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Evaluation of TP53 codon 72 polymorphism in esophageal cancer susceptibility in Eastern Anatolia Region of Turkey

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ABSTRACT

The tumor suppressor *TP53* gene plays a key role in the regulation of cell cycle. Polymorphisms in this gene have been associated with many cancers including esophageal cancer (EC). Many studies in other populations have demonstrated that codon 72 polymorphism of *TP53* gene contribute to the prediction of EC risk, especially in Asians. The aim of this study was to explore the effect of codon 72 polymorphism on the EC risk in eastern Turkey.

The codon 72 polymorphism was genotyped by real time polymerase chain reaction (qPCR) with TaqMan SNP geno typing assay in 79 patients and 80 healthy control subjects.

No statistically significant difference was observed in distribution of genotype and allele frequencies. Heterozygous Arg/Pro (CG) was the most frequent genotype in both patients and controls. Homozygous Arg/Arg (GG) genotype frequency was higher in patients than controls, but not statistically significant ($p>0.05$). However, tumor location in the lower part of the esophagus was significantly higher in non-C carriers (GG, Arg/Arg) compared to C-carriers (CG/CC) ($p=0.01$). G-carriers were also more likely to have poorer survival compared to patients with CC genotype ($p=0.04$).

Our results suggest that the Codon 72 polymorphism was not associated with the EC in eastern Turkey. However, GG genotype (Arg/Arg) may have a role in tumor development at the lower location of the esophagus. Additionally, G carriers may exist the poorer survival compared to the non-G carriers (CC). Therefore, it is thought that individuals with CC genotype (Pro/Pro) may have better survival.

Keywords: Esophageal cancer, *p53*, codon 72, polymorphism

Introduction

Esophageal cancer (EC) is the eighth most common cancer and the sixth most common cause of cancer death globally (1-2). EC incidence is higher in China, Korea, Japan, India, South Africa, Singapore, Russia, Iran and Turkmenistan than in other countries. EC has low moderate risk in Turkey, but epidemiological studies have identified higher incidence in Eastern Anatolian Region of Turkey (2-4). EC is classified into histologically two main subtypes as esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) (5). Whilst ESCC is the primary type in the Eastern countries and developing countries, EAC incidence increases in the industrialized Western countries (6). EC is a complex disease influenced by multiple factors such as genetic factors, lifestyles and environmental factors (7,8). Since in some other populations single nucleotide polymorphisms (SNPs) have been shown to be associated with EC risk, it suggests that certain SNPs can increase or decrease susceptibility to EC and potentially be used as biomarkers of EC (9-11).

Polymorphisms of p53 are variable in EC progression, however this variable was not determined in all populations. *TP53* codon 72 polymorphism (rs1042522) is one of the most extensively studied SNPs, encoding proline (CCC, Pro72) or arginine (CGC, Arg72) in exon 4 codon 72. There are many population based studies that have shown the association between codon 72 polymorphism and EC except Turkey (12-15).

In addition to genetic factors, environmental factors such as smoking, alcohol consumption, low fruit, low vegetable intakes and hot food consumption have also pivotal role in the development of EC (7). These factors for ESCC

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Table 1. Frequency distribution of tumor and clinical parameters of patients with EC

Characteristics		N (%)
Cancer in family	Positive	42 (53)
	Negative	30 (38)
	Unknown	7 (9)
Histological type	ESCC	53 (67)
	EADC	16 (20)
	Unknown	10 (13)
Location	Upper	2 (3)
	Middle	38 (48)
	Lower	21 (27)
	Unknown	18 (23)
Hemoglobin (g/dl)	<11	17 (22)
	11-18	60 (75)
	>18	0 (0)
	unknown	2 (3)
Albumin (g/L)	<35	15 (19)
	35-52	43 (54)
	>52	0 (0)
	Unknown	21 (27)
CA-19-9 (u/ml)	≤37	46 (58)
	>37	8 (10)
	Unknown	25 (32)
CEA (ng/ml)	≤5	46 (58)
	>5	6 (8)
	unknown	27 (34)

CA-19-9 cancer antigen; CEA carcinoembryogenic antigen

show differences depending on the geographical regions. While smoking and alcohol consumption are the most risk factors in Western countries, low fruit and vegetable intake, hot food and beverage consumption, opium consumption, have been implicated in countries with high incidence of ESCC, such as China, Kenya and Iran (16-19). Van herbed cheese consumption, baking bread in the tandoor and heavy smoking are indicated as major risk factors for esophageal cancer in the Eastern region of Turkey (20). The major risk factors for EAC development are Barrett's esophagus, chronic gastroesophageal reflux, obesity and smoking (21).

In this present report, we investigated the association between p53 codon 72 polymorphism, clinical parameters, and demographic characteristics of EC patients and healthy control groups in eastern Turkey population

Materials and Methods

Seventy-nine subjects with EC and 80 healthy controls were enrolled in the study. The gender in the control group were well corresponded and age was relative matched with those in the EC group (Table 1). Ethics committee approval was received for this study from the SBU Van Education and Research Hospital (2017/7). The blood samples of cancer group were collected from patients who diagnosed with EC after endoscopic and pathological examinations in the hospital. The subjects in the control group were healthy individuals diagnosed with different cause without any tumor before blood collection. Written informed consent was obtained from all participants. A questionnaire was used to collect demographic information including age at the time of diagnosis and history of screening and clinical information such as tumor characteristics and type.

Blood samples were collected from EC patients and healthy controls in sterile ethylenediamine

Table 2. Demographic and clinical characteristics of patients with EC and the control group

Characteristics		Cases n(%)	Controls n(%)	P	OR (95%CI)
No.		79	80		
Age	Mean	58	53	-	
	<55	30 (38)	43 (54)		0.53 (0.28-0.99)
	≥55	49 (62)	37 (46)	0.07	
Gender	Male	35 (44)	37 (46)	0.93	1.08 (0.58-2.02)
	Female	44 (56)	43 (54)		
BMI (kg/m²)*	<25	45 (57)	14 (17)		
	25-30	27 (34)	53 (66)	<0.001*	
	>30	1 (1)	12 (10)		
	Unknown	6 (8)	1 (1)		
Reflux	Negative	31 (39)	65 (81)		
	Positive	43 (54)	15 (19)	<0.0001*	0.17 (0.08-0.34)
	Unknown	5 (6)	0 (0)		
Alcohol history	Negative	3 (4)	0 (0)		
	Positive	72 (91)	80 (80)	>0.05	
	Unknown	4 (5)	0 (0)		
Smoking history	Negative	43 (54)	59 (74)		
	Positive	32 (41)	21 (26)	0.04*	0.48 (0.24-0.94)
	Unknown	4 (5)	0 (0)		
Tandoor fumes, for only in women (patient n=44, control n=58)	Negative	7 (16)	44 (76)		
	Positive	34 (77)	14 (24)	<0.0001*	0.05 (0.02-0.12)
	Unknown	3 (7)	0 (0)		
Van Herbed cheese	Never	4 (5)	15 (19)		
	Every morning	67 (85)	65 (81)	0.02*	0.26 (0.08-0.82)
	Unknown	8 (10)	0 (0)		
Hot black tea consumed	Warm	16 (20)	21 (26)		
	Hot	59 (75)	58 (73)	0.57	0.75 (0.36-1.58)
	Unknown	4 (5)	1 (1)		
Type of nutrition	Usually red meat	54 (69)	60 (75)		
	Usually organic and olive oil	20 (25)	20 (25)	0.92	0.9 (0.44-1.85)
	Unknown	5 (6)	0 (0)		
Fruit, times/week	≤3	28 (35)	26 (33)		
	>6	47 (59)	53 (66)	0.68	1.21 (0.63-2.36)
	unknown	4 (5)	1 (1)		

BMI, body mass index. Significant level = $p< 0.05$ by Fisher Exact test (column value <5), Chi square test (column value >5) *indicating a significant difference

tetra acetic acid (EDTA) tubes for DNA extraction. DNA was extracted from peripheral blood using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. DNA samples were genotyped using TaqMan single-nucleotide polymorphism genotyping assay for rs1042522 (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's instructions. The results were analyzed on a StepOne Plus Real Time PCR system using the TaqMan assay program of StepOne software version 2.3 (Applied Biosystems).

Data analysis was carried out using GraphPad Prism-7 (San Diego, CA). Chi square analyses, followed by Fisher's exact test wherever required,

Table 3. Genotype and allele frequencies of codon 72 polymorphism in EC and control subjects

Genotype	Patients	Controls	p	OR(95%CI)
	n (%)	n (%)		
Arg/Arg	21 (27)	16 (20)		
Arg/Pro	36 (46)	34 (42)	0.26	
Pro/Pro	20 (25)	30 (38)		
Unknown	2 (3)	0 (0)		
G-carriers	57 (74)	50 (63)	0.17	1.71 (0.86-3.38)
Non- G	20 (26)	30 (37)		
Allel				
G-allel	78 (51)	66 (41)	0.12	1.46 (0.94-2.28)
C-allel	76 (49)	94 (59)		

Significant level = $p< 0.05$ by Fisher Exact test (column value <5), Chi square test (column value >5)

Table 4. Comparison of Clinical and Demographic Parameters With Codon 72 Genotypes According to C-Carriers vs. Non-C Carriers

Variable	C-carriers (CC, GC) (mean±SEM) (n)	Non-C (GG) (mean±SEM) (n)	p
BMI (kg/m2)	23.42 ± 0.39 (52)	24.47 ± 0.74 (19)	0.26
Hemoglobin (g/dl)	12.15 ± 0.31 (54)	12.18 ± 0.58 (21)	0.77
Albumin (g/l)	35.51 ± 1.44 (42)	37.92 ± 1.61 (13)	0.61
Globulin (g/l)	33.79 ± 1.65 (19)	30.86 ± 3.48 (7)	0.13
CA-19-9 (u/ml)	86.17 ± 46.31 (36)	91.14 ± 74.2 (16)	0.80
CEA (ng/ml)	44.86 ± 38.62 (35)	5.45 ± 3.66 (16)	0.10

CA-19-9 cancer antigen; CEA carcinoembryogenic antigen; Mann whitney U test (column value <30), Unpaired t test (column value >30)

were used to compare the frequencies of polymorphism between patients and healthy controls. The test was also applied for identifying the deviations from the Hardy–Weinberg proportion. Differences were assessed using unpaired t test and Mann-Whitney U test. The chi-square test was used to evaluate associations between categorical variables. Survival analysis was assessed using Kaplan-Meier analysis (the Gehan-Breslow-Wilcoxon test). Odds ratios with 95 % confidence intervals were given wherever appropriate. All tests were two-tailed, and results were considered significant when p value was <0.05.

Results

From Oct 2017 to Oct 2018, 159 blood samples, including 79 EC samples and 80 healthy controls, were collected from the Dursun Odabası Medical Center of Van Yuzuncu Yıl University. Length of follow-up for survival was up to 3 years and was based on hospital records. According to pathology report, we separated our patient's samples into two subgroups: ESCC and EAC. Most of the patients were diagnosed as ESCC (67%). The histological and demographic characteristics, such as histological type, age at diagnosis, smoking and hot tea consumption were determined as shown in Table 1 and 2. The mean age of patients with EC were 58. Forty eight cases (62%) were 55 years or older; forty two cases (53%) had cancer history in their family; 43 (54%) were positive for reflux; 38 (48%) had middle-tumor localization (Table 1 and 2). The age, BMI, reflux, smoking status, tandoor fumes and Van herbed cheese (some cheese every morning) differences between the EC group and control group were statistically significant ($p<0.05$, Table 2). There was not significant difference in gender, alcohol, hot black tea consumed, nutrition and fruit between cases and controls ($p>0.05$, Table 2). The gender in the patient group was well corresponded in the control group (Table 2). The most of the cancer group were all just diagnosed patients without any tumor-connected treatment before blood collection. However, tumor location in the lower part of the esophagus was significantly higher in non-C carriers (GG,

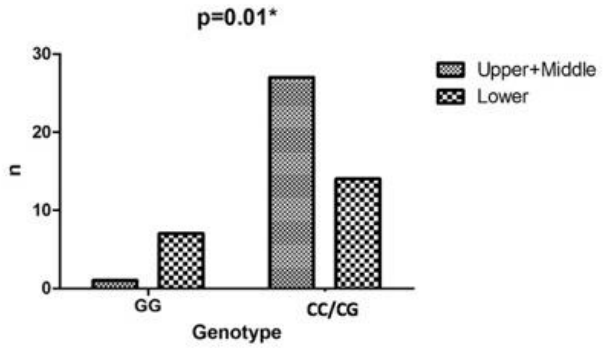

Fig. 1. Comparison of tumor locations with TP53 gene polymorphism in patients

Arg/Arg) compared with C-carriers (CG/CC) in patients (n=61, *p=0.01*, Fig. 1).

Blood samples from 79 subjects with EC and 80 healthy controls were analyzed to determine the frequency of *TP53* gene codon 72 polymorphism. The allele and genotype frequencies in patients and controls are listed in Table 3. We detected G allele frequencies as 51% and 41% for the patient group and the control group, respectively and C allele frequencies as 49% and 59% for the patient group and the control group, respectively. Codon 72 polymorphism genotype frequencies in the patients was determined as 21 (27%) with Arg/Arg, 36 (46%) with Arg/Pro and 20 (25%) with Pro/Pro, while the distributions of Arg/Arg, Arg/Pro and Pro/Pro genotypes in controls were 16 (20%), 34 (42%) and 30 (38%), respectively. Additionally, G carriers of this polymorphism were higher in EC patients than controls, but differences in the allele and genotype frequencies were not statistically significant (*p>0.05*). These results indicate that TP53 codon 72 polymorphism was not associated with EC risk.

The codon 72 polymorphism of *TP53* gene was not directly associated with BMI, hemoglobin, albumin, globulin and tumor antigens of EC patients (*p>0.05*, table 4).

Kaplan-Meier survival charts were drawn and median life span was determined. Overall survival is shown in Figure 2. Gehan test was used to compare the life span of the two groups (G-carriers and non-G carriers). Median overall survival was 65 age for patients with G-carriers (Arg/Arg, Pro/Arg) and 72 age for patients with non-G carriers (Pro/Pro). Kaplan-Meier analysis using the Gehan-Breslow-Wilcoxon test showed CC genotype (non-G carriers) was positively associated with overall survival (OR= 1.11, 95% CI= 0.65-1.57, p=0.04).

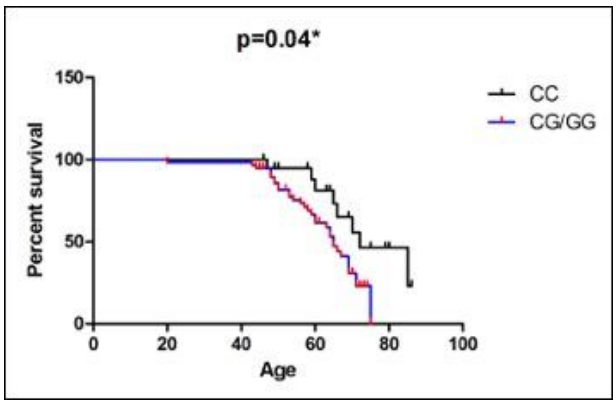

Fig. 2. Overall survival in patients with non-G carriers versus G-carriers (Kaplan-Meier survival plots compared by Gehan-Breslow-Wilcoxon test)

Discussion

TP53 codon 72 polymorphism is the most common variation that is responsible for cancer susceptibility in many population. Although the relationship between this polymorphism and EC development has been shown in previous studies in different populations, its effect on Eastern Anatolia Region of Turkey has not been clarified. There are also contradictory results about the effects of TP53 codon 72 in the literature depending on the detection type, histological type, sample numbers and populations. Therefore, understanding the effects of TP53 codon 72 with population-based studies is important. In our study, we could not find a significant relationship between codon 72 polymorphism and EC. Nevertheless, a significant association was observed between tumor location and the polymorphism with non-C allele carriers (GG genotype). Our result showed that non-C allele carriers were more susceptible to the lower tumor location compared to C-allele carriers. On the other hand; non-G carriers (CC genotype) compared to G-carriers was positively associated with overall survival.

EC initiating molecular mechanisms are still not fully understood. The TP53 gene has been defined as an important tumor suppressor due to its high mutation prevalence in many cancers (22). The genotype frequencies of codon 72 polymorphism in the population based studies of Das et al. (13] and Hong et al. (23) were Arg/Arg 20 %, Arg/Pro 43 % and Pro/Pro 37 %, Arg/Arg 26.3 %, Arg/Pro 44.9 % and Pro/Pro 28.9 % compared to Arg/Arg 27 %, Arg/Pro 46 % and Pro/Pro 25 % in patients with EC in our study population. Although genotype frequencies are similar, no significant difference was found between patients and controls in our study. Two polymorphic variants (Arg72 and Pro72) of *TP53* may affect

cancer risk or treatment, because the two variants are functionally different (24,25). Therefore, some studies in different populations have demonstrated the association of the Arg72 variant with EC risk, while others with the Pro72 variant. A meta-analysis study reported that Arg72 carriers are significantly associated with reduced EC risk in especially Asian populations (25). In previous studies, some of the researchers suggested that *TP53* codon72 polymorphism (Arg allele or Pro allele) was significantly associated with subtypes risk of EC (23, 26-34). Other some researchers did not detect any significant association between EC and *TP53* codon 72 polymorphism. These differences may due to the heterogeneity of tumor subtypes and different ethnicities (35-38). The results of our study were consistent with the studies, in which codon 72 polymorphism was identified to be not significant association in patients with EC in comparison with the control group. However, the genotype frequencies in our population were not consistent with the results of these studies. Although the Arg72 carriers were found to be high (51%) in our study, it was not statistically significant. The main reason of the contradictory results between our study and previous studies may be the differences between the populations.

Generally, esophageal cancer is known to be more common in men, but it is observed that the gender distribution is different in Turkey. The results of our study showed that EC is more common in women, similar to the study of Celik et al. and the same study showed that several demographic parameters were significantly associated with EC risk (20). Regarding the Van herbed cheese, smoking, hot tea and tandoor fume our results were also statistically consistent with the other study conducted in Turkey. However, the codon 72 polymorphism in patients with EC was not significantly associated with the clinical and the demographic parameters except tumor location and overall survival. A relationship between codon 72 polymorphism and tumor location in gastric cancer has been reported (39), but such an outcome has not yet been reported in the EC. Our results suggest that tumor location in the GG genotype group is mostly in the lower part of the esophagus, while tumors of individuals with other genotypes mostly located in the upper and middle part of the esophagus (p=0.01). Previous EC studies reported that the p53 Pro/Pro genotype was associated with shorter survival (30,40). In contrast to these studies, we observed that the same genotype (Pro/Pro) was associated with longer survival in EC patients in our population (p=0.04).

This is the first study that has examined the association between TP53 codon 72 polymorphism and EC in Eastern part of Turkey. In this study, no association was found between TP53 codon 72 polymorphism and EC risk in Turkey. However, the polymorphism was significantly associated with tumor location and overall survival in patients. Our findings suggest that Arg/Arg genotype of the TP53 gene may increase the risk of developing the tumor in lower location of esophagus and patients with CC genotype (Pro/Pro) may have better survival.

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