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Association Between *CYP1A1* Polymorphisms and Esophageal Cancer Susceptibility: A Case-control Study

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Abstract. *Background/Aim: Environmental and genetic factors (such as polymorphisms) contribute to the development of esophageal cancer (EC), but the disease's molecular genetic markers are not fully understood. The purpose of this study was to investigate previously unstudied cytochrome P450 (CYP)1A1 polymorphisms (rs2606345, rs4646421 and rs4986883) in EC. Materials and Methods: We performed real-time polymerase chain reaction (qPCR) to identify CYP1A1 polymorphisms (rs2606345, rs4646421, and rs4986883) in 100 patients and 100 controls. Results: Smoking and tandoor fumes were significantly higher in all EC and esophageal squamous cell carcinoma (ESCC) patients compared to the control group (p<0.0001). The risk of EC was two-fold higher in hot tea drinkers compared to non-drinkers, but this factor was not significant for ESCC or esophageal adenocarcinoma (EAC) (p>0.05). The rs4986883 T>C polymorphism was not found in our population. The rs2606345 C allele was significantly associated with EC risk in men, and C-carriers who drank hot black tea had a nearly threefold higher risk of EC than non-drinkers. In addition, EC risk in hot black tea drinkers was approximately 12 times higher in rs4646421 A carriers than in non-A carriers, and approximately 17 times higher in the presence of both rs2606345 C allele and rs4646421 A allele. Furthermore, the rs2606345 AA genotype may act as a protective factor for the rs4646421 GG genotype. Conclusion: Among the CYP1A1 polymorphisms, rs2606345 may increase the risk of EC only in men. The risk of EC in hot tea drinkers may increase in the presence of rs4986883 and rs2606345 polymorphisms.*

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Esophageal cancer (EC) is a type of cancer that is caused by genetic and environmental factors. There are two subtypes of EC, with esophageal squamous cell carcinoma (ESCC) being the most common in developing countries and esophageal adenocarcinoma (EAC) being the most common in industrialized countries (1). In Turkey, EC is more common, particularly in the eastern Anatolia region, with ESCC being the most common subtype (2-8). The environmental risk factors unique to each population are the most important reason for such disparities in results. Environmental risk factors such as smoking, alcohol, diet, excessive hot drinks, and carcinogen exposure all play a role in the development of EC. Xenobiotic metabolizing enzymes play an important role in the metabolism of environmental carcinogens and many studies have been conducted on the relationship between variants in genes in this pathway and EC. It has been reported that xenobiotic metabolic activation and detoxification take place primarily in the liver but can also take place locally in the esophageal mucosa and other tissues (1). Phase I and phase II enzymes, also known as xenobiotic metabolizing enzymes (XMEs), are the two main enzyme families involved in metabolic activation and detoxification (1, 9). The cytochrome P450 (CYP) family of phase I enzymes is one of those involved in the xenobiotic metabolism of endogenous compounds and carcinogens in humans. Furthermore, when the activity of CYP enzymes changes, drug metabolism can change dramatically (10). The CYP enzyme family consists of three members: CYP1 family (CYP1A1, CYP1A2, and CYP1B1), CYP2 family (CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP218, CYP219, CYP2D6, CYP2E1), and CYP3 family (CYP3A4, CYP3A5, CYP3A7) (11). One of the most important CYPs involved in the metabolism of chemical carcinogens is CYP1A1, which functions in phase I detoxification. The primary function of CYP1A1 is to catalyze the first step in the metabolism of polycyclic aromatic hydrocarbons (PAH), one of the carcinogens (1).

The *CYP1A1* gene has 7 exons and 6 introns and is located on 15q22-24 (12). Polymorphisms in this gene have been associated to increased enzyme activity and individual susceptibility to a variety of cancers, including EC (1, 13-20). *CYP1A1* single nucleotide polymorphisms (SNPs) have

Table I. *Basic characteristics of the study participants.*

Variable	Controls n (%)	EC n (%)	ESCC n (%)	EAC n (%)	*p*-Value	Odds ratio (95%CI)
	N=100	N=100	N=69	N=20		
Sex					a=0.66	0.9 (0.5-1.5)
Female	55 (55)	58 (58)	46 (67)	5 (25)	b=0.12	0.6 (0.3-1.2)
Male	45 (45)	42 (42)	23 (33)	15 (75)	**c=0.01**	**3.7 (1.2-10.8)**
Age					a=0.77	1.1 (0.6-1.9)
<55	44 (44)	42 (42)	32 (46)	3 (15)	b=0.76	0.9 (0.5-1.7)
≥55	56 (56)	58 (58)	37(54)	17(85)	**c=0.02**	**4.5 (1.2-16.2)**
Hot black tea						
Negative	45 (46)	30 (32)	22 (33)	6 (30)	**a=0.04**	**1.9 (1.02-3.3)**
Positive	52 (54)	64 (68)	45 (67)	14 (70)	b=0.08	1.8 (0.9-3.8)
Unknown	3	6	2		c=0.18	2.0 (0.7-5.7)
Van Herbed cheese						
Never or rarely	16 (16)	7 (8)	5 (8)	2 (11)	a=0.07	2.3 (0.9-5.9)
Every morning	81 (84)	83 (92)	60 (92)	17 (89)	b=0.1	2.4 (0.8-6.8)
Unknown	3	10	4	1	c=0.73	1.7 (0.4-7.9)
Smoking						
Negative	76 (76)	48 (51)	34 (51)	9 (45)	**a=0.0003**	**3.0 (1.7-5.6)**
Positive	24 (24)	46 (49)	33 (49)	11 (55)	**b=0.0007**	**3.1 (1.6-5.9)**
Unknown		6	2		**c=0.005**	**3.9 (1.43-10.5)**
Tandoor fumes, for only in women						
Negative	35 (64)	6 (11)	3 (7)	2 (40)	**a<0.0001**	**14 (5.1-38.5)**
Positive	20 (36)	48 (89)	42 (93)	3 (60)	**b<0.0001**	**24.5 (6.7-89.4)**
Unknown		4	1		c=0.36	2.6 (0.4-17.1)
Reflux						
Negative	69 (71)	56 (60)	41 (61)	11 (58)	a=0.1	1.6 (0.9-2.9)
Positive	28 (29)	37 (40)	26 (39)	8 (42)	b=0.2	1.6 (0.8-3.0)
Unknown	3	7	2	1	c=0.3	1.8 (0.7-4.9)
EC in family*						
Negative	100 (100)	72 (79)	48 (76)	18 (90)	d=0.22	0.4 (0.07-1.71)
Positive	0 (0)	19 (21)	15 (24)	2 (10)		
Unknown		9	6			
Tumor localization						
Upper	-	1 (1)	1 (2)	0 (0)	-	-
Mid		44 (53)	40 (68)	2 (10)		
Lower		38 (46)	18 (30)	18 (90)		
Unknown		17	10			

EC: Esophageal cancer; CI: confidence interval; ESCC: esophageal squamous cell carcinoma; EAC: esophageal adenocarcinoma; [a]EC *vs.* controls; [b]ESCC *vs.* controls; [c]EAC *vs.* controls; [d]ESCC *vs.* EAC. *No first-degree family history of EC. Bold indicates statistically significant value.

been widely studied in many cancers, but the results have remained very inconsistent (21). The *CYP1A1* intronic polymorphism rs2606345 (IVS1+606, C>A) has been linked to depressive symptoms in premenopausal and perimenopausal women, testicular germ cell tumor, brain tumor, and gallbladder cancer (22-25). Furthermore, it has been reported that this SNP influences drug response by decreasing *CYP1A1* expression in epileptic patients, and that the 'A' allele and 'AA' genotype are associated with recurrent seizures and altered anti-epileptic response in epileptic women (26, 27). In addition to the study that found rs4646421 (IVS1-728 G>A) polymorphism to be associated with laryngeal squamous carcinoma, another study found that the TT genotype increased the risk of developing chronic Hepatitis B virus infection by a factor of four (28, 29). By changing the sequence in which suppressor factors bind in the gene's intron 1 region, the CYP1A1 rs4646421 polymorphism can prevent enzyme inhibition and increase the risk of cancer (30). However, other studies have not identified the rs4646421 polymorphism as a risk factor for cancer (31-33). There were only three studies that examined the 3'UTR variant rs4986883 (m3=3205 T/C) SNP and found that it was not associated with breast cancer, cardiovascular risk, and cervical cancer risk (34-36).

Table II. *Genotype and allele frequencies between EC patients and controls.*

SNPs	Genotype	Allele	Patients n (%)	Controls n (%)	*p*-Value	Odds ratio (95%CI)
rs4646421	GG		78 (79)	75 (75)		1[a]
	GA		20 (20)	22 (22)	0.83	1.14 (0.58-2.27)
	AA		1 (1)	3 (3)	0.37	3.1 (0.32-30.68)
		G	176 (89)	172 (86)		1[a]
		A	22 (11)	28 (14)	0.47	1.30 (0.72-2.37)
rs4986883	TT		100 (100)	100 (100)	-	-
	TC		0	0		
	CC		0	0		
rs2606345	AA		34 (34)	39 (39)		1[a]
	AC		48 (48)	39 (39)	0.35	0.71 (0.38-1.32)
	CC		17 (17)	22 (22)	0.92	1.13 (0.52-2.47)
		A	116 (59)	117 (58.5)		1[a]
		C	82 (41)	83 (41.5)	0.93	1 (0.67-1.49)
			Male Patients n (%)	Male Controls n (%)		
		A	50 (43)	52 (58)	**0.036**	**0.55 (0.32-0.97)**
		C	66 (57)	38 (42)		

Bold indicates statistically significant value. SNP: Single nucleotide polymorphism; CI: confidence interval. [a]Reference group.

Several studies have been conducted to date to investigate the relationship between *CYP1A1* gene polymorphisms and EC (16, 18-20, 37-41). These studies, however, were unrelated to the rs2606345, rs4646421, and rs4986883 polymorphisms. There have been no studies on EC and *CYP1A1* polymorphisms in Turkey. The aim of this case-control study was to investigate at *CYP1A1* polymorphisms in regions where EC is most common in Turkey and determine how they relate to individual susceptibility to EC

Materials and Methods

Study population. This is a case–control study conducted in the period between February 2019 and February 2020. One hundred patients with histologically confirmed EC and 100 healthy controls (age- and sex matched), living in Eastern Turkey were included in the study. The cases were all incident EC patients detected at Van Yuzuncu Yıl University's Dursun Odabas Education and Research Hospital. As controls, healthy volunteers who were admitted to the same hospital, were approximately the same age and sex as the cases and had no personal or first-degree family history of EC were chosen at random. Clinical values (albumin, globulin, *etc.*) measured in routine examination were used in the study. The face-to-face method was used within the content of the research to investigate possible environmental exposure information such as dietary preferences, hot tea preferences, alcohol consumption, and smoking status of the participants. The survival analysis was performed from the time of diagnosis to the time of death. Table I describes the clinical and demographic characteristics of EC patients and healthy controls. The Van Yuzuncu Yıl University's non-interventional clinical research ethics committee approved this case–control study (01.02.2019/02), and all participants provided written informed consent. Furthermore, the research was carried out in accordance with the Helsinki Declaration.

DNA extraction and genotyping. From each participant, 5 ml of peripheral blood samples were collected into ethylenediaminetetraacetic acid (EDTA) coated tubes. The DNA was isolated from blood samples using the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The concentration and the purity of the extracted DNA were assessed by measuring the optical density at 260 and 280 nm using NanoDrop™ spectrophotometer (Thermo Fisher Scientific).

SNPs (rs2606345, rs4646421 and rs4986883) of the *CYP1A1* gene were genotyped using the TaqMan single-nucleotide polymorphism genotyping assays (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's instructions. PCR was performed in a final volume of 10 μl with approximately 10 ng DNA as template, 5 μl of 2XTaqMan Genotyping Master Mix, and 0.25 μl of 40XTaqMan Genotyping Assay Mix (Applied Biosystems); the reaction's volume was then completed with dH_2O. The 96-well plate was loaded into StepOne Plus Real Time PCR system (Applied Biosystems), and conditions were as follows: 10 min at 95˚C for initial denaturation followed by 40 cycles of 95˚C for 15 s and 60˚C for 1 min. The results were analyzed on an Applied Biosystems StepOnePlus Real Time PCR system with the TaqMan assay program from StepOne software version 2.3. (Applied Biosystems).

Statistical analysis. GraphPad Prism-6 was used for all statistical analyses (La Jolla, CA, USA). The Chi square test or Fisher's exact test was used to compare the allele and genotype frequencies of *CYP1A1* polymorphisms. To assess associations between categorical variables, the chi-square test was used. For survival analysis, the Kaplan-Meier method (the Gehan-Breslow-Wilcoxon test) was used. All tests were two-tailed, and a *p*-value of 0.05 was considered

Table III. *Genetic association models of rs2606345 and rs4646421 polymorphisms in esophageal cancer (EC) patients and controls.*

Genetic model	EC *versus* Control		ESCC *versus* Control	
	Odds ratio (95%CI)	*p*-Value	Odds ratio (95%CI)	*p*-Value
rs2606345				
Homozygous model (CC *vs.* AA)	0.89 (0.4-1.9)	0.92	0.71 (0.3-1.7)	0.59
Heterozygous model (AC *vs.* AA)	1.41 (0.8-2.6)	0.36	1.32 (0.7-2.6)	0.53
Dominant model (CC/AC *vs.* AA)	1.22 (0.7-2.2)	0.59	1.10 (0.6-2.1)	0.89
Recessive model (CC *vs.* AC/AA)	0.73 (0.4-1.5)	0.49	0.61 (0.3-1.4)	0.33
rs4646421				
Homozygous model (AA *vs.*GG)	0.32 (0.03-3.1)	0.37	0.45 (0.05-4.5)	0.64
Heterozygous model (GA *vs.* GG)	0.87 (0.4-1.7)	0.83	1.64 (0.7-3.8)	0.35
Dominant model (AA/GA *vs.* GG)	0.81 (0.4-1.6)	0.64	1.56 (0.7-3.5)	0.38
Recessive model (AA *vs.* GA/GG)	0.33 (0.03-3.2)	0.62	1.79 (0.2-18.3)	0.85

ESCC: Esophageal squamous cell carcinoma; CI: confidence interval.

Table IV. *Comparison of genotype combination of rs2606345 and rs4646421 in patients with esophageal cancer (EC) and controls.*

Variable	rs2606345AA+ rs4646421GG n (%)	rs2606345 AC+ rs4646421 GA n (%)	rs2606345 CC+ rs4646421 AA n (%)	*p*-Value	Odds ratio (95%CI)
EC patients	34 (68)	15 (30)	1 (2)	0.57	1[a] 0.69 (0.29-1.69)[b] 2.61 (0.26-26.35)[c]
Controls	39 (72)	12 (22)	3 (6)	0.62	
Male EC patients	16 (76)	4 (19)	1 (5)	0.73	1[a] 1.41 (0.33-5.95)[b] 0.94 (0.05-16.36)[c]
Male controls	17 (71)	6 (25)	1 (4)	1	
Female EC patients	18 (62)	11 (38)	0 (0)	0.25	1[a] 0.44 (0.14-1.44)[b] 4.11 (0.18-91.15)[c]
Female controls	22 (73)	6 (20)	2 (7)	0.49	

SNP: Single nucleotide polymorphism; CI: confidence interval. [a]Reference group (rs2606345AA+rs4646421GG); [b]reference group *versus* rs2606345 AC+rs4646421 GA; [c]reference group *versus* rs2606345 CC+rs4646421 AA.

statistically significant. To estimate associations between SNPs and EC risk, the odd ratios (OR) and 95% confidence intervals (95%CI) were calculated.

Results

Characteristics of study participants. The characteristics of the study participants are shown in Table I. The present study recruited 100 EC patients (69 ESCC patients, 20 EAC patients, and 11 other histological type or unknown type) and 100 healthy controls. The mean age of patients and controls was 56.58 and 54.12 years, respectively. Fifty-eight patients (58%) were female; 58 patients (58%) were 55 years or older; 37 patients (40%) were positive for reflux; 19 patients (21%) had cancer history in their family; 44 patients (53%) had middle-tumor localization. There were no significant differences among EC and control groups regarding sex, age, reflux, and Van herbed cheese ($p>0.05$). Smoking and exposure to tandoor fumes were significantly higher in all EC and ESCC patients compared with the control group ($p<0.0001$). It was observed that drinking hot tea increased the risk of EC by about 2-fold (OR/95%CI=1.9/1.02-3.3, $p=0.004$), but it was not significant for ESCC and EAC ($p>0.05$). In ESCC patients (68%), the tumor was usually found in the middle esophagus, but in EAC patients, it was more commonly found in the lower esophagus (90%).

Genotypic distribution and allelic frequencies. The genotype and allele frequencies of the *CYP1A1* polymorphisms among control and EC subjects are summarized in Table II. The genotype distribution of *CYP1A1* rs2606345 and rs4646421 SNPs was consistent with Hardy-Weinberg equilibrium in all groups ($p>0.05$).

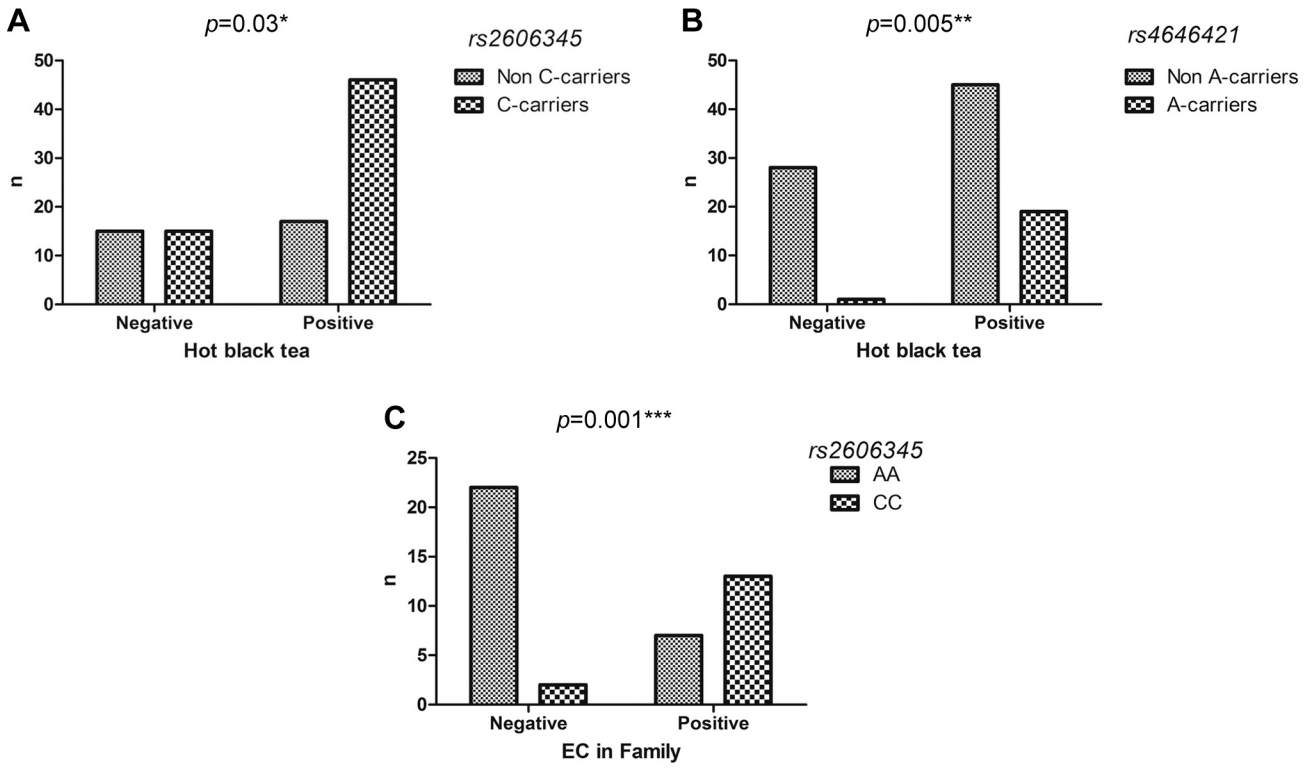

Figure 1. *Association of rs2606345 and rs4646421 polymorphisms with family history and hot black tea in patients with esophageal cancer (EC). (A) rs2606345 polymorphism (OR/95%CI=2.7/1.1-6.7); (B) rs4646421 polymorphism (OR/95%CI=11.8/1.5-93.3); (C) rs2606345 polymorphism (OR/95%CI=20.4/3.7-113.5).*

There was no significant difference in genotype and allele frequencies of *CYP1A1* SNPs (rs2606345, rs4646421, and rs4986883) between patients and controls ($p>0.05$). rs4986883 T>C polymorphism was never observed in either patients or controls, all participants had the TT genotype. The rs2606345 minor genotype (CC) frequencies were 17 and 22%, minor allele (C) frequencies were 41 and 41.5% for the patients and controls, respectively. The rs4646421 minor genotype (AA) frequencies were 1 and 3%, minor allele (A) frequencies were 11 and 14% for the patients and controls, respectively. We also analyzed the association between *CYP1A1* SNPs and genotype/allele frequencies in male and female EC patients separately. There was no significant difference in genotype frequencies when rs2606345 and rs4646421 SNP frequencies were compared separately in males and females (data not shown). The C allele frequency for rs2606345, on the other hand, was found to be significantly higher in male patients than in male controls (Table II, OR/95%CI=0.55/0.32-0.97, $p=0.036$), but it was not statistically significant in females (data not shown). As shown in Table II, the rs2606345 minor allele (C) frequencies were 42 and 57% for the male control group and male patients, respectively.

CYP1A1 polymorphisms and EC risk. Homozygous, heterozygous, dominant, and recessive genetic association models were applied to examine the associations of the rs2606345 and rs4646421 polymorphisms with EC risk (Table III). No association between *CYP1A1* polymorphisms (rs2606345 and rs4646421) and EC risk was observed in all genetic models ($p>0.05$). Similarly, comparison of male patients' and controls' genetic models revealed no significant differences in *CYP1A1* polymorphisms (rs2606345 and rs4646421) and EC risk (data not shown, $p>0.05$).

The rs2606345 and rs4646421 polymorphism genotype combinations were not significantly associated with an increased risk of EC ($p>0.05$, data not shown). Interestingly, all patient and control samples with the AA genotype for rs2606345 also had the GG genotype for rs4646421 (data not shown). However, in both men and women, this genotype association (AA+GG) was not associated with IPF risk (Table IV, $p>0.05$). There was no significant difference between all patients and all controls/male patients and male controls/female patients and female controls when the combination of rs2606345 AA and rs4646421 GG was compared to other genotypes (Table IV, $p>0.05$).

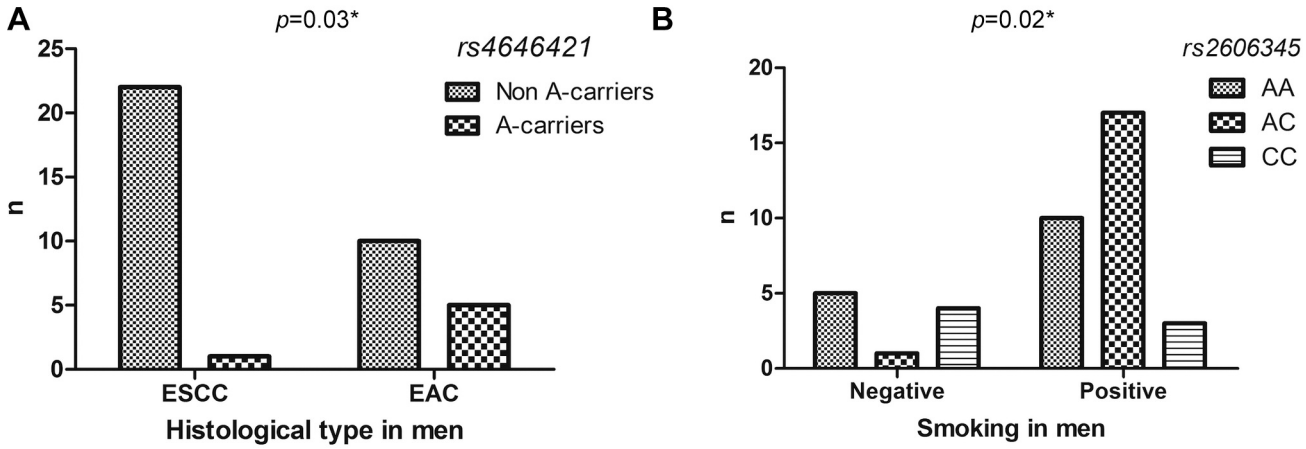

Figure 2. *Histological type and smoking in men. (A) rs4646421 polymorphism (OR/95%CI=11/1.1-106.9); (B) rs2606345 polymorphism.*

CYP1A1 polymorphisms and clinical/demographic parameters in EC patients. There was no statistically significant relationship between EC risk and *CYP1A1* polymorphisms (rs2606345 and rs4646421) based on sex, age, Van Herby cheese, smoking, tandoor fumes, reflux, family history, or tumor localization ($p>0.05$, data not shown). However, we observed that both polymorphisms increased the risk of EC when drinking hot black tea. In those who drink hot black tea daily, the risk of EC was approximately three times higher in rs2606345 C carriers than in non-C carriers (Figure 1A, OR/95% CI=2.7/1.1-6.7, $p=0.03$), and it was about twelve times higher in A carriers than non-A carriers of the rs4646421 polymorphism (Figure 1B, OR/95%CI=11.8/1.5-93.3, $p=0.005$). Furthermore, people with the rs2606345 CC-genotype and a family history of EC cancer had a 20-fold higher risk of EC than people with the AA-genotype (Figure 1C, OR/95%CI= 20.4/3.7-113.5, $p=0.0001$).

When the above significant factors (hot black tea and family history) were examined separately for men and women, they were not statistically significant ($p>0.05$, data not shown). In males, however, there was a significant difference between histological types for the rs4646421 polymorphism and smoking for the rs2606345 polymorphism. rs4646421 non-A carriers had significantly more ESCC histological types than A-carriers, while A-carriers had more EAC types (Figure 2A, OR/95%CI=11/1.1-106.9, $p=0.03$). Smokers with the rs2606345 AC genotype had a significantly higher risk of EC when compared to other genotypes (Figure 2B, $p=0.02$).

Sex, age, Van herbed cheese, smoking, tandoor fumes, reflux, family history, tumor localization, histological type, and *CYP1A1* polymorphism genotype combinations were found to have no significant association (Table V, $p>0.05$). We previously stated that drinking hot black tea approximately doubles the risk of EC (Table I), and this risk nearly triples in rs2606345 C carriers (Figure 1A). This risk is approximately four times higher in rs4646421 A carriers than in rs2606345 C carriers (Figure 1B). Furthermore, the coexistence of rs2606345 C-carrier and rs4646421 A carrier increased the risk by approximately 6 times when compared to rs2606345 C carriers alone (Table V, OR/95%CI=16.8/1.99-140.8, $p=0.002$).

Association of CYP1A1 polymorphisms with survival analysis of EC patients. The median life span was calculated using Kaplan-Meier survival charts. The Gehan test was used to compare the two groups' life spans. The median age of rs2606345 A-carriers and rs4646421 non G-carriers was higher than that of the other genotypes (non A-carriers and G-carriers). The Gehan-Breslow-Wilcoxon test and Kaplan-Meier analysis, on the other hand, revealed that genotypes were not associated with overall survival (Figure 3A and B, $p>0.05$).

Discussion

SNPs are the most common variants in the human genome and are well-established cancer predictive and prognostic biomarkers (42). SNPs in a large number of genes have been examined in case-control studies for EC, but some of them have been shown association with EC whereas others have yielded controversial results. *CYP1A1* is one of the genes studied in EC, and studies have shown a strong relationship between the rs1048943 polymorphism in this gene and EC (15). rs2606345, rs4646421, and rs4986883 SNPs in the *CYP1A1* gene in EC, on the other hand, have never been studied. We conducted this case-control study to determine the association of *CYP1A1* rs2606345, rs4646421, and rs4986883 SNPs with EC risk.

In our study, no significant differences in sex, age, reflux, or Van herbed cheese were found between the EC and

Table V. *Basic characteristics and genotype combination of rs2606345 and rs4646421 in esophageal cancer (EC) patients.*

Variable	rs2606345AA+ rs4646421GG n (%)	rs2606345 C-carriers+ rs4646421A-carriers n (%)	*p*-Value	Odds ratio (95%CI)
Sex				
Female	16 (47)	7 (33)	0.31	1.78 (0.57-5.5)
Male	18 (53)	14 (67)		
Age				
<55	11 (32)	10 (48)	0.26	0.53 (0.17-1.61)
≥55	23 (68)	11 (52)		
Hot black tea				
Negative	15 (47)	1 (5)	**0.002**	**16.76 (1.99-140.8)**
Positive	17 (53)	19 (95)		
Van Herbed cheese				
Never or rarely	2 (6)	2 (11)	0.61	0.53 (0.07-4.15)
Every morning	30 (94)	16 (89)		
Smoking				
Negative	16 (50)	12 (60)	0.48	0.67 (0.21-2.07)
Positive	16 (50)	8 (40)		
Tandoor fumes, for only in women				
Negative	18 (56)	7 (35)	0.14	2.39 (0.75-7.57)
Positive	14 (44)	13 (65)		
Reflux				
Negative	21 (66)	12 (60)	0.68	1.27 (0.4-4.03)
Positive	11 (34)	8 (40)		
EC in family				
Negative	22 (76)	15 (75)	0.94	1.05 (0.28-3.93)
Positive	7 (24)	5 (25)		
Tumor localization				
Upper	1 (4)	0 (0)	0.69	-
Mid	13 (46)	9 (53)		
Lower	14 (50)	8 (47)		
Histological type				
ESCC	25 (83)	13 (68)	0.29	2.31 (0.59-9.02)
EAC	5 (17)	6 (32)		
EC patients	34 (47)	21 (46)	0.92	1.04 (0.49-2.18)
Control	39 (53)	25 (54)		
ESCC	25 (39)	13 (34)	0.78	1.23 (0.53-2.85)
Control	39 (61)	25 (66)		

Bold indicates statistically significant value. ESCC: Esophageal squamous cell carcinoma; EAC: esophageal adenocarcinoma; CI: confidence interval.

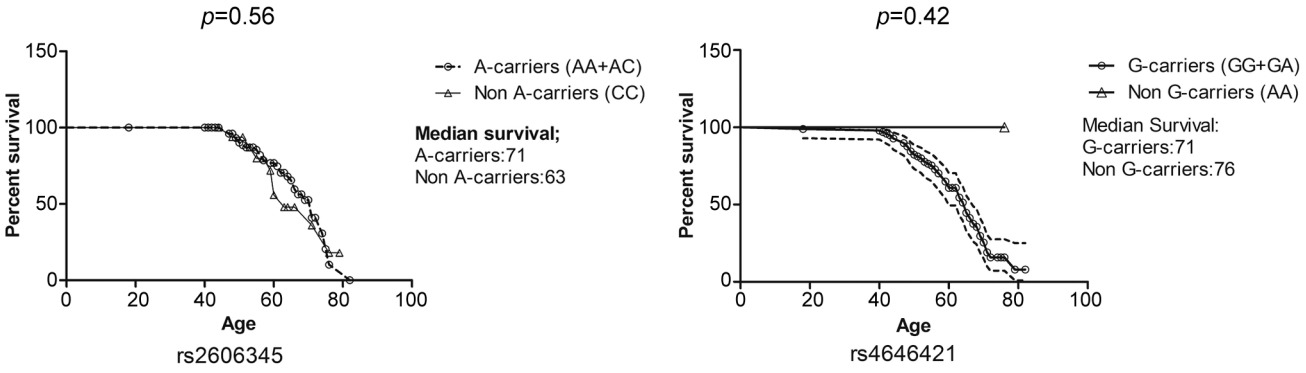

Figure 3. *Overall survival according to CYP1A1 single nucleotide polymorphisms in patients with esophageal cancer. (A) rs2606345 polymorphism; (B) rs4646421 polymorphism.*

control groups, but exposure to cigarette and tandoor fumes was significantly higher in all EC and ESCC patients compared to the control group (Table I, $p<0.05$). Although not all smokers develop cancer or smoking-related diseases, secondary individual factors such as diet, polymorphisms, lifestyle, and exposure to other environmental toxins have been reported as risk factors for these diseases in smokers (43). We found that drinking hot tea doubled the risk of EC ($p=0.04$), whereas we did not observe any significant association when ESCC and EAC were examined separately (Table I, $p>0.05$). Our findings on smoking and tandoor fumes in EC patients were consistent with some previous studies. However, the findings of studies on the relationship between hot tea, reflux, and Van herbed cheese and EC risk are contradictory (2, 3, 44-47). These findings suggest that smoking and tandoor fumes may be risk factors for EC, but more research on hot tea/foods, reflux, and Van herbed cheese is needed. The differences between the studies about hot tea results may be due to data not being recorded with the same objectivity. In future studies, it may be necessary to create a common data set that can be objectively evaluated (how often it is drunk, how hot it is drunk, *etc.*) while collecting information about hot tea consumption.

CYP1A1 SNPs have been extensively studied with various cancers due to the important role of *CYP1A1*'s chemical carcinogen metabolism in phase I detoxification (1, 13-20), and they are also among the most studied in EC (15, 16, 19, 20, 37-39). Another study reported that CYP1A1 polymorphisms have no effect on cancer risk (48). However, there was no study that focused on the role of rs2606345, rs4646421, and rs4986883 SNPs in EC. Accordingly, this study explored the association of *CYP1A1* rs2606345, rs4646421, and rs4986883 SNPs with the risk of EC. We observed that allele and genotype frequencies of the three SNPs were not associated with EC (Table II, $p>0.05$). When we analyzed the male EC cases in our study, the C (minor) allele frequency for rs2606345 in male patients was significantly higher than that in male controls (Table II, $p=0.036$). A previous case-control study reported that rs2606345 is associated with brain tumor, and minor genotypes may increase the risk of brain tumors, especially in female patients (24). Another study about gallbladder and bile duct cancers found that the major allele increased the risk of these cancers (25). However, it has been reported that rs2606345 is not a risk factor for breast cancer in women (49). The controversial results between the studies may be due to different variables such as cancer types and sexes, although minor allele or genotype are the common point in some studies (24, 25, 49). As a result, the rs2606345 C allele may be associated with an increased risk of EC in men.

The SNP rs2606345 has been associated with an increased risk of lung cancer in both smokers and nonsmokers (50). We discovered a significant association between rs2606345 heterozygote genotype (AC) and EC risk depending on smoking history (Figure 2B, $p=0.02$). Previous research has not examined the association between rs2606345 and hot black tea drinkers in cancers. The results of our study showed that drinkers of hot black tea carrying the rs2606345 C allele had an about three times greater risk of EC than non-C carriers (Figure 1A). According to our findings, this risk was approximately 12 times higher in rs4646421 A carriers than in non-A carriers (Figure 1B). Furthermore, compared to other genotype combinations, the risk of EC in hot black tea drinkers was approximately 17 times higher in the presence of both rs2606345 C allele and rs4646421 A allele (Table V). There was no research examining the relationship between the two SNPs (rs2606345 and rs4646421) and cancer survival. The current study found no association between genotypes of rs2606345 and rs4646421 and overall survival ($p>0.05$, Figure 3).

Our healthy sample findings were most similar to the rs4646421 genotype and allele frequencies found in healthy Italy (Tuscan) and European populations, but very different from the frequencies found in healthy Tunisian and Oceania populations (51, 52). These SNP studies have yielded contradictory results, with some reporting no risk for certain cancers and others providing evidence that it may increase the risk for certain cancers (28-33). According to our findings, rs4646421 SNP is not a risk factor for EC (Table III). We also observed that genotypes were distributed differently among the histological types; non-A carriers were primarily detected in ESCC whereas A carriers were common in EAC (Figure 2A and B).

The rs4986883 SNP has been studied for its association with cardiovascular risk, cervical cancer, and breast cancer, but no studies have found a significant link between this SNP and these diseases (34-36). The rs4986883 T>C polymorphism was not found in our population, and all participants had the TT genotype (Table II).

We determined that the coexistence of the rs2606345 AA and rs4646421 GG genotype was not a risk factor for EC compared to other genotypes ($p>0.05$, Table IV). It was noteworthy, however, that all patient and control samples with rs2606345 AA genotype also had rs4646421 GG genotype. These findings suggest that the rs2606345 AA (wild-type) genotype, regardless of sex, may act as a protective factor for the rs4646421 GG (wild-type) genotype. There has been no other study that shows the coexistence of these two polymorphisms' genotypes (AA+GG).

The results our study suggest that smoking and tandoor fumes are an important factor in the development and progression of EC. rs4986883 T>C polymorphism was not found in our population. The rs2606345 C allele is significantly associated with EC risk in males, with carriers of the C-allele having approximately three times the risk of EC as non-C carriers in hot black tea drinkers. In addition,

the results indicate that EC risk in hot black tea drinkers is approximately 12 times higher in rs4646421 A carriers than in non-A carriers and is approximately 17 times higher in the presence of both rs2606345 C allele and rs4646421 A allele. It was found that the rs2606345 AC genotype can significantly increase the risk of EC in smokers. Furthermore, the rs2606345 AA (wild-type) genotype, regardless of sex, may act as a protective factor for the rs4646421 GG (wild-type) genotype. To determine the potential clinical implications of these findings, future studies with larger sample sizes, as well as functional studies on *CYP1A1* polymorphisms, are required.

Conflicts of Interest

The Authors declare that there are no conflicts of interest in relation to this study.

Authors' Contributions

Research design: ZK; patient and questionnaire summary: ZK, SG; experimental work: ZK, SG; statistical analysis: ZK; article writing: ZK; manuscript checking and discussing: ZK, SG.

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