
No	130 (78.3)	192 (92.3)	
Smoking status at diagnosis, *n* (%)			
Never	124 (74.7)	150 (72.1)	$\chi^2 = 0.31$; OR, 0.87; 95% CI, 0.55–1.39; $p = 0.57$
Current	42 (25.3)	58 (27.9)	
Menopausal status at diagnosis, *n* (%)			
Premenopausal	68 (41)	106 (51)	$\chi^2 = 3.70$; OR, 1.49; 95% CI, 0.99–2.26; $p = 0.054$
Postmenopausal	98 (59)	102 (49)	
Age at menarche, mean (SD)			
<13 age	84 (50.6)	126 (60.6)	$\chi^2 = 3.73$; OR, 1.50; 95% CI, 0.99–2.26; $p = 0.053$
≥13 age	82 (49.4)	82 (39.4)	
BMI, mean (SD)			
<25	36 (21.7)	72 (34.6)	$\chi^2 = 7.51$; OR, 1.91; 95% CI, 1.19–3.04; $p = 0.006$
≥25	130 (78.3)	136 (65.4)	

Table 2. Distribution of *HER*2 Alleles and Genotype Frequencies among Breast Cancer Patients and the Control Group

	Cases, n *(%)*	*Controls*, n *(%)*	*OR (95% CI)*	p-*value*
HER2 genotype				
*HER2 Bsm*AI				
Ile/Ile	128 (77.1)	166 (79.8)	1.0[a] ref	
Ile/Val	34 (20.5)	40 (19.2)	1.10 (0.66–1.83)	0.70
Val/Val	4 (2.4)	2 (1)	2.59 (0.46–14.68)	0.27
Ile/Val+Val/Val	38 (47.5)	42 (52.5)	1.17 (0.71–1.92)	0.52
HER2 alleles				
Ile allele frequency	290 (87.3)	372 (89.4)	1.0[a] ref	
Val allele frequency	42 (12.7)	44 (10.6)	0.8 (0.43–1.54)	0.53

[a]Reference.
n, number of alleles and genotypes; OR, odds ratio; CI, confidence interval.

formed for breast cancer risk factors: BMI ($<25\,kg/m^2$ and $\geq 25\,kg/m^2$); age at menarche (<13 and ≥13 years); premenopausal and postmenopausal (menopausal status); and first-degree family history of breast cancer (yes/no). All *p*-values were two sided. We used the SPSS software, v11.5 (Statistical Package for the Social Science release 11.5.1, EAD TOOLS; EAD Technologies, Chicago, IL).

Results

In total, 166 patients with breast cancer and 208 healthy controls were included in this study. The distributions of major risk factors for breast cancer are shown in Table 1. BMI and family history were different between cases and controls ($\chi^2 = 7.51$; OR, 1.91; 95% CI, 1.19–3.04; $p = 0.006$; and $\chi^2 = 15.10$; OR, 3.32; 95% CI, 1.77–6.23; $p = 0.00$, respectively). However, no significant differences were found between cases and controls for cigarette smoking, age at menopause, and age at menarche (Table 1).

Table 2 depicts the genotype distribution and allele frequencies for *HER2* in breast cancer patients and the control group. Using the χ^2 test, the distribution of the *HER2* I655V genotypes in all patients with breast cancer and healthy controls was consistent with Hardy–Weinberg equilibrium ($p = 0.64$ and $p = 0.97$, respectively). In the examination of the numerical and percentage findings related to *HER2* I655V alleles, it was determined that the Ile allele frequency in the control group was 372 (89.4%) and in the case group was 290 (87.3%); the polymorphic Val allele frequency was 44 (10.6%) in the control group and 42 (12.7%) in the case group ($p = 0.53$). The frequencies of the AA, AG, and GG genotypes in the control and case groups were 79.8%, 19.2%, and 1%, and 77.1%, 20.5%, and 2.4%, respectively. In the statistical analysis related with the genotype ratios, patients with breast cancer showed no significant differences compared to controls ($p = 0.50$; data not shown). Besides, regarding the AG + GG genotype ratios having G polymorphic allele, there were no differences between breast cancer and control groups ($p = 0.52$) (Table 2).

In the evaluation of relationships between other potential risk factors for breast cancer and findings about the *HER2* I655V genotypes, it was determined that family history, age at menarche, and BMI had no effect on the risk for breast cancer carrying polymorphic G allele as homozygote or heterozygote (Table 3). Although no interactions between the *HER2* polymorphism and major risk factors were statistically significant, the inverse association with breast cancer risk was stronger in some subgroups. In particular, ORs were significantly

Table 3. Risk of Breast Cancer According to the *HER*2 I655V Polymorphism and Major Risk Factors

	Ile/Ile (controls/cases)	*Ile/Val+Val/Val (controls/cases)*	*OR (95% CI)*	p-*value*
Age				
<40	24/18	10/8	1.06 (0.35–3.24)	0.90
40–49	58/30	18/10	1.07 (0.44–2.61)	0.87
50–59	58/56	12/8	0.69 (0.26–1.81)	0.45
>60	26/24	2/10	7.5 (1.28–43.68)	0.02
Family history				
Yes	14/24	2/12	3.5 (0.68–17.96)	0.13
No	152/104	38/22	0.95 (0.54–1.65)	$p = 0.85$
Age at menarche				
<13 age	96/62	26/26	1.13 (0.60–2.14)	0.69
≥13 age	70/66	12/16	1.41 (0.62–3.21)	0.40
BMI (kg/m^2)				
<25	58/30	14/6	0.82 (0.28–2.37)	0.72
≥25	108/98	28/32	1.25 (0.70–2.24)	0.43

BMI = weight (in kilograms)/height (in meters).
OR, odds ratio; CI, confidence interval; BMI, body mass index.

increased for women at older ages than for women under the age of 40 (OR, 7.5; 95% CI, 1.28–43.68; $p = 0.02$) (Table 3).

Discussion

In this study, we analyzed the influence of *HER2* gene polymorphism on breast cancer in Turkish population. The current case–control study gives support to previous findings suggesting that the Val genotype for the *HER2* I655V polymorphism confers an increased risk for breast cancer, particularly in subgroups of women (Xie *et al.*, 2000; Millikan *et al.*, 2003; Montgomery *et al.*, 2003; Rutter *et al.*, 2003; Pinto *et al.*, 2004). However, previous studies on the association between breast cancer risk and *HER2* polymorphism have been highly inconsistent (Baxter and Campbell, 2001; Keshava *et al.*, 2001; Wang-Gohrke and Claude, 2001; Hishida *et al.*, 2002; Kalami-Sarvestani *et al.*, 2004; Benusiglio *et al.*, 2005).

In our study, women older than 60 have demonstrated that homozygosity or heterozygosity for the valine allele (Ile/Val and Val/Val) was associated with an increased risk of late onset breast cancer (OR, 7.5; 95% CI, 1.28–43.68; $p = 0.02$). Our findings are in agreement with previously published data (Hauptmann *et al.*, 2003; Pinto *et al.*, 2004). On the other hand, some previous studies found no significant association between the *HER2* polymorphism at position 655 and the risk of late onset breast cancer (Xie *et al.*, 2000; Millikan *et al.*, 2003; Montgomery *et al.*, 2003; Rutter *et al.*, 2003).

We found no statistically significant difference in the allelic and genotypic frequencies of *Bsm*AI polymorphism of the *HER2* gene between breast cancer patients and control groups. Our findings are in agreement with previously published data (Baxter and Campbell, 2001; Wang-Gohrke and Claude, 2001; Hishida *et al.*, 2002; Kalami-Sarvestani *et al.*, 2004; An *et al.*, 2005; Cox *et al.*, 2005; Nelson *et al.*, 2005). Further, we could not find evidence to support heterogeneity in the association between the *HER2* I655V polymorphism and breast cancer risk according to BMI, age at menarche, or family history.

There were no significant differences between breast cancer risk and other risk factors, including cigarette smoking, age at menopause, and age at menarche. But we determined that family history (OR, 3.3; 95% CI, 1.771–6.237; $p = 0.00$) and BMI (OR, 1.91; 95% CI, 1.19–3.04; $p = 0.006$) were significant risk factors for the development of breast cancer. There were differences approaching statistical significance in the percentage of women, menopausal state, and age of menarche between the cancer and controls groups. However, the statistical results (p value) on menopausal state and age of menarche show that these factors were not risks for breast cancer.

The often-conflicting findings of these reports might be caused by some confounding factors, such as ethnicity, selection of control group, case characterization, sample size, and gene–gene and gene–environment interactions.

In this study, which was conducted in a Mersin sample, the *HER2* I655V polymorphism allele and genotype frequencies for the Turkish population were determined, and this polymorphism was shown to be a risk factor for breast cancer, particularly among older women. In addition to the study of Akısık and Dalay in the Marmara region, our findings in Mersin, located in the Eastern Mediterranean region, have important contributions on whether there is a relationship in Turkish population between the *HER2* gene I655V polymorphism and breast cancer risk. The determination of the frequencies of this gene polymorphism for the Mersin sample will not only be a significant contribution for the Turkish population, but also provide important information for the determination of other illnesses related to this gene polymorphism in the future.

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