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Investigation of the relationship between *β2-adrenergic receptor (β2-AR)* polymorphism and gastric cancer

β_2-Adrenerjik reseptör (β_2-ar) polimorfizmi ile mide kanseri arasındaki ilişkinin incelenmesi

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SUMMARY

Objective: Gastric cancer is a multifunctional disease. Emotional stress, physiological and neuroendocrine changes in cancer patients can lead to the activation of the hypothalamic-pituitary-adrenal axis and the release of hormone dependent hormones such as catecholamine. In particular, it has been reported that catecholamine induction directly affects the biological behavior of tumor cells via *β2-Adrenergic receptor (β2-AR)* mediated signaling. In this study, it was aimed to investigate polymorphism of *β2-AR* gene (rs1042717) in gastric cancer patients.

Method: Polymorphism in the *β2-AR* gene (rs1042717) was determined by Real-Time PCR method in 60 gastric cancer patients and 50 healthy controls. The results were evaluated using logistic regression and Chi-square ($\chi 2$) test.

Results: The comparison of gastric cancer patients and controls determined a statistically significant relationship for alcoholic drink consumption ($p<0,05$). There was a statistically significant relationship between *β2-AR* (rs1042717) polymorphism and stomach cancer ($p<0.05$). There was a statistically significant relationship between mutant (AA) genotype with wild type (GG) and heterozygous (AG) polymorphic genotypes when evaluated in *β2-AR* polymorphism gastric cancer patients and control group ($\chi 2$: 19,38, $p: 0.001$). Similarly, there was a statistically significant correlation between heterozygous (AG) with wild type (GG) and mutant (AA) polymorphic genotypes in gastric cancer ($\chi 2$: 14, 27, $p: 0,001$).

Conclusions: In this study, it was found that the AG genotype is predominant in gastric cancer patients and controls, and that the AA genotype has a protective effect against gastric cancer.

Keywords: *β2-adrenergic receptor*, gastric cancer, polymorphism, rs1042717

ÖZET

Amaç: Mide kanseri multi-fonksiyonel bir hastalıktır. Kanser hastalarında duygusal stres, fizyolojik ve nöroendokrin değişiklikler, hipotalamik-pitüiter-adrenal eksenin aktivasyonuna ve katekolamin gibi strese bağlı hormonların serbest bırakılmasına neden olabilmektedir. Özellikle katekolaminin uyarılması ile *β_2-Adrenerjik reseptör (β_2-AR)* aracılı sinyal ileti yoluyla tümör hücrelerinin biyolojik davranışlarını doğrudan etkilediği bildirilmiştir. Bu çalışmada, mide kanserli hastalarda *β_2-AR* gen (rs1042717) polimorfizminin araştırılması amaçlanmıştır.

Yöntem: *β_2-AR* genindeki polimorfizim (rs1042717) 60 mide kanseri hastası ve 50 sağlıklı kontrol de Real-Time PCR metoduyla belirlendi. Elde edilen sonuçlar, lojistik regresyon ve Khi-kare ($\chi 2$) testi kullanılarak değerlendirildi.

Bulgular: Mide kanseri hastaları ve kontroller alkol kullanma açısından değerlendirildiğinde, istatistiksel olarak anlamlı bir ilişki belirlendi ($p<0,05$). *β2-AR* (rs1042717) polimorfizmi ile mide kanseri arasında istatistiksel olarak anlamlı bir ilişki bulundu ($p<0,05$). *β2-AR* (rs1042717) polimorfizmi mide kanseri hastaları ve kontrol grubunda değerlendirildiğinde, mutant (AA) genotipi ile yabanıl tip (GG) ve heterozigot (AG) polimorfik genotipleri arasında istatistiksel olarak anlamlı bir ilişki olduğu görüldü (χ^2: 19,38, p: 0,001). Benzer şekilde heterozigot (AG) ile yabanıl tip (GG) ve mutant (AA) polimorfik genotipleri kıyaslandığında ise mide kanserinde istatistiksel olarak anlamlı bir ilişki olduğu saptandı (χ^2: 14,27, p:0,001).

Sonuç: Bu çalışmada, mide kanseri hastaları ve kontrollerde en çok AG genotipinin hakim olduğu ve ayrıca, *AA* genotipinin mide kanserine karşı koruyucu bir etkiye sahip olduğu görülmüştür.

Anahtar sözcükler: β_2-adrenerjik reseptör, mide kanseri, polimorfizm, rs1042717

INTRODUCTION

Gastric cancer (GC) is the fourth most common malignancy and the second primary cause of cancer mortality, resulting in >800,000 mortalities worldwide annually (1,2). Although the incidence and mortality of gastric cancer have decreased markedly in most areas of the world over the past several decades, control of gastric cancer at the advanced stage remains difficult (3). In addition to environmental factors, genetic variants are significant in gastric cancer (4).

Recently, numerous studies have evaluated the interaction between gastric cancer and *β-Adrenergic receptor (β-AR)* (5). The *β-AR* is member of the 7-transmembrane receptor family and composed of 413 amino acid residues. *β-ARs* are divided into β_1, β_2, and β_3 subtypes (6). They belong to the superfamily of G-protein-coupled receptors (GPCR) and are characterized by forming a pocket containing binding sites for agonists and competitive antagonists (7). The *β_2-AR* is the most common adrenergic receptor in carcinogenic processes. The gene encoding this receptor, *β_2-AR*, as already stated, is a member of GPCR superfamily. The receptor is directly associated with one of the ultimate effectors class C L-type calcium channel (8). The gene encoding this G-protein-coupled *β_2-AR* is located on the chromosome 5q31-33 and is highly polymorphic. To date, nine distinct polymorphisms in the *β_2-AR* gene have been reported (9). Several of the polymorphisms of this gene have been associated with various diseases, including asthma, obesity (6,8,10,11), and cancer (12).

In this study, we aimed to investigate polymorphism of *β2-AR* gene (rs1042717) in gastric cancer patients.

MATERIAL AND METHODS

Study population

The study was approved by the local ethic committee and informed consent was obtained from all patients. All subjects agreed to participate in the study and completed a short questionnaire, which included questions about their occupation, tobacco use, alcohol consumption and family history of cancer. In the present study, a total of 110 individuals (60 GC and 50 control subjects) were investigated. Blood samples were collected from the 60 patients who were diagnosed with GC patients at the Department of General Surgery, Cumhuriyet University, Faculty of Medicine (Sivas, Turkey). The diagnosis of GC was histologically confirmed and the tumor types were classified according to WHO guidelines, (https://www.iarc.fr/en/publications/pdfs-online/pat-gen/bb2/bb2-chap3.pdf) No age and sex restrictions were applied for the selection of healthy volunteers, who were free of any chronic diseases, lived in the same geographic area, and had no history of cancer. All cases and controls were born and lived in Turkey.

DNA isolation

Peripheral blood samples (2ml) were obtained and collected into citrate-containing tubes from all subjects. The DNA was extracted from whole blood using the salting out procedure as soon as the samples reached the laboratory (13).

β2-AR genotyping

The *β2-AR* rs1042717 (G/A) polymorphism was determined by using SNPsig Real-time Genotyping kit (Jena Bioscience) with dual

labeled fluorescent probes (FAM-VIC). Real-time PCR condition was initial denaturation (95°C, 2 min), 15 cycles of denaturation (95°C, 2 min), and first extension (60°C, 60 s) followed by 40 cycles of second denaturation (95° C, 15s) and second extension steps (68°C, 60 s). Fluorogenic data were collected through the green (FAM) and yellow (VIC) channels at the end of each cycle of second extension. Following SNP analysis, the amplification marked with FAM showed the G allele, the wild type allele. The amplification marked with VIC showed the A allele, that is, the mutated allele. We genotyped amplified PCR product for *β_2-AR* rs1042717 (G/A) polymorphism by allelic discrimination assay according to the manufacturer's instruction (Figure 1). In addition, heterozygous (AG), mutant (AA) and wild type (GG) genotype distributions of the disease are shown in figure 2.

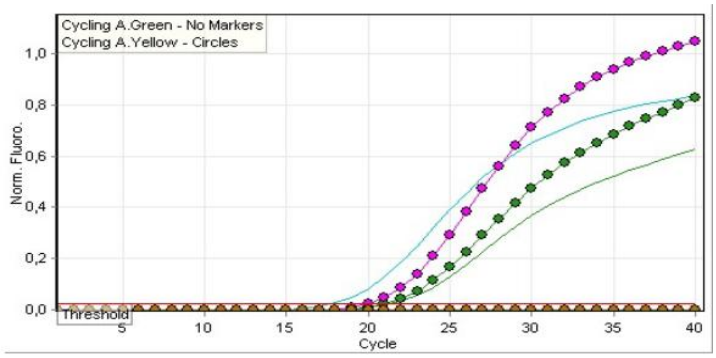

Figure 1. The genetic variant of *β_2-AR* polymorphism (rs1042717) was detected by real-time PCR and allele discrimination

No	Color	Name	Genotype	Green channel	Yellow channel
1		Case 1	Heterozygous (AG)	There is reaction	There is reaction
2		Case 2	Mutant (AA)	There is no reaction	There is reaction
3		Case 3	Wild Type(GG)	There is reaction	There is no reaction
4		Control		There is no reaction	There is no reaction

Figure 2. Genotype distributions of rs1042717 polymorphism

Statistical analysis

All statistical analyses were performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA). Genotype-associated odds ratios (ORs), their corresponding 95% confidence intervals (CIs), and associated P-values were estimated via unconditional logistic regression. Differences in the distributions of demographic characteristics between the cases and control subjects were evaluated using Student's t-test. $\chi 2$ or Fischer's exact test (two-sided) were used to compare the sex distribution, to test the association between the genotypes and alleles in relation to the controls, and to test for deviation of the genotype distribution from Hardy-Weinberg equilibrium. Pearson's $\chi 2$ test was used to determine whether there were any significant differences in allele and genotype frequencies between patients and control subjects. Logistic regression procedures were performed to assess the interaction between age, sex and all genotypes.

RESULTS

In this study, the association between the *β_2-AR* gene polymorphism rs1042717 (G/A) and GC was investigated in a Turkish population. The polymorphism in the *β_2-AR* gene, rs1042717 has been determined in 60 patients with GC and in 50 healthy control subjects using the RT-PCR method. Table 1 shows the demographic characteristics of the study population. The distribution of sex, age, and ethnic origin of the study population was similar. The mean age of the GC patients and control subjects were 59.91±10.42 years (males, 60,12±9,81; females,

59,00±13,30) and 61.18±9.30 years (males, 60,18±9,48; females, 67,28±5,12), respectively. The percentage of males and females in the cases were 81.7 and 18.3%, respectively (Table I). No statistically significant differences were identified between the cases and control subjects by age and sex (*p>0.05*). There were no statistically significant associations between the cases and control subjects for smoking history (OR=0.93; 95% CI, 0.44-1.98; *p*=0,862; Table II). But, there was statistically significant differences were identified regarding alcohol consumption among the cases and control subjects (OR=19,37; 95% CI, 2,47-151,68; *p*=0.001) (Table II). Comparison between GC cases and control subjects indicated no statistically significant differences in family history of cancer (OR=0,39; 95% CI, 0,13-1,15; *p=0.083;* Table II). There was a statistically significant relationship between β_2-AR (rs1042717) polymorphism and gastric cancer (*p<0.05*). When β_2-AR (rs1042717) polymorphism is assessed in gastric cancer patients and control group; There was a statistically significant relationship between AA genotype and GG + GA genotypes (χ2: 19,38, *p: 0,001*). Similarly, when the heterozygous AG and GG + AA genotypes were compared, a statistically significant correlation was found in gastric cancer (χ^2: 6,35, *p: 0.012*). When AA genotype distributions were analyzed, it was found that there were 4 (6.7%) and 21 (42.0%) gastric cancer patients and a statistically significant correlation was found between them (χ2: 14,27, *p: 0,001*) (Table III).

Table 1. Demographic information on gastric cancer patients and controls

	Control, n (%)	**Gastric Cancer, n(%)**
Sample Size	50	60
Sex		
Males	43 (86,0)	49 (81,7)
Females	7 (14,0)	11 (18,3)
Age (years)		
Range	46-85	40-85
Means ± SD		
Males	60,18±9,48	60,12±9,81
Females	67,28±5,12	59,00±13,30
Smoking History		
Smoker	25 (58,1)	29 (48,3)
Males	25 (58,1)	27 (47,9)
Females	0 (0,0)	2 (18,2)
Alcoholic Drink Consumption		
Drinker	1 (2,3)	17 (28,3)
Males	1 (2,3)	15 (30,6)
Females	0 (0,0)	2 (18,2)
Family History of Cancer	11 (22,0)	6 (10,0)

Table 2. Distribution of selected variables in gastric cancer cases and control subjects

Variable	Gastric cancer (n=60)	Control (n=50)	*P*-value	Odds ratio
	N (%)	N(%)		
Smoking Status				
No	31(51,7)	25(50,0)		
Yes	29(48,3)	25(50,0)	0,862	0,93 (0,44-1,98)
Drinking Status				
No	43(7.0)	49(98,0)		
Yes	17(28,3)	1(2,0)	**0,001**	19,37 (2,47-151,68)
Family History of Cancer				
No	54(90,0)	39(78,0)		
Yes	6 (10,0)	11(22,0)	0,083	0,39 (0,13-1,15)

Table 3. Stratification analyses between *β2-Adrenergic receptor (β2-AR)* genotypes and gastric cancer risk.

	Control (n=50)(%)	Gastric Cancer (n=60)(%)	X^2	*p*-value	Crude OR (95% CI)	Adjusted OR (95% CI)
Rs1042717 (G /A)						
G	33(%32,6)	68(%67,4)	Ref			
A	67(%56,3)	52(%43,7)	12,36	**0,001**	0,38(0,21-0,68)	-
Codominant						
GG	4(%8,0)	12(%20,0)	Ref			
GA	25(%50,0)	44(%73,3)	0,72	0,393	0,58(0,17-2,01)	0,59(0,18-2,02)
AA	21(%42,0)	4(%6,7)	14,27	**0,001**	0,06(0,01-0,30)	0,25(0,11-0,54)
Dominant						
GG	4(%8,0)	12(%20,0)	Ref	-	-	
GA+AA	46(%92,0)	48(%80,0)	3,16	0,075	0,34(0,10-1,15)	0,35(0,11-1,16)
Recessive						
GG+GA	29(%58,0)	56(%93,3)	Ref	-	-	
AA	21(%42,0)	4(%6,7)	19,38	**0,001**	0,09(0,03-0,31)	0,31(0,17-0,56)
Overdominant						
GG+AA	25(%50,0)	16(%26,7)	Ref	-	-	
GA	25(%50,0)	44(%73,3)	6,35	**0,012**	2,75(1,24-6,10)	2,75(1,24-6,10)

DISCUSSION

Gastric cancer is the result of a multifactorial complex process, for which a multistep modelof carcinogenesis is currently accepted. Additionally to the infection with Helicobacter pylori, that plays a major role, environmental factors as well as genetic susceptibility factors are significant players at different stages in the gastric cancer process. The differences in population origin, demographic structure, socioeconomic development, and the impact of globalization lifestyles experienced in Latin America in the last decades, all together offer opportunities for studying in this context the influence of genetic polymorphisms in the susceptibility to gastric cancer (14). The current understanding of host genetic polymorphisms and gastric cancer susceptibility is based largely on studies in Asians and Caucasians (from Europe and North America) populations. Moreover, ethnicity has been proposed as a factor modifying the risk of cancer (15).

The human *β_2-AR* gene on chromosome 5 is abundantly expressed in cardiac myocytes and vascular smooth muscle cells. It has three major missense polymorphisms, which encode amino acids 16, 27, and 164 of the extracellular N-terminus of the β2-adrenoceptor (16,17). Each of these polymorphisms represents a single base pair substitution. Four of these polymorphisms result

in amino acid substitutions at amino acids 16, 27, 34, and 164, whereas the other five are silent mutations located at amino acids 84, 175, 351, 366, and 413 (18). Polymorphisms of β-adrenergic receptors (β1-receptor and β2-receptor) have been reported that alter the function of the receptor (19-22). Our study aimed to determine the allelic and genotypic frequency distribution of polymorphisms of rs1042717 in the *β2-AR* gene and to compare findings with other ethnic groups. The frequencies of *G* and *A* alleles were 32,6% and 56,3% respectively, and the frequencies of the *GG*, *GA* and *AA* genotypes were 8,0%, 50,0%, and 42,0% respectively in the study population. The distribution of three genotypes fitted the Hardy–Weinberg equation ($\chi 2 = 0.854$, $p = 0.330$) (Table 2). In the current study, of 60 GC cases and 50 control subjects, the crude OR of GC patients for alcohol drinking status of cancer was 19,37, indicating an association between GC incidence and alcohol drinking status (p=0,001; Table II). To the best of our knowledge, GC and alcohol drinking status association has not been proposed in previous studies. In addition, GC patients and control subjects were evaluated by logistic regression analysis for smoking habits. No statistically significant difference was identified between GC patients and smoking habits in the Turkish population investigated in the current study (p=0,083; Table II). The *β2-AR* rs1042717 polymorphism in GC patients has been investigated in various different regions of the world; however, to the best of our knowledge, no research has been performed in Turkey. This study was the first study to evaluate the polymorphic variants of *β2-AR* rs1042717 polymorphism and risk of GC. In the present study, a statistically significant difference was identified in the AA and AG genotypes distribution between GC patients and control subjects in the investigated Turkish population (OR=0,09; 95% CI, 0,03-0,31; p=0,001; Table III). In this study, it was found that the AG genotype is predominant in gastric cancer patients and controls, and that the AA genotype has a protective effect against gastric cancer. In the human population, the *β2-AR* shows significant genetic variability in its structure. These differences in structure result in differences in the way the receptor functions or are regulated. Early studies have suggested that these different polymorphic forms of the receptor may influence the severity of GC.

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