

ORIGINAL ARTICLE**Single Nucleotide Polymorphisms in the Hypoxia-inducible Factor-1 α (HIF-1 α) Gene in Human Sporadic Breast Cancer**Isil Apaydin,^a Ece Konac,^b H. Ilke Onen,^b Murat Akbaba,^c Ercument Tekin,^d and Abdullah Ekmekci^b^aDepartment of Pathology, Faculty of Medicine, ^bDepartment of Medical Biology and Genetics, ^dDepartment of General Surgery, Faculty of Medicine, Gazi University, Besevler, Ankara, Turkey^cPolyclinic of Second General Surgery, Ankara Oncology Education and Research Hospital, Demetevler, Ankara, Turkey

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Background. DNA sequence variations in hypoxia-inducible factor-1 α (HIF-1 α) gene, which have been demonstrated to be correlated with tumor angiogenesis, may yield changes both in the production outcomes and in the activities of the gene. In this study, we investigated the relationship between three single nucleotide polymorphisms (SNPs) [C1772T and G1790A in exon 12 and C111A in exon 2 of the HIF-1 α gene] in the HIF-1 α gene coding regions and development of sporadic breast cancer in the Turkish population. These three polymorphisms result in an amino acid change from proline 582 to serine, from alanine 588 to threonine and from serine 28 to tyrosine, respectively.

Methods. Genomic DNA was isolated from 102 sporadic breast cancer patients and 102 healthy female controls. All three HIF-1 α gene regions were amplified by PCR, and genotypes were determined by RFLP and DNA sequencing.

Results. There were no significant differences between patients and controls in terms of the distribution of C1772T and G1790A polymorphisms of HIF-1 gene ($p > 0.05$). As for HIF-1 α C111A polymorphism, we did not find CA and AA variants of the gene in either controls or patients. Multivariable logistic regression analysis was performed between CC and CT + TT genotypes of C1772T polymorphism. No significant differences were found between these two genotypes in terms of clinicopathological characteristics of the patients including age at enrollment, age at menarche and first delivery, number of full-term pregnancies, body mass index, use of oral contraceptives and postmenopausal hormones, family history of breast and ovarian cancers, menopausal status, histopathological features, oophorectomy, smoking habits, and alcohol consumption ($p > 0.05$).

Conclusions. Our results suggest that none of the polymorphisms studied in the HIF-1 α gene influence susceptibility to sporadic breast cancer. The present study is the first case-control study that investigates the association of HIF-1 α polymorphisms with sporadic breast cancer in the Turkish population. © 2008 IMSS. Published by Elsevier Inc.

Key Words: HIF-1 α , Polymorphism, Risk factors, Sporadic breast cancer, Turkish population.

Introduction

Breast cancer is the most prevalent cancer type in the world today due to its high incidence rate and relatively good prognosis (1). Although, there are several well-established

risk factors identified for breast cancer such as the inherited genetic predisposition, age of menarche and menopause, reproductive history, body mass index (BMI), prolonged use of oral contraceptives and hormone replacement therapy, and alcohol consumption, the etiology of breast cancer is still not fully understood (2,3).

Hypoxia-inducible factor-1 α (HIF-1 α) is a key regulator of cellular response to hypoxia and has been suggested to play an important role in tumor progression and metastasis

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through activation of >60 target genes that are particularly involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism and invasion (4–6). HIF-1 α protein levels and transcriptional activity are tightly regulated in response to oxygen levels (7). HIF-1 α is overexpressed in human cancers as a result of intratumoral hypoxia as well as genetic alterations (6). Experimental evidence has indicated an important role for HIF-dependent gene expression in tumor growth (4). Tumors derived from cells lacking HIF-1 α or HIF-1 β , a constitutively expressed subunit of HIF-1 gene, show significantly reduced vascularization and in most studies, reduced growth rates compared to parental cells (4,8). Indeed, a large number of clinicopathological studies confirm that HIF-1 α is overexpressed in the cytoplasm and nuclei of 40–80% of human carcinoma cases, including head-neck, colon, breast, stomach, pancreas, prostate, kidney, esophagus, endometrial, and non-small cell lung cancer (9–13). This makes HIF-1 α an attractive target for the development of anticancer agents (14).

Genetic polymorphisms are responsible for interindividual variation and diversity and have been considered as the main genetic elements involved in the development of common and complex diseases (15). Single nucleotide polymorphisms (SNPs) are commonly one of the genetic alterations. Yamada et al. (16) examined all regions of the HIF-1 α and found a total of 35 SNPs in the gene. Polymorphisms of the gene have not been associated with some diseases (17); however, other diseases have been associated with production of HIF1 protein and reported to be involved in susceptibility to several disorders (16). In addition, a number of studies have reported associations between HIF-1 α gene polymorphisms and several types of cancer like non-small cell lung (18), head and neck (18,19), renal cell carcinoma (20), androgen-independent prostate (21), colorectal (22), and cervical and endometrial cancers (23). On the other hand, there are many studies reporting an association between genetic polymorphisms and susceptibility to breast cancer. Gerger et al. (24) studied a comprehensive panel of genetic polymorphisms with breast cancer risk. These polymorphisms were involved in a variety of cancer-related pathways including folate metabolism (MTHFR), inflammation (IL10), angiogenesis (VEGF), cell proliferation and apoptosis (CCND1, FAS, FASL, TGFB1), cellular signaling (GNB3, ITGA2, ITGB3), degradation of extracellular matrix (MMP1 and MMP3), prostaglandin synthesis (PTGS2), and detoxification of compounds (SULT1A1) (24). Krippel et al. found that carriers of the VEGF 936T allele were less frequent in breast cancer patients than in healthy subjects, indicating that this genetic variant may be protective against breast cancer (25). In addition, Balasubramanian et al. discussed the possible role of angiogenesis-related polymorphisms in various cancer types including breast cancer in their review (26). It has been shown that a SNP at codon 388 of fibroblast growth factor

receptor 4 (FGFR4) genes may predispose breast cancer patients for significantly accelerated disease progression (27). Furthermore, this polymorphism is a marker for breast cancer progression in patients with adjuvant systemic therapy, particularly chemotherapy, and thus may indicate therapy resistance (28). In another study it has been suggested that the presence of the 5A polymorphism at the MMP3 promoter may be one of the risk factors for the development and/or progression of cancer, especially in mammary tumors (29). Genetic variation in tumor necrosis factor- α (TNF- α) and heat-shock proteins (hsp70–2), determining factors in the immune response to tumor cells, may represent not only markers for the increased risk of breast carcinoma but also may predict the clinical outcome (30). TNF receptor II polymorphisms may also predict the late onset of breast carcinoma, relapse and death for patients with breast carcinoma (31). On the other hand, plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphism has not been associated with appearance and/or progression of breast cancer (32).

Although HIF-1 α plays pivotal roles in the development and progression of several cancer types including breast cancer (33), there has been only one published study so far (34) regarding the relationship between HIF-1 α polymorphisms and breast cancer. Therefore, in the present study we investigated the association of three SNPs, which were located in coding regions of the gene—one in exon 2 (S28Y) and two in exon 12—(P582S, A588T) with sporadic breast cancer in the Turkish population.

Materials and Methods

Study Population

One hundred and two women (mean age 46.6 years; range, 25–65 years) for whom the existence of sporadic breast carcinoma has been histopathologically confirmed were enrolled in the study from June 2005 to January 2007. All patients came from the Departments of General Surgery, Faculty of Medicine, Gazi University and Polyclinic of Second General Surgery, Ankara Oncology Education and Research Hospital. We studied 102 consecutive healthy female (mean age 45.5 years; range, 29–60 years) blood donors with no previous history of breast and ovarian cancer in first-degree relatives. Furthermore, none of the control individuals had history of oral contraceptive and postmenopausal hormone use, oophorectomy, cigarette smoking, and alcohol intake. The study was approved by the Ethics Committee of Gazi University. All cases and controls became subjects of this study after submitting their written consent. The study populations were Caucasian. Age at enrollment, age at menarche and first delivery, number of full-term pregnancies, body mass index, menopausal status, use of postmenopausal hormone and oral contraceptives, history of breast and ovarian cancer types in first-degree

relatives (stages I–IV), and grades of tumor based on the International Federation of Gynecology and Obstetrics (FIGO) staging method with histopathological classification, information on smoking habits, alcohol intake, and oophorectomy (a protective procedure against breast cancer) were obtained from the patients' files.

Clinical characteristics of the patients and their matched controls are summarized in Table 1.

DNA Extraction and Genotyping

Genomic DNA was isolated from peripheral blood by using a DNA extraction kit (Heliosis; Metis Biotechnology, Ankara, Turkey) according to the manufacturer's instructions. We set the PCR cycling conditions for the C1772T (rs11549465) and G1790A (rs11549467) regions as explained by Ollerenshaw et al. (20). For the C1772T, the forward primer used was 5'-AAG GTG TGG CCA TTG TAA AAA CTC-3', whereas the reverse primer was 5'-GCA CTA GTA GTT TCT TTA TGT ATG-3' (Gene bank accession no. AH006957; nucleotides 425–770). Amplifications of the C1772T and G1790A regions of the HIF-1 α gene were

carried out by placing in a Mastercycler gradient (Eppendorf, Hamburg, Germany) thermal cycler, a total volume of 50 μ L PCR mixture containing 50 ng genomic DNA, 2.5 mM MgCl₂, 100 μ M dNTP, 50 pmol/ μ L of each primer and 1.0 U/ μ L Taq DNA polymerase. The C1772T and G1790A nucleotide substitutions lead to a change from a proline to serine at codon 582 and an alanine to threonine at codon 588, respectively. The 1772C allele was cut into two fragments of 228 bp and 118 bp, whereas the 1772T allele remained uncut (346 bp). The polymorphism was analyzed by digesting the PCR products (346 bp) with restriction endonuclease, *Hph*I (Fermentas, Vilnius, Lithuania) and loading them onto 3% agarose gel (containing 0.5% ethidium bromide). For the G1790A, the polymorphism was analyzed by digesting the same PCR products with restriction endonuclease, *Acc*I (Fermentas) and loading them onto 3% agarose gel. The products were separated by electrophoresis and visualized by Gel Logic 100 gel image system (Kodak, Rochester, NY). The G allele produces a single cut site to give two bands, 201 bp and 145 bp. The substitution from a G to an A abolishes an *Acc*I cut site (346 bp).

The C111A nucleotide substitution leads to a change from a serine to tyrosine at codon 28. For the C111A, the forward primer used was 5'-GGA TAA GTT CTG AAC GTC GA-3', whereas the reverse primer was 5'-ATC CAG AAG TTT CCT CAC AC-3' (Gene bank accession no. AH006957; nucleotides 177–363). Amplifications of the C111A regions of the HIF-1 α gene were carried out by placing in a Mastercycler gradient (Eppendorf) thermal cycler, a total volume of 50 μ L PCR mixture containing 50 ng genomic DNA, 2.5 mM MgCl₂, 100 μ M dNTP, 3% dimethylsulfoxide (DMSO), 50 pmol/ μ L of each primer and 1.0 U/ μ L Taq DNA polymerase. The conditions for the PCR reaction were denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 5 min. The polymorphism was analyzed by digesting the PCR products (187 bp) with restriction endonuclease, *Bgl*II (Fermentas) and loading them onto 3% agarose gel. The products were separated by electrophoresis and visualized by Gel Logic 100 gel image system (Kodak). The 111C allele was cut into two fragments of 143 bp and 44 bp, whereas the 111A allele remained uncut (187 bp).

RFLP results were confirmed by sequencing the PCR products from randomly selected samples (25% of study population) using an automated sequencing system (ABI Prism 310 Genetic Analyzer; Applied Biosystems Divisions, Foster City, CA). For C1772T and G1790A polymorphisms, reverse primers were used, but for C111A polymorphism, forward primer was used (Figure 1).

Statistical Analysis

Tests for the Hardy-Weinberg equilibrium, which revealed normal distribution of the population, were performed by

Table 1. Clinical characteristics of the patients and controls

Characteristics	Cases (n = 102)	Controls (n = 102)
Age at enrollment (years) ^a	46.6 $\pm$ 10.5	45.5 $\pm$ 9.1
(Range, years)	(25–65)	(29–60)
Age of menarche ^a	13.4 $\pm$ 1.2	13.1 $\pm$ 1.4
Age of first delivery ^a	22.3 $\pm$ 4.4	23.4 $\pm$ 4.9
Number of full-term pregnancies ^b	3.0 $\pm$ 1.8	2.4 $\pm$ 0.9
Body mass index <25 kg/m ^{2c}	102 (100)	102 (100)
Oral contraceptive use ^c		
0	78 (76)	102 (100)
1–5 years	10 (10)	0 (0)
5–10 years	9 (9)	0 (0)
>10 years	5 (5)	0 (0)
Family history of breast and ovarian cancers ^c		
No	102 (100)	102 (100)
Menopausal status ^c		
Premenopausal	56 (55)	50 (49)
Postmenopausal	46 (45)	52 (51)
Postmenopausal hormone use ^c		
Yes	6 (6)	0 (0)
No	96 (94)	102 (100)
Oophorectomy ^c		
No	93 (91)	102 (100)
Lateral	1 (1)	0 (0)
Bilateral	8 (8)	0 (0)
Cigarette smoking ^c		
Yes	11 (11)	0 (0)
No	91 (89)	102 (100)
Alcohol intake		
Yes	5 (5)	0 (0)
No	97 (95)	102 (100)

^aYears (mean $\pm$ SD).

^bNumbers (SD).

^cNumbers (percentages).

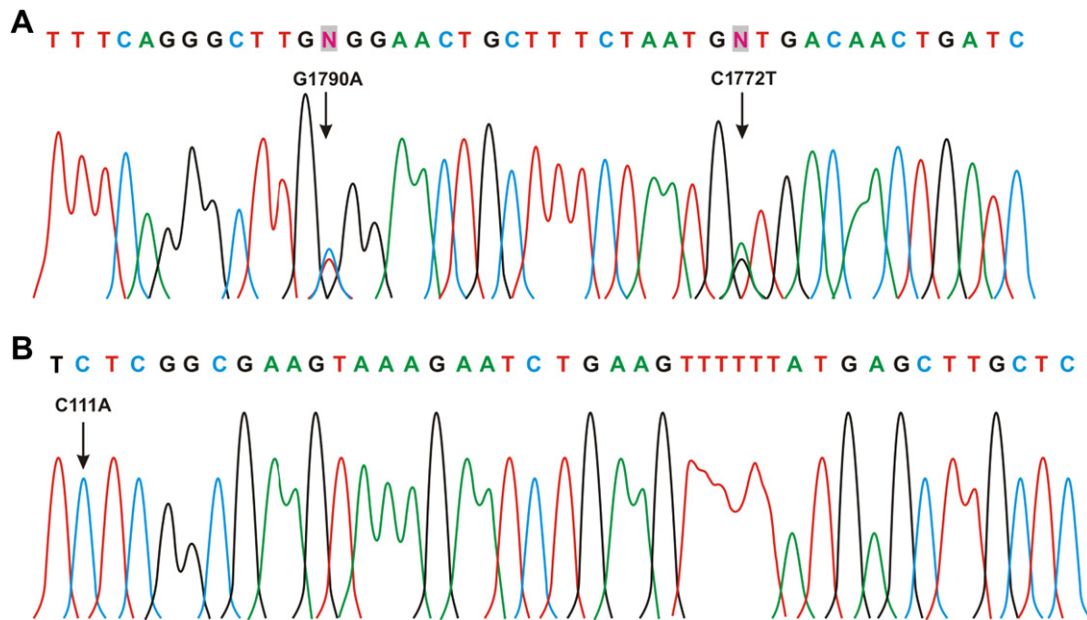


Figure 1. (A) Electropherograms of direct DNA sequence analysis of a heterozygote control for both C1772T and G1790A polymorphisms (CT genotype of C1772T and GA genotype of G1790A polymorphisms of HIF-1 α gene). (B) Electropherograms of direct DNA sequence analysis of homozygote CC genotype patient for C111A polymorphism of HIF-1 α gene. Color version of this figure available online at www.arcmedres.com

the χ^2 test. Pearson's two-way χ^2 tests were used first to compare patients and controls for clinical characteristics and then for genotype and allele frequencies. When the assumption of the χ^2 was violated, Fisher's exact test was performed. The relationships between genotypes and/or alleles and sporadic breast cancer risks were determined by obtaining the odds ratios (ORs) through a logistic regression method [OR, 95% confidence interval (CI)]. The association between the C1772T polymorphism and clinicopathological characteristics of the sporadic breast cancer was assessed using the multivariable logistic regression method. All p values are two sided; p values <0.05 were considered statistically significant. All analyses were conducted by using SPSS V.11.5 (SPSS, Inc., Chicago, IL).

Results

All three SNP (C1772T, G1790A, C111A) genotype frequencies in both sporadic breast cancer patients and controls were in agreement with Hardy-Weinberg equilibrium ($p > 0.05$). The baseline clinical characteristics of patients and controls are summarized in Table 1. There were no significant differences between patients and controls in terms of age at enrollment, age of menarche and first delivery, number of full-term pregnancies, body mass index, family history of breast and ovarian cancers, menopausal status and alcohol consumption ($p > 0.05$). Although significant differences were found between patients and controls in terms of the use of oral contraceptives ($p < 0.001$) and postmenopausal hormone ($p = 0.029$), cigarette smoking

($p < 0.001$) and oophorectomy ($p = 0.002$), these results were not clinically significant due to the criteria we set for the selection of the controls. These clinical characteristics were only observed in patients (Table 1).

Of the 102 healthy control cases, 68 were type CC, 29 were type CT and 5 were type TT. CC genotype was more frequent among sporadic breast cancer patients (77%) than among controls (67%). However, there were no significant differences in genotype ($p = 0.179$) and allele ($p = 0.057$) frequencies for HIF-1 α C1772T polymorphism between sporadic breast cancer patients and controls (Table 2). ORs were calculated by logistic regression analysis in comparison with the most common homozygote genotype observed in controls. No significant difference was found for genotypic frequencies of the HIF-1 α C1772T polymorphism between sporadic breast cancer patients with CT, TT, CT + TT genotypes and those with CC genotype ($p > 0.05$). Carriers of a 1772T allele (CT + TT genotypes) were more frequent among controls (33%) than among patients (23%). The OR per copy of the "T" allele was 0.59 (95% CI: 0.34–1.02, $p = 0.057$) (Table 2). Multivariable logistic regression analysis was performed between CC and CT + TT genotypes of C1772T polymorphism due to the insufficient number of TT homozygotes. Occurrence of sporadic breast cancer was not associated with clinicopathological features ($p > 0.05$) (Table 3).

As for HIF-1 α G1790A genotypes, of the 102 healthy control cases, 98 were type GG and 4 were type GA. All 102 patients were type GG (Table 4). The frequencies of G allele were 100% in patients and 98% in the control group. The frequencies of A allele were 2% in controls

Table 2. Distribution of C1772T polymorphism of HIF-1 α gene in sporadic breast cancer patients and control subjects

Genotypes	Patients <i>n</i> = 102 (%)	Controls <i>n</i> = 102 (%)	<i>p</i> value ^a	OR ^b (95% CI)	<i>p</i> value ^b
CC	79 (77)	68 (67)	0.179	1.00 (reference)	
CT	21 (21)	29 (28)		0.62 (0.33–1.19)	0.151
TT	2 (2)	5 (5)		0.34 (0.06–1.83)	0.257
CT + TT	23 (23)	34 (33)		0.58 (0.31–1.08)	0.086
C allele	179 (88)	165 (81)	0.057	1.00 (reference)	
T allele	25 (12)	39 (19)		0.59 (0.34–1.02)	0.057

OR, odds ratio; CI, confidence interval.

^a χ^2 analysis.^bCalculations were performed CC vs. CT, TT, CT + TT.

and null in the patient group (Table 4). As there were no GA and AA genotypes among sporadic breast cancer patients and only 4 GA genotypes among the controls, it became meaningless to run a multivariable logistic regression analysis to estimate *p* values with clinicopathological characteristics.

None of the sporadic breast cancer patients and controls analyzed exhibited C to A nucleotide substitution at point 111, and all individuals were homozygous CC (Table 5). Therefore, no comparison could be made between the CC genotype patients and CA and AA variants; hence, *p* values with clinicopathological characteristics were not estimated.

Discussion

Breast cancer is a multifactorial disease involving a complex interplay between genetic and environmental factors. There are many association studies between SNPs and breast cancer due to the polygenic model of breast cancer susceptibility. Genes involved in angiogenesis and hypoxias are suitable candidates for possible breast cancer susceptibility genes. Our study is the first to investigate whether HIF-1 α -related polymorphisms play a role in sporadic breast cancer in the Turkish population.

In our study there were no significant differences between sporadic breast cancer patients and controls in terms of the distributions of C1772T and G1790A genotypes and alleles (*p* > 0.05). Ollerenshaw et al. (20) found a highly significant increase in both the GA and CC genotypes in their patients with renal cell carcinoma and a significant decrease in the GG and CT genotypes. They found that T allele was more frequent both in patients and controls. In contrast, C allele is more frequent in our population in terms of the distribution of C1772T polymorphism. In addition, Ollerenshaw et al. (20) found a significant difference between the G and A alleles, with an increase in the A allele and a decrease in the G allele in the patients. However, we found no significant differences between patients

Table 3. Distribution of clinicopathological characteristics of sporadic breast cancer patients according to CC and CT + TT genotypes of C1772T polymorphism of HIF-1 α gene

Characteristics	CC	CT + TT	<i>p</i> value
Age at enrollment	46.0 $\pm$ 10.5	47.2 $\pm$ 10.6	0.822
Age of menarche	13.4 $\pm$ 1.1	13.3 $\pm$ 1.1	0.517
Age of first delivery	22.3 $\pm$ 4.4	22.2 $\pm$ 4.5	0.512
Number of full-term pregnancies	3.2 $\pm$ 1.6	2.9 $\pm$ 2.0	0.204
BMI <25 kg/m ²	79 (77)	23 (23)	1.000
Oral contraceptive use			
0	61 (78)	17 (22)	
1–5 years	8 (80)	2 (20)	0.843
5–10 years	7 (78)	2 (22)	
> 10 years	3 (60)	2 (40)	
Family history of breast and ovarian cancers			
No	79 (77)	23 (23)	1.000
Menopausal status			
Premenopausal	43 (77)	13 (23)	0.859
Postmenopausal	36 (78)	10 (22)	
Postmenopausal hormone use			
Yes	3 (50)	3 (50)	0.126
No	76 (79)	20 (21)	
TNM classification			
Clinical stage			
I	33 (73)	12 (27)	
II	41 (80)	10 (20)	0.668
III	5 (83)	1 (17)	
Lymph nodes			
N0	21 (78)	6 (22)	
N1	32 (73)	12 (27)	0.457
N2	12 (92)	1 (8)	
N3	14 (78)	4 (22)	
Metastasis			
M0	73 (76)	23 (24)	0.333
M1	6 (100)	0 (0)	
Pathological grade			
1	17 (81)	4 (19)	
2	39 (72)	15 (28)	0.383
3	23 (85)	4 (15)	
Oophorectomy			
No	73 (78)	20 (22)	
Lateral	0 (0)	1 (100)	0.216
Bilateral	6 (75)	2 (25)	
Cigarette smoking			
Yes	7 (64)	4 (36)	0.262
No	72 (79)	19 (21)	
Alcohol intake			
Yes	4 (80)	1 (20)	1.000
No	75 (77)	22 (23)	

All values indicate numbers (percentages).

and controls in terms of the distribution of G1790A alleles because the frequencies of G alleles were 100% in patients and 98% in the control group. This discrepancy may be due to the ethnic composition differences between the studies, different sample sizes, differences between retrospective study designs, limited information available on control groups and the fact that the mechanism by which the C1772T polymorphism affects serum levels of HIF-1 α may differ during the progression of breast and renal cell

Table 4. Distribution of G1790A polymorphism of HIF-1 α gene in sporadic breast cancer patients and control subjects

Genotypes	Patients <i>n</i> = 102 (%)	Controls <i>n</i> = 102 (%)	OR* (95% CI)	<i>p</i> value*
GG	102 (100)	98 (96)	1.00 (reference)	
GA	0 (0)	4 (4)	0.19 (0.02–1.67)	0.212
AA	0 (0)	0 (0)	Not calculated	–
G allele	204 (100)	200 (98)	1.00 (reference)	
A allele	0 (0)	4 (2)	0.19 (0.02–1.69)	0.123

OR, odds ratio; CI, confidence interval.
Calculations were performed GG vs. GA.

cancers. Tanimoto et al. (18) found CC and CT genotypes of C1772T polymorphism, but none of the subjects had the homozygous genotype TT. In our study, we found all the variants of the polymorphism, but TT homozygotes were quite small in number in our population. Furthermore, none of the subjects had a homozygous genotype AA for G1790A polymorphism in either study. In another study (21), the distribution of HIF-1 α genotypes for C1772T in androgen-independent prostate cancer patients and controls were consistent with our findings. In a case-control study among Swedish colorectal cancer patients and healthy individuals (22), the distributions of C1772T and G1790A genotypes and alleles were similar to our findings. Fransen et al. (22) observed a significant high risk, possibly due to a higher transactivating capacity of the polymorphic variants in patients who were heterozygous for the C1772T polymorphism, with more severe ulcerative growth pattern compared to the patients who were homozygous C1772C. Furthermore, patients carrying one or more polymorphic alleles in either the HIF-1 α C1772T or the G1790A polymorphism display a significantly higher risk for the development of ulcerative colorectal cancers (22). Kuwai et al. (35) found that the T allele of the C1772T polymorphism was not detected in patients with colorectal carcinoma and controls. In our previous study, T allele was the least observed in ovarian cancer patients and healthy individuals (23). However, there was a statistically significant increase in the percentages of carriers of T allele (CT, TT, and CT + TT genotypes) in cervical and endometrial cancer patients, which points to the fact that carrying the T allele is a risk factor in the development of cervical and endometrial cancers (23). In the present study, the allele and genotype

frequencies of all three polymorphisms of the HIF-1 α gene were in close agreement with those found in our previous study on ovarian, endometrial and cervical cancers. The association of the HIF-1 α polymorphism with sporadic breast cancer has recently been published (34). It was found that the minor alleles were T and A in mentioned polymorphisms, and there were no significant associations identified with the likelihood of breast cancer. Our findings are similar to those of Schneider et al. (34).

We also studied the association between the C1772T polymorphism and well-known risk factors, namely, age at menarche and first delivery, number of full-term pregnancies, body mass index, use of oral contraceptives and postmenopausal hormones, family history of breast and ovarian cancer, menopausal status, oophorectomy, and smoking habits and alcohol consumption. Owing to the small number of TT homozygote individuals, we followed a more conservative calculation strategy and combined carriers of heterozygous and rare homozygous genotypes (CT + TT). It was suggested that body size may represent a complex set of factors that influence the interaction of the estrogen, insulin, and inflammatory pathways among Hispanic and non-Hispanic pre- and postmenopausal Caucasian women (36). However, we found no association between C1772T polymorphism and the mentioned clinicopathological characteristics for sporadic breast cancer ($p > 0.05$). In addition, we did not find any statistically significant associations among C1772T polymorphism, age at enrollment and histopathological features ($p > 0.05$). Any imbalances that may exist in the clinical variables are often observed in cases that present family history of breast cancer. However, neither the patients nor the control group in our study were reported to have a family history of breast cancer.

As for C111A polymorphism of the HIF-1 α gene, “A” allele was not detected in patients and controls, i.e., all samples were found to be CC genotype in our population. Nevertheless, distribution of the C111A polymorphism was consistent with the report of Yamada et al. (16) and our previous study (23).

HIF-1 α expression is correlated with diagnostic and prognostic indicators for early relapse and metastatic disease, thus making HIF-1 α a potential prognostic biomarker in proteomic assessments of breast cancer (37). HIF-1 α is expressed in the early stages of mammary carcinogenesis and expression correlates with areas of nearby necrosis in the ductal carcinoma in situ (DCIS) (9,11,38). The HIF-1 α subunit is regulated by O₂-dependent hydroxylation of two conserved proline residues at positions 402 and 564, by prolyl hydroxylase domain protein 2 (PHD2). This allows subsequent recognition by the von Hippel-Lindau (VHL) tumor suppressor protein, which targets HIF for degradation by the ubiquitin-proteasome pathway (39–41). The HIF-1 α subunit is also regulated by O₂-dependent hydroxylation of asparagine residue 803 by factor-inhibiting HIF-1

Table 5. Distribution of C111A polymorphism of HIF-1 α gene in sporadic breast cancer patients and control subjects

Genotypes	Patients <i>n</i> = 102 (%)	Controls <i>n</i> = 102 (%)
CC	102 (100)	102 (100)
CA	0 (0)	0 (0)
AA	0 (0)	0 (0)
C allele	204 (100)	204 (100)
A allele	0 (0)	0 (0)

(FIH-1), which blocks the binding of the 300-kDa coactivator protein (p300) and CREB binding protein (CBP) (40,41). Under hypoxic conditions, prolyl hydroxylation of HIF is inhibited, allowing it to escape VHL-mediated degradation (39). The polymorphisms studied (proline to serine for C1772T and an alanine to threonine for G1790A), which were located within or near the amino-terminal transactivation domain (N-TAD), were not O₂-dependent hydroxylation sites of HIF (39). Therefore, HIF protein level and transcriptional activity would not be expected to be affected, and therefore the HIF pathway would be predicted to be regulated in a normal fashion. In addition, proline hydroxylation is a general feature of the cellular response to changes in oxygen, and this modification may be used in other signaling pathways. Once HIF-1 α is induced, whether these polymorphisms would have additional effects on tumor evolution and progression, including metastasis, would remain to be investigated. Semenza (40) recently suggested that a growing number of proteins and small molecules have been identified that regulate HIF-1 activity by modulating the physical or functional interaction of PHD2, VHL, FIH-1, receptor for activated C kinase 1 (RACK1), or hsp90 with HIF-1 α .

Tanimoto et al. (18) showed the elevated transactivation capacity of variant forms of HIF-1 α , which implied a role of HIF-1 α polymorphisms in generating individually different tumor progression potential. However, the role for these polymorphisms in vivo is still unclear because in patients with HIF-1 α variants, angiogenic factors such as VEGF could be upregulated not only in tumor cells but also in stromal cells via the enhancement of HIF-1 α transactivation (42). Therefore, polymorphic variant forms of HIF-1 α may comprehensively promote tumor angiogenesis in terms of tumor–stroma interactions. It has so far proved difficult to extrapolate the results of knockout experiments of HIF-1 α or angiogenic factors to genetic polymorphisms because genetic polymorphisms influence all the cells of the body including tumor and stromal cells.

In conclusion, we found no associations between any of the polymorphisms examined and the overall sporadic breast cancer risk. However, our results are expected to contribute to the efforts to better understand the phenotypic effects of SNPs, which are based on direct genetic effects and gene–gene and gene–environment interactions. The role of HIF-1 α gene polymorphisms in sporadic breast cancer remains to be further studied in larger series of different populations. Moreover, it may be worth investigating the possible association of HIF-1 α gene expression levels in relation to genotyping with sporadic breast cancer for the selection of the treatment strategies. It is known that both intratumoral hypoxia and genetic alterations cause overexpression of HIF-1 α gene. Therefore, to investigate the effects of polymorphic vs. non-polymorphic HIF overexpression on HIF target genes and metastasis and decide on the use of specific cancer treatment, we suggest that

O₂-dependent hydroxylation regions and functional polymorphic regions of HIF-1 α gene should be considered together.

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