Genetic Variations in the Hypoxia-Inducible Factor-1α Gene and Lung Cancer

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Hypoxia-inducible factor-1 (*HIF-1*), an important genetic component of angiogenesis, becomes stable as a response to tumor hypoxia and facilitates tumor survival. The polymorphisms of the *HIF-1α* gene may cause changes in the activity of this protein, which serves as a transcription factor for many genes in tumorigenesis. In this study, we have investigated the relationship between seven *HIF-1α* polymorphisms [C > T substitution in intron 8 (rs10873142), T418I (rs41508050) in exon 10, P564P (rs41492849), L580L (rs34005929), P582S (rs11549465), A588T (rs11549467) in exon 12 and dinucleotide GT repeats in intron 13 (rs10645014)] among lung cancer patients in the Turkish population. Genomic DNA was isolated from 141 lung cancer cases and 156 controls and subjected to PCR for amplification. Genotyping was carried out with RFLP and DNA sequencing methods. There was no significant difference between the lung cancer cases and controls in terms of the distribution of genotyping frequencies of seven *HIF-1α* polymorphisms ($P > 0.05$). No significant relationship was found between the C > T substitution in intron 8 and P582S haplotypes and development of lung cancer. In addition, there were no significant associations between the genotypes and clinopathological characteristics of the cases examined. These findings show that polymorphisms in the *HIF-1α* gene do not confer susceptibility to lung cancer. Exp Biol Med 234:1109–1116, 2009

Key words: DNA sequencing; *HIF-1α*; lung cancer; polymorphism; Turkish population

Introduction

Hypoxia is an important environmental regulator of tumor angiogenesis and growth. Many of the adaptations to hypoxia are mediated by the activation of specific genes through hypoxia-inducible factor (*HIF*) (1). *HIF-1* is a heterodimeric, helix-loop-helix transcription factor that consists of *HIF-1α* and *HIF-1β* subunits. In normoxic conditions, *HIF-1α* is hydroxylated at specific proline residues. Under hypoxic conditions, proline hydroxylation is inhibited, preventing the association with von Hippel-Lindau tumor suppressor protein (pVHL). As a result, *HIF-1α* accumulates and dimerizes with *HIF-1β*, which then activates a specific set of genes by binding to hypoxia response elements (HREs) in their promoter regions (2). Consequently, it has been suggested that *HIF-1α* is an endogenous marker of cellular hypoxia and that activation of the *HIF-1* transcription pathway characterizes a variety of pathological conditions, including cancer (3).

Polymorphisms can regulate the expression, structure and stability of *HIF-1α* mRNA and/or protein (4). There have been several polymorphism studies of *HIF-1α* for various cancer types in different populations. Significant associations have been reported between *HIF-1α* polymorphisms and lung (3), head and neck (5), renal cell (6, 7), prostate (8, 9), androgen-independent prostate (10), colorectal (11), cervical and endometrial (12), as well as breast (13) cancers. However, other studies have detected no associations between *HIF-1α* polymorphisms and colorectal (14), esophageal (15), prostate (16), breast (17, 18) and bladder (19) carcinomas.

With more than one million deaths per year, lung cancer is one of the most common cancer types in terms of mortality for both males and females in the developing world (20). Several environmental and genetic factors have been reported to affect lung cancer risk (21). As a genetic risk factor, *HIF-1α* polymorphisms may play a critical role in lung cancer risk. However, only one study to-date has examined the relationship between *HIF-1α* P582S, A588T and GT repeat polymorphisms and lung cancer (3). In the present study, we investigated whether C/T substitution in intron 8, T418I in exon 10, P564P, L580L, P582S, A588T in exon 12 and dinucleotide GT repeat polymorphisms in

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Table 1. Patients' and Control Characteristics

	Lung cancer cases *n* (%) (*n* = 141)	Controls *n* (%) (*n* = 156)	*P* value
Gender			0.080
Male	119 (84.3)	142 (91.0)	
Female	22 (15.7)	14 (9.0)	
Smoking status			< 0.001*
Yes	125 (88.6)	54 (34.6)	
No	16 (11.4)	102 (65.4)	
Pack-years (PY)[a]			< 0.001*
Light (≤50 PY)	73 (58.4)	45 (83.3)	
Heavy (>50 PY)	52 (41.6)	9 (16.7)	
Age			0.003*
<60	72 (51)	106 (67.9)	
≥60	69 (49)	50 (32.1)	
Histological type			
Squamous cell carcinoma	62 (44)		
Large cell undifferentiated carcinoma	29 (20.6)		
Small cell carcinoma	29 (20.6)		
Adenocarcinoma	18 (12.7)		
Missing	3 (2.1)		
Clinical stage[b]			
SCLC			
LS (limited stage)	13 (44.8)		
ES (extended stage)	16 (55.2)		
NSCLC			
I	8 (7.1)		
II	5 (4.5)		
III	54 (48.2)		
IV	45 (40.2)		

[a] We categorized the subjects as light or heavy smokers based on the mean of cumulative tobacco consumption among the control group.
[b] SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.
* *P* values < 0.05.

intron 13 of *HIF-1α* influence lung cancer risk in the Turkish population.

Materials and Methods

Subjects. The study was conducted within Gazi University, Faculty of Medicine, Department of Medical Biology and Genetics. Cases were recruited from Gazi University, Faculty of Medicine, Department of Chest Diseases, between 2005 and 2008. In total, 141 individuals diagnosed with any type of lung cancer were characterized as the case group. Clinical characteristics of each case were obtained from medical records. All cases had reported the absence of a history of lung cancer in their first and second degree relatives and that they had not been treated by chemotherapy or radiotherapy. A total of 156 healthy subjects who visited the clinics for a routine health check-up were recruited as controls. We adopted the TNM system of American Joint Committee on Cancer for classifying non small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Four stages from I to IV were taken for NSCLC and two stages, namely limited stage (LS) and extended stage (ES), were taken for SCLC. Details on patient and control characteristics are listed in Table 1. All individuals gave written informed consent to participate in this study and Gazi University Ethics Committee approved the study.

Genotyping Analysis. Genomic DNA was collected from peripheral blood samples using the Heliosis Kit (Metis Biotechnology, Ankara, Turkey) according to the manufacturer's instructions. We genotyped the investigated regions of *HIF-1α,* using Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) and DNA sequencing methods. Nomenclature for the analyzed *HIF-1α* polymorphisms is shown in Table 2 (Methodical Nomenclature Recommended by Human Genome Variation Society, www.hgvs.org).

For C > T (rs10873142) nucleotide substitution in intron 8, PCR amplification was performed with the forward 5′-TTC TGT TCC TGG GTT ATC TCA C-3′ and reverse 5′-CCT TTA ATG CAA CAA TGC CTA C-3′ primers, both of which were designed by ourselves. A total volume of 50 μl mixture contained 50 ng template DNA, 2.5 mM $MgCl_2$, 100 pmol/μl of each primer, 100 mM dNTP and 1 U/μl Taq DNA polymerase. The PCR conditions were initial denaturation at 95°C for 5 minutes, followed by 30 cycles at 95°C for 30 seconds, 65°C for 90 seconds and extension at 72°C for 5 minutes. The PCR products were digested with *Rsa*I restriction endonuclease (Roche Diag-

Table 2. Nomenclature for Analyzed *HIF-1α* Polymorphisms

Loci[a]	Position	db SNP	Common nomenclature used in paper	Methodical nomenclature
+41224 C > T	Intron 8	rs10873142	C > T	c.1029–145C > T
+42570 C > T	Exon 10	rs41508050	p.T418I	c.1253C > T
+45267 C > T	Exon 12	rs41492849	p.P564P	c.1692C > T
+45315 G > A	Exon 12	rs34005929	p.L580L	c.1740G > A
+45319 C > T	Exon 12	rs11549465	p.P582S	c.1744C > T
+45337 G > A	Exon 12	rs11549467	p.A588T	c.1762G > A
+49340 ins (GT)13–18	Intron 13	rs10645014	Dinucleotide GT repeat	c.2202+47–2202+48ins2

[a] Calculated from the transcriptional start site.

nostics GmbH, Mannheim, Germany) at 37°C for 16 hours, followed by electrophoresis in a 2% agarose gel containing 0.5% ethidium bromide. The PCR products were visualized on a Logic 100 gel image system (Kodak, NY, USA). The restriction fragments were 864 bp for the T allele, and 707 bp and 157 bp for the C allele. This is the first study to use the PCR-RFLP method for the analysis of this region of *HIF-1α*.

For the C > T nucleotide substitution in exon 10 (rs41508050, which gives rise to Thr/Ile substitution at codon 418), the designed PCR amplification primers were forward 5′-AAT TTG GTG ACA TTT TGT TG-3′ and reverse 5′-TGA GGG GAG CAT TAC ATC-3′. The PCR reaction was carried out in a final volume of 50 µl, containing 50 ng template DNA, 2.5 mM $MgCl_2$, 100 pmol/µl of each primer, 100 mM dNTP and 1 U/µl Taq DNA polymerase. Thermal cycling conditions were 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 seconds, 65°C for 90 seconds and then 72°C for 5 minutes. This PCR reaction produced a 492 bp long fragment which was digested with *Cai*I restriction endonuclease (MBI Fermentas, Vilnius, Lithuania) at 37°C for 16 hours. After the incubation time, the fragments were separated on a 2% agarose gel that was stained with ethidium bromide and visualized on a Gel Logic 100 image system (Kodak, NY, USA). The restriction fragments were 492 bp for the T allele, and 436 bp and 56 bp for the C allele. Again, this is the first study to use PCR-RFLP method for this region of *HIF-1α*.

Amplifications were performed with a Mastercycler gradient (Eppendorf, Hamburg, Germany) thermal cycler. A 346 bp long PCR product which contains the P564P (rs41492849), L580L (rs34005929), P582S (rs11549465) and A588T (rs11549467) SNPs was produced, using the following primers: forward 5′-AAG GTG TGG CCA TTG TAA AAA CTC-3′ and reverse 5′-GCA CTA GTA GTT TCT TTA TGT ATG-3′ (6). Amplifications of the exon 12 region of *HIF-1α* were performed in a final volume of 50 µl, containing 50 ng genomic DNA, 2.5 mM $MgCl_2$, 100 mM dNTP, 50 pmol/mL of each primer and 1.0 U/mL Taq DNA polymerase. Thermal cycling was based on PCR conditions laid out in Ollerenshaw *et al.* (6) and with an initial denaturation for 5 minutes at 95°C, followed by 30 cycles of denaturation for 30 seconds at 95°C, primer annealing for 60 seconds at 58°C and synthesis for 60 seconds at 72°C. The final primer extension was conducted for 5 minutes at 72°C (12). After PCR purification and cycle sequencing, all samples were genotyped (ABI Prism 310 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA).

A DNA fragment containing intron 13 of *HIF-1α* was amplified with the following primers: forward 5′-Fam-TGG TTC ACT TTT TCA AGC AGT AGG-3′ and reverse 5′-CTA TTT GGA AGA AAC GTG GAA ACA-3′. The 25 µl reaction volume contained 25 ng genomic DNA, 3 mM $MgCl_2$, 50 mM dNTP, 10 pmol/mL of each primer and 1.0 U/mL Taq DNA polymerase. PCR conditions were set as initial denaturation for 5 minutes at 95°C, 30 cycles of denaturation for 30 seconds at 95°C, annealing for 60 seconds at 65°C and synthesis for 60 seconds at 72°C, followed by a 5-minute final extension at 72°C. The fluorescence-labeled PCR products were analysed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sizes of the PCR products were determined by Genescan v.3.0 software (Applied Biosystems, Foster City, CA, USA). ROX-500 (Applied Biosystems, Foster City, CA, USA) was used as an internal size standard.

The locations of these seven SNPs are shown in Figure 1 in relation to the genomic structure of the *HIF-1α* gene.

Statistical Analysis. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) Version 11.5 (SPSS Inc., Chicago, IL, United States). Hardy-Weinberg equilibrium tests were conducted for each SNP using the Genepop Version 4.0 (22). Nominal data were evaluated by the Pearson χ^2 test, Fisher's Exact Test and Univariate Logistic Regression Analysis. The most prevalent homozygous genotype among controls was selected as the reference group. Odds ratios (ORs) and confidence intervals (95% CIs) were calculated for each genotype and allele. Multivariate unconditional logistic regression analysis with adjustment for age, gender, smoking habits and genotype distribution was performed to calculate adjusted ORs at 95% CIs. The Linkage Disequilibrium (LD) values for the five pairs of SNPs (rs10873142, rs41508050, rs11549465, rs11549467, rs10645014) were calculated using Haploview Version 4.1

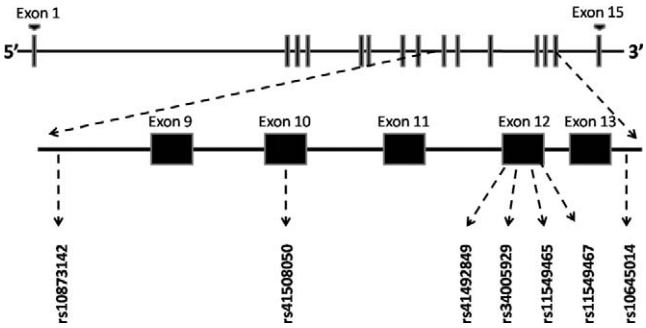

Figure 1. The location of each of the studied seven polymorphic variations of *HIF-1α* relative to the overall gene.

(Website: http://www.broad.mit.edu/mpg/haploview). One haplotype block was identified (23). SNPStats software program was used for the estimation of the haplotype frequencies (24). Values of $P < 0.05$ were accepted as statistically significant.

Results

There were significant differences between cases and controls in terms of smoking habits ($P < 0.001$), pack/year ($P < 0.001$) and age ($P = 0.003$) (Table 1). DNA samples of the subjects were analyzed for seven *HIF-1α* polymorphisms. None of the analyzed subjects exhibited P564P (+45267 C > T, rs41492849) and L580L (+45315 G > A, rs34005929) variations in exon 12 and all subjects were homozygous CC for P564P and GG for L580L. The genotype frequencies of intron 8 C > T (+41224 C > T, rs10873142) and exon 10 T418I (+42570 C > T, rs41508050), exon 12 P582S (+45319 C > T, rs11549465) and A588T (+45337 G > A, rs11549467) variations were in Hardy-Weinberg equilibrium ($P > 0.05$). Nonetheless, for the intron 13 dinucleotide GT repeat polymorphism (+49340 ins (GT)13–18, rs10645014), the distribution of genotypes in the control group ($P = 0.9418$) did not differ significantly from expected values, but there was a significant deviation from Hardy-Weinberg equilibrium in the case group ($P = 0.0239$).

As shown in Table 3, for the C > T nucleotide substitution in intron 8, there were no significant differences in genotype ($P = 0.716$) and allele ($P = 0.486$) frequencies between cases and controls. For this SNP, TT genotype was more frequent than the CC in all subjects. For the T418I (+42570 C > T) polymorphism, no TT variants of the gene were identified in any of the subjects. Also, CT heterozygotes were few in cases (1.4%) and controls (1.9%), thus no significant differences in genotype frequencies were found between cases and controls ($P > 0.05$). For the P582S (+45319 C > T) polymorphism, the TT genotype was not identified in any of the cases, but it was evident in a small percentage of controls (1.3%). This SNP was not associated with lung cancer risk in terms of genotype ($P = 0.137$) or allele distributions ($P = 0.142$). The other selected SNP of *HIF-1α* gene, A588T (+45337 G > A), did not demonstrate an association with the likelihood of lung cancer in our study ($P > 0.05$). There was only one subject (0.7%) who had the genotype GA in cases and two (1.3%) in controls. The AA genotype was not evident in cases or controls. For the intron 13 dinucleotide GT repeat (+49340 ins (GT)13–18) polymorphism, we observed different alleles with several GT repeat lengths ranging from 13 to 18 (Fig. 2). Because of the complexity of analyses with each repeat number, we decided to determine a cutoff point that contained the largest subject size both in controls and in cases. This cutoff point was used to divide alleles into two categories: short (S ≤ 14 GT repeat) and long alleles (L > 14 GT repeat). After this division, the dinucleotide GT repeat genotype frequencies were SS = 64.5%, SL = 31.2% and LL = 4.3% for the lung cancer cases (Table 3). For the control group, the corresponding frequencies were SS = 57.7%, SL = 37.8% and LL = 4.5% (Table 3). Genotype frequencies for the intron 13 dinucleotide GT repeat polymorphism did not differ statistically between the two groups ($P = 0.469$). In addition, the frequencies of S and L alleles were similar in each group, thus no association with lung cancer was revealed ($P = 0.296$). We next examined the relationship between the *HIF-1α* polymorphisms and age, gender and smoking habits of the subjects. Again we found no significant differences between genotypes and each of these factors in cases and controls ($P > 0.05$, data not shown).

Gene structure and a linkage disequilibrium plot for *HIF-1α* are shown in Figure 3. However, rs10873142 was not in linkage disequilibrium with rs41508050, rs11549467, rs10645014. In addition, rs41508050 was not in linkage disequilibrium with rs10873142, rs11549465, rs11549467, rs10645014. Since we observed that only two SNPs—rs10873142 and rs11549465—were in linkage disequilibrium, we continued our haplotype study with these two SNPs. The distributions of *HIF-1α* haplotypes along with estimations of ORs in both patients and controls are presented in Table 4. No correlation between intron 8 C > T (rs10873142) and exon 12 P582S (rs11549465) was demonstrated in either cases or controls ($P > 0.05$).

Discussion

HIF-1α is a preliminary stage molecule in angiogenesis and it may play a role in the development of lung malignancies (5, 25). Several studies have reported that genetic factors, as well as polymorphisms, could contribute to the lung cancer susceptibility (26). To our knowledge, the present study is the first to investigate whether the seven *HIF-1α* polymorphisms we have studied are correlated with the development of lung cancer and to investigate the role of *HIF-1α* intron 8 C > T polymorphism in lung cancer.

We found that the genotype and allele distributions were very similar in cases and controls ($P > 0.05$) (Table 3). Hlatky *et al.* (27) found the minor allele "C" to be more prevalent among stable angina patients than among acute

Table 3. Genotype and Allele Frequency Distributions of the *HIF-1α* Polymorphisms in Lung Cancer Patients and Controls

Loci	Genotypes	Cases $n = 141$ (%)	Controls $n = 156$ (%)	*P* value	OR (CI 95%)*	*P* value
+41224 C > T, Intron 8	**C > T** *(rs10873142)*					
	TT	78 (55.3)	79 (50.6)	0.716	1	
	CT	51 (36.2)	63 (40.4)		0.82 (0.51–1.33)	0.421
	CC	12 (8.5)	14 (9.0)		0.87 (0.38–2.00)	0.739
	CT+CC[a]	63 (44.7)	77 (49.4)	0.420	0.83 (0.53–1.31)	0.420
	TT+CT[b]	129 (91.5)	142 (91.0)	0.888	1.06 (0.47–2.37)	0.888
	Alleles					
	T	207 (73.4)	221 (70.8)	0.486	1	
	C	75 (26.6)	91 (29.2)		0.88 (0.61–1.26)	0.486
+42570 C > T, Exon 10	**T418I** (*rs41508050)*					
	CC	139 (98.6)	153 (98.1)	1.000	1	
	CT	2 (1.4)	3 (1.9)		0.73 (0.12–4.46)	1.000
	Alleles					
	C	280 (99.3)	259 (98.9)	0.676	1	
	T	2 (0.7)	3 (1.1)		0.62 (0.10–3.72)	0.676
+45319 C > T, Exon 12	**P582S** (*rs11549465)*					
	CC	110 (78.0)	111 (71.2)	0.137	1	
	CT	31 (22.0)	43 (27.6)		0.73 (0.43–1.24)	0.240
	TT	0 (0)	2 (1.3)		NC	
	CT+TT[c]	31 (22.0)	45 (28.8)	0.176	0.70 (0.41–1.18)	0.176
	CC+CT[d]	141 (100.0)	154 (98.7)	0.500	NC	
	Alleles					
	C	251 (89.0)	265 (84.9)	0.142	1	
	T	31 (11.0)	47 (15.1)		0.70 (0.43–1.13)	0.142
+45337 G > A, Exon 12	**A588T** *(rs11549467)*					
	GG	140 (99.3)	154 (98.7)	1.000	1	
	GA	1 (0.7)	2 (1.3)		0.55 (0.05–6.13)	1.000
	Alleles					
	G	281 (99.6)	310 (99.4)	1.000	1	
	A	1 (0.4)	2 (0.6)		0.55 (0.05–6.12)	1.000
+49340 ins (GT)13–18, Intron 13	**Dinucleotide GT repeat** *(rs10645014)*					
	SS	91 (64.5)	90 (57.7)	0.469	1	
	SL	44 (31.2)	59 (37.8)		0.74 (0.45–1.20)	0.220
	LL	6 (4.3)	7 (4.5)		0.85 (0.27–2.62)	0.774
	SL+LL[e]	50 (35.5)	66 (42.3)	0.227	0.75 (0.47–1.20)	0.227
	SS+SL[f]	135 (95.7)	149 (95.5)	0.922	1.06 (0.35–3.22)	0.922
	Alleles					
	S	226 (80.1)	239 (76.6)	0.296	1	
	L	56 (19.9)	73 (23.4)		0.81 (0.55–1.20)	0.296

* OR, odds ratio; CI, confidence interval; NC, not calculated.
[a] Comparing of subjects with CT+CC genotypes versus TT genotype.
[b] Comparing of subjects with TT+CT genotypes versus CC genotype.
[c] Comparing of subjects with CT+TT genotypes versus CC genotype.
[d] Comparing of subjects with CC+CT genotypes versus TT genotype.
[e] Comparing of subjects with SL+LL genotypes versus SS genotype.
[f] Comparing of subjects with SS+SL genotypes versus LL genotype.

myocardial infarction (MI) patients. Although it is difficult to compare our results with those of Hlatky *et al*. (27) due to analysis of different diseases, the genotype and allele distributions could be compared. When the minor allele frequency is separated into patient ancestry, the Europeans, Asians and Hispanics studied by Hlatky *et al*. revealed nearly similar frequencies. This finding is close to our "C" allele frequency in the Turkish population. Also, for the C to T transition at nucleotide 1253 (reported herein as T418I polymorphism) of *HIF-1α* in exon 10, Hlatky *et al*. (27), in conformity with our findings, did not observe the TT genotype. The minor allele "T" frequency of T418I was 0.6% in controls, 0.4% in patients with MI and 1% in patients with angina (27). Our "T" allele frequencies for this region were 1.1% for controls and 0.7% for cases (Table 3). While the minor allele "T" is absent in patients with African-American ancestry, the Asians present 1.7%, whereas Europeans and Hispanics present 0.5% T allele (27). The latter is similar to our "T" allele frequency of 0.7% in cases.

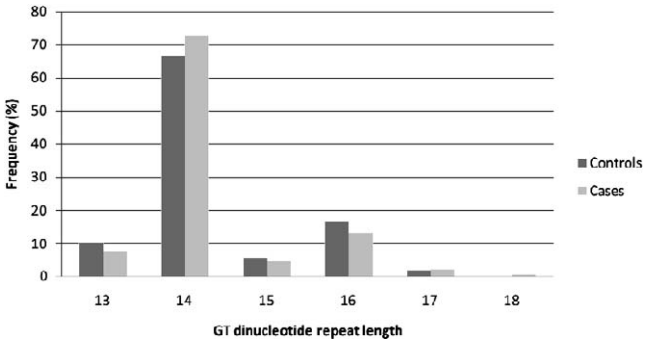

Figure 2. Frequency distribution of GT repeat length in 141 lung cancer patients and 156 controls.

We further analyzed exon 12 of the *HIF-1α* gene. All subjects were homozygous in terms of "C" allele for P564P (c.1692C > T) and "G" allele for L580L (c.1740G > A). Since there are no past studies genotyping these two polymorphisms by RFLP, we were not able to compare our results with those of other studies. For analysis of P582S (C to T transition at nucleotide 1744) and A588T (G to A transition at nucleotide 1762) in exon 12, there were no significant differences between lung cancer cases and controls in terms of the distribution of these two common polymorphic variants of the *HIF-1α* gene ($P > 0.05$) (Table 3). Previous studies found the prevalence of the rare homozygous AA (A588T) or TT (P582S) genotypes to be 0.2%–0.3% in the general population (5). Although there was a correlation between these two SNPs and increased tumor microvessel density and higher stage disease, no statistically significant results were indicated for the genotype distributions of the *HIF-1α* gene in head and neck squamous cell carcinoma cases and controls (5). For the P582S polymorphism, no variants were detected in a Japanese case-control study for colorectal carcinoma (14). Even though there was an association with the tumor size and lymph node metastasis, Ling *et al*. (15) found no significant differences in P582S genotype distributions between esophageal squamous cell carcinoma patients and healthy controls in the Chinese population. Ling *et al*. (15) did not find the TT genotype for P582S polymorphism. In addition, the genotype frequencies observed in the patients and controls were 11.58% versus 10.58%, respectively, for genotype CT (15). A significant difference was observed in genotype distribution between androgen-independent prostate cancer patients and control subjects for the P582S polymorphism (10). Koukourakis *et al*. (3) reported 34.3% heterozygous CT and 3.1% homozygous TT in 32 non small cell lung cancer patients. Our findings regarding the distribution of P582S polymorphism were consistent with those of Kuwai *et al*. (14), Chau *et al*. (10), Ling *et al*. (15) and Koukourakis *et al*. (3). For colorectal cancer, the distributions of P582S and A588T polymorphisms found by Fransen *et al*. (11) were similar to our results. When the colorectal cancer patients were categorized by their clinical parameters for ulcerative or polyploidy tumor phenotype, for the P582S, the heterozygous CT patients displayed a statistically significant higher risk for ulcerative tumors compared with homozygous CC patients (11). Also, for the ulcerative colorectal cancer patients, there was a statistically significant risk when they had one or more polymorphic alleles in their P582S and A588T regions of the *HIF-1α*

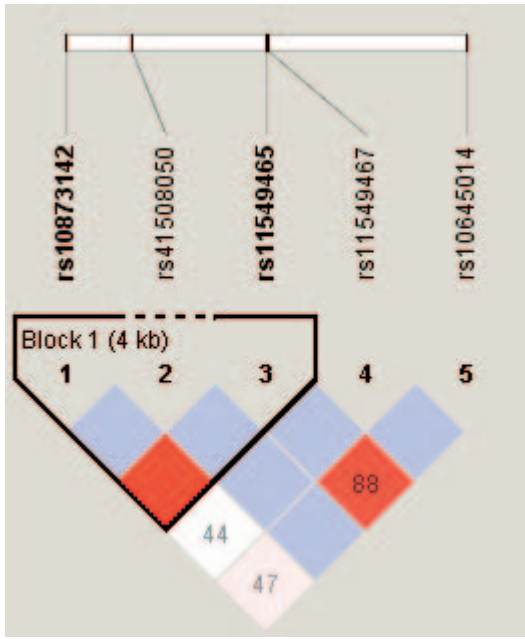

Figure 3. Gene structure and linkage disequilibrium plot for *HIF-1α*. The locations of the genotyped SNPs (rs10873142, rs41508050, rs11549465, rs11549467, rs10645014) relative to the introns and exons are indicated. The linkage disequilibrium plot at the bottom displays D' values (percent) for each pair of SNPs in the *box* at the *intersection* of the *diagonals* from each SNP. A color version of this figure is available in the online journal.

Table 4. *HIF-1α* SNPs (Intron 8 C > T, rs10873142 and Exon 12 P582S, rs11549465) Haplotypes Comparison in the Cancer and Control Groups

Haplotypes	Cases ($2n = 282$) (%)	Controls ($2n = 312$) (%)	OR (95% CI)[a]	*P* value
TC	207 (73)	221 (71)	1	
CC	31 (11)	47 (15)	0.70 (0.43–1.15)	0.161
CT	44 (16)	44 (14)	1.07 (0.68–1.69)	0.780

[a] OR, odds ratio; CI, confidence interval.

gene (11). In our past study on Turkish ovarian, cervical and endometrial cancer patients, we had found a significantly higher risk when the cervical and endometrial cancer patients had CT and TT genotypes for P582S polymorphism (12). In the present study, the distribution of the genotype TT in the P582S was lower in subjects (1.3% in controls and zero in cases), which is consistent with another past case-control study that we carried out in breast cancer (17). In another population-based study, this polymorphism was not associated with breast cancer risk in Korean women (18). Frequency of the *HIF-1α* P582S SerSer (or TT) genotype was 0.5% in cases and 0.1% in controls (18). The distribution of the genotypes differed slightly from our findings in the sense that Lee *et al*. (18) found the minor allele "T" carrier (CT+TT) frequencies for cases and controls to be less than those we report for the same groups. Kim *et al*. (13) examined the impact on prognosis of *HIF-1α* gene polymorphism and protein expression in the Korean population and found that *HIF-1α* overexpression was associated with the 582S polymorphic allele in breast cancer patients (13). Recently, Foley *et al*. (9) identified the heterozygous CT genotype for P582S as an increased risk factor for clinically localized prostate cancer. For the renal cell carcinoma, Ollerenshaw *et al*. (6) reported that the GA (for A588T) and CC (for P582S) genotypes among cases were associated with higher risk whereas GG and CT genotypes were associated with decreased risk when compared with the controls. In contrast to our findings, they found that "T" allele was more frequent in both cases and controls. Despite the absence of "A" allele in the A588T in our study, Ollerenshaw *et al*. (6) found a higher frequency of the "A" allele in cases. Besides, haplotype analysis revealed that T-A combination was significantly increased in cases and this state was commented as the strongest transcriptional activation under normoxic and hypoxic conditions. All of these dissimilarities may have emerged due to the ethnic composition differences, different cancer types and/or different sample sizes and selection criteria. In accordance with Koukourakis *et al*. (3), Fransen *et al*. (11) and Apaydin *et al*. (17), we did not detect the AA genotype in cases and controls for the A588T.

As for dinucleotide GT repeat polymorphism in intron 13 of the *HIF-1α* gene, deviation from Hardy-Weinberg equilibrium in cases may indicate the association of the polymorphism either with the disease or population stratification. We did not find any associations between the lung cancer cases and controls for this region ($P > 0.05$) (Table 3) (Fig. 2). Suzuki *et al*. (28) studied this polymorphism in Sherpas who are local residents of the Himalayas and who are adapted to hypoxia. When compared with the Japanese population, Sherpas have fewer GT repeats and this may play a role in their ability to respond to the hypoxic conditions at high altitudes. Also, the alleles with 14/17, 17/17 and 16 GT repeats were not evident in the Japanese or Sherpas who were tested (28). Koukourakis *et al*. (3) demonstrated some polymorphic variation association with protein expression in lung cancer. They reported that the dinucleotide repeat GT polymorphisms affected the *HIF-1α* expression immunohistochemically in non small cell lung cancer patients. The authors noted that more repeats of dinucleotide polymorphism were associated with *HIF-1α* overexpression in cancer cells and led to slow degradation and accumulation of the protein in the hypoxic tumor environment (3). The findings of Koukourakis *et al*. as regards to GT repeats are quite distinct from our findings. The fact that no control population was included in the study by Koukourakis *et al*. as well as the low sample size of the said study might have contributed to this distinction between the two studies. Furthermore, the goals of the two studies are quite distinct. Koukourakis *et al*. analyzed the expression of the *HIF-1α* downstream proteins whereas our analysis is based on SNPs. We also calculated the haplotype frequencies between lung cancer risk and intron 8 C > T and exon 12 P582S for both cases and controls and found no significant differences between cases and controls ($P > 0.05$) (Table 4) (Fig. 3). Furthermore, for the seven *HIF-1α* polymorphisms examined in the present study, we did not find statistically significant differences among gender, smoking habits, age and genotype distributions in lung cancer ($P > 0.05$).

In conclusion, it is well known that angiogenesis is one of the basic milestones in solid tumor growth. The molecular events that are critical for angiogenesis in cancer need further analysis for the adoption of the appropriate treatment methods, in particular, anti-angiogenic therapies. The results of this study suggest that the seven *HIF-1α* polymorphisms did not relate to lung cancer risk in the Turkish population. Perceiving the phenotypic effects of SNPs may be complicated because of complex molecular pathways, related gene-gene and protein-protein interactions. Nevertheless, it would be promising to carry these polymorphism studies to a more advanced level through mRNA and/or protein expression studies for selected genes in order to ascertain their function in the progression of different tumor types, in order to develop more effective strategies for prevention and therapy.

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