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Research Article

INVESTIGATION OF THE ASSOCIATION BETWEEN pre-miR-423 AND pre-miR-608 GENE POLYMORPHISMS AND COLORECTAL CANCER IN A TURKISH POPULATION

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ABSTRACT. This study investigated the potential role of pre-miR-423 and pre-miR-608 gene polymorphisms in colorectal cancer (CRC) within the Turkish population. The study involved 100 healthy individuals with an average age of 52.6 and 100 individuals diagnosed with colorectal cancer with an average age of 56.89, comprising the patient group. Molecular analysis of blood samples from both groups was conducted using the Real-Time PCR method. The relationship between genotypes and alleles concerning the disease was examined through chi-square trend tests. The results revealed a statistically significant difference in genotype distribution for the pre-miR-423 A/C gene polymorphism between CRC patients and the control group (p=0.039). The CC genotype was more frequent in patients than in the control group, and it was found that the CC genotype increased the risk of colorectal cancer by 1.9% (OR=1.935, 95% CI: 1.031-3.632, p=0.040). However, for the pre-miR-608 C/G gene polymorphism, there was no statistically significant difference in genotype distribution between CRC patients and the control group (p=0.395). This study is the first to explore the potential association between pre-miR-423 and pre-miR-608 SNPs and CRC in the Turkish population. Further large-scale studies from different centers are necessary to validate these findings.

Keywords: *Colorectal cancer, pre-miR423, pre-miR608, polymorphism*

INTRODUCTION

The incidence of colorectal cancer is high in Western Europe, North America, and Australia, which are developed countries [1]. In recent years, it has been increasing rapidly in countries with a low risk of colorectal cancer (CRC), such as East Asia and Eastern Europe [2]. China, in particular, has seen an increase in CRC incidence and mortality [3]. CRC ranks 7th among Turkey's 10 most common cancers, with approximately 5000 new cases and 3200 CRC-related deaths yearly [4].

Two molecular pathways, including 'chromosomal instability' and 'microsatellite instability,' are involved in the development of colorectal cancer [5]. In elucidating the molecular pathogenesis of the disease, miRNA molecules, which do not encode proteins and negatively regulate the expression of genes, have been defined.

Due to the relatively recent recognition of the prevalence and significance of miRNAs in humans, only a few studies demonstrate human polymorphisms associated with miRNAs and their effects [6]. In these studies, miRNA target sites in the genome have been estimated using different numerical approaches and only a small proportion of these estimated target sites have been experimentally confirmed. Human miRNAs are first transcribed as precursor miRNA (pre-miRNA) and pre-miRNA then undergoes several processes to form mature miRNAs [7]. miRNAs mature in at least 2 stages. First, they are transcribed as pri-miRNA consisting of several hundred nucleotide sequences. They then undergo the so-called pre-miRNA structural change, which occurs in a sequence of about 70 nucleotides, forming a coiled structure [8]. The conservation of pre-miRNA sequences in the genome demonstrates the importance of this

biological system. Pre-miRNA and mature miRNA polymorphism modify biological events by influencing miRNA-activated processes and target selection. miRNA expression and serum miRNA concentrations differ markedly between tumors [8]. MiRNAs are involved in the regulation of many oncogenic and tumor suppressor pathways involved in CRC pathogenesis [9]. Proteins in signaling pathways (such as Wnt/beta-catenin, phosphatidylinositol-3 kinase pathway, KRAS, p53) that are involved in CRC development are affected by miRNA regulation [9]. The Wnt/beta-catenin signaling pathway is essential in early CRC development. Mutation of the APC gene, the most critical event in the initiation of colorectal carcinogenesis, is detected in 60% of colorectal adenomas and carcinomas and leads to the activity of the Wnt/beta-catenin pathway [10].

SNPs in miRNA genes affect the formation and expression of mature miRNA and may also be responsible for cancer development and clinical manifestations. Abnormal expression of miRNAs has been observed to function as biomarkers and functional regulators in tumourigenesis and prognosis of CRC [11]. Similarly, many recent studies have shown that SNPs in pre-miRNAs and miRNA binding sites are associated with CRC risk and disease progression. Furthermore, a study that screened 400 human pre-miRNAs and their flanking genes showed that SNPs in pre-miRNA genes were less numerous compared to their flanking region. This suggests pre-miRNAs may be extremely well-conserved and functional [12]. Studies have found that colorectal cancer is associated with the pre-miR-423 rs6505162 and pre-miR-608 rs4919510 SNP regions.

pre-miR-423 is an RNA fragment located on chromosome 17q11.2 in the human genome. This fragment is located in the first intron of the NSRP1 (Nuclear Speckle Splicing Regulatory Protein) gene and regulates alternative splicing of mRNAs. Furthermore, the expression of mature miRNAs derived from miR423 is altered in many cancer types [13, 14].

miR608, which is thought to be a tumor suppressor miRNA, is located on chromosome 10q24, and its function is not fully understood [15]. 22 bp of the 25 bp sequence make up the mature miRNA. miR608 contains a single SNP region, rs4919510 [15]. The C-G polymorphism is common in many populations. Heterozygous loss in its prelocus has been reported to cause many human cancers, including colorectal, prostate, pancreatic, and brain. In the studies, rs4919510 SNP targets in the miR-608 gene region are considered BCL-XL, SEPT9, and CDK [16]. The alteration of miR-608 target sites may be directly related to CRC cell development. In a study conducted with patients diagnosed with CRC in the Chinese population, 10 different pre-miRNA SNP regions were screened [17]. In another study, they concluded that the pre-miR-608 rs4919510 SNP region was not a risk factor for developing colorectal cancer in African-American patients diagnosed with colorectal cancer [18].

In this study, we examined the potential association between pre-miR-423 (rs6505162) and pre-miR-608 (rs4919510) gene polymorphisms and the development of colorectal cancer in patients diagnosed with this condition.

MATERIALS AND METHODS

The study group included 100 patients aged between 18 and 65 diagnosed with colorectal cancer at Mersin University Faculty of Medicine Hospital, Department of General Surgery. The control group comprised 100 healthy individuals without any known medical conditions. The control group was selected to be compatible with the patient group regarding number and gender distribution. The study obtained approval from the Ethics Committee of Mersin University. All individuals in the patient and control groups were requested to complete and sign the informed consent form. This form was designed in compliance with the Ethics Committee's guidelines, signifying their agreement to participate in the study.

DNA Isolation and Genotyping

For DNA isolation from the patient and control groups, 6-7 ml of venous blood was placed in 15 ml centrifuge tubes containing 1 ml of 2% ethylenediethyltetetraacetic acid (EDTA). Blood was stored at +4ºC until DNA isolation and extraction using a High Pure PCR Template Preparation Kit (Roche, Switzerland). Amplification, genotyping, and analysis of the gene regions of pre-miR403 and pre-miR-608 polymorphisms were performed using a Bioneer ExiCycler96 model real-time PCR device and ExiData V3.54.8 software (Bioneer, USA).

Real-Time PCR Preparation of The Reaction Mix

The real-time PCR reaction mix was dispensed into 96-well transparent polypropylene plate wells. A negative control well without sample DNA was used in each reaction plate to determine if the prepared mixture was contaminated. After distributing the reaction mix into the wells, the wells were covered with real-time PCR film. The plate was placed on the heating block and the reaction was started by opening the pre-prepared template file for the execution method. After approximately two and a half hours of experiment, genotype determination was performed. For each polymorphism, 43 µl PCR grade water, 5 µl sample DNA (approx. 50 ng/uL), 1 µl primer/probe set (10 pmol/uL), 1 µl qPCR PreMix (Cont: Taq DNA Polymerase, 10X reaction buffer, dye (xylene cyanol), stabilizer (sorbitol), Tween 20, dNTP. (Bioneer AccuPower GreenStar qPCR PreMix, USA) Real-time PCR conditions were used. The characteristics of the primers and PCR conditions are detailed in Tables 1 and 2.

Table 1. *Primers used and their sequences*

Primers	Sequences	Length
pre-miR-423 (A/C)		
Forward Primer:	5’-ACGTTGGATGTTTTCCAAAAGCTCGGTCTG -3’	30
Reverse Primer:	5’-ACGTTGGATGCAAGCGGGGAGAAACTCAAG -3’	30
Pre-miR-608 (C/G)		
Forward Primer:	5’- ACGTTGGATGAAGATCCACTGGGCCAAGGT -3’	30
Reverse Primer:	5'- ACGTTGGATGATGGAAGCTCTTGGAGATGC -3'	30

Table 2. *Real-Time PCR conditions, materials used, and quantities*

Q-PCR Reaction Mixture		**Reaction Volume**
Example	Negative Control	5ml
	Sample DNA	5ml
Forward Primer		1ml
Revers Primer		1ml
PCR Grade Water		43ml
Total		50ml
Line	Temperature	Operating Time
Line1: First Denaturation	95	5 minutes
Line2: Denaturation	95	5 seconds
Line3: Extension	60	40 seconds
Scan	Target paint/filter: FAM/TAMRA	

Statistical Analysis

Power analysis was used to determine the sample size of patients and control group for pre-miR423 and pre-miR-608 gene polymorphisms between colorectal cancer patients and the control group.

Power analysis was used to determine the sample size of the patients and control group for pre-miR423 and pre-miR-608 gene polymorphisms between colorectal cancer patients and the control group. Independent samples t-test was used to test whether there was a difference between the groups regarding age. Associations of genotypes and alleles with the disease were

analyzed using chi-squared or likelihood ratio tests. "Hardy-Weinberg equilibrium of the patient and control groups with respect to genotypes was checked. Descriptive statistics were reported as mean ± standard deviation for continuous variables and as frequencies and percentages for categorical variables. Statistical analyses were performed using the SPSS v.11.5 software package, and results were considered significant if $p<0.05$ in statistical analyses.

RESULTS

Distribution of Allele and Genotype Ratios of the pre-miR-423 (A/C) rs6505162 Gene Polymorphism between Control Group and Patients and its Association with CRC

Statistical analysis revealed that the patient and control groups were not in Hardy-Weinberg equilibrium with respect to pre-miR-423 gene polymorphism ($p<0.001$).

Table 3. *Hardy Weinberg Equilibrium Control (pre-miR-423)*

	Genotypes	Observed value	Expected value	P Value
Control	**AA**	48 (%48)	34,22	<0,001
	CA	21 (%21)	48,56	
	CC	31 (%31)	17,22	
Patient	**AA**	36 (%36)	20,70	<0,001
	CA	19 (%19)	49,60	
	CC	45 (%45)	29,70	

As there was an interaction between genotypes in the patient and control groups, calculations were made using the chi-squared test for trend.

When the genotype frequencies of the pre-miR-423 A/C (rs6505162) polymorphism were analyzed in CRC patients and the control group,

The frequency of AA genotype was 36% in CRC patients and 48% in the control group;

The frequency of CA genotype was 19% in CRC patients and 21% in the control group;

The frequency of CC genotype was 45% in CRC patients and 31% in the control group.

When the results were analyzed proportionally in terms of allele frequency,

In the control group

An allele was found to be 58.5% and C allele was found to be 41.5%.

In patients with CRC

An allele was found to be 45.5% and C allele was found to be 54.5% ($p=0.012$) (Table 3).

A statistically significant difference in genotype distribution for pre-miR-423 was found between CRC patients and controls ($p=0.039$). The proportion of patients with CC genotype was 1.935 times higher than those with CC genotype in the control group (OR=1.935, (1.031-3.632), $p=0.040$). According to this result, the risk of CRC was increased in individuals with the CC genotype. A statistically significant difference was observed in the allele distribution between the patient and control groups ($p=0.012$).

Table 4. *Distribution of pre-miR-423 (rs6505162) Polymorphism Genotype and Allele Ratios between Patient and Control Group (N: number of alleles and genotypes)*

Genotype	Control N (%)	Patient N (%)	P Value	OR	Confidence Interval (95%)	P Value
AA	48 (48%)	36 (36%)	0,039	Reference		
CA	21(21%)	19 (19%)		1,206	0,566-2,570	0,627
CC	31 (31%)	44 (45%)		1,935	1,031-3,632	0,040
Allele						
A	117 (58,5%)	91 (45,5%)	0,012	0,592	0,399-0,879	0,009
C	83 (41,5%)	109 (54,5%)				

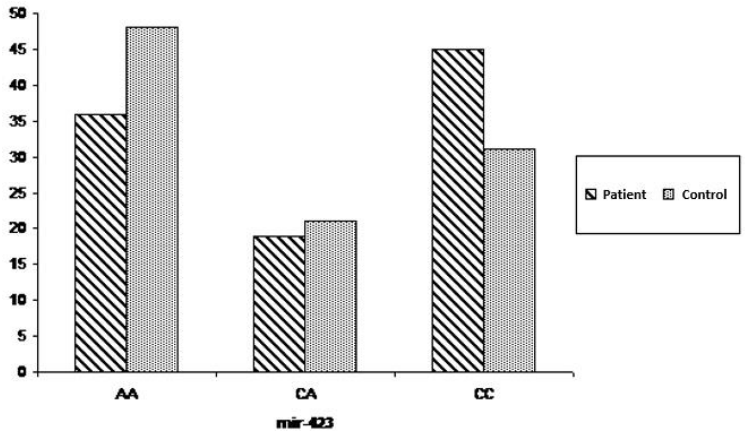

Fig 1. *Distribution of Genotype Ratios of pre-miR-423 A/C (rs6505162) Polymorphism between Control Group and CRC Patient Group*

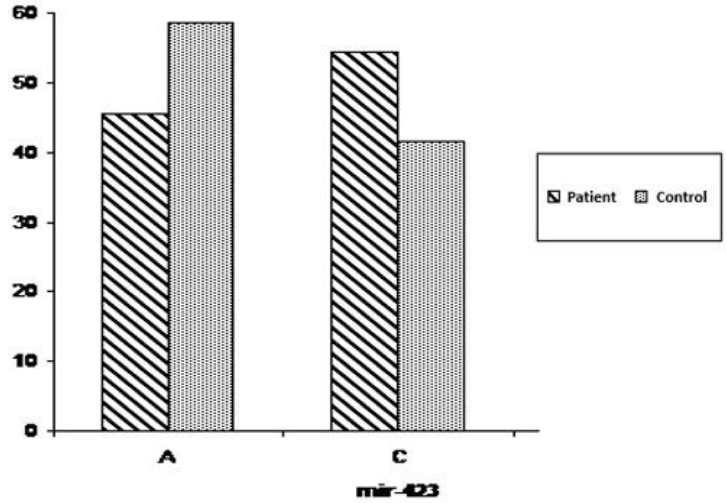

Fig 2. *Distribution of Genotype Ratios of pre-miR-423 A/C (rs6505162) Polymorphism between Control Group and CRC Patient Group*

Distribution of Allele and Genotype Ratios of pre-miR-608 (C/G) rs4919510 Polymorphism Between Control Group and Patients and Its Relationship with CRC

The statistical analysis found that the patient and control groups were not in 'Hardy Weinberg's equilibrium regarding pre-miR-608 gene polymorphism (p<0.001).

Table 5. *Hardy Weinberg Equilibrium Control (Before miR-608)*

	Genotypes	Observed Value	Expected Value	P Value
Control	**CC**	43	26,01	<0,001
	GC	16	49,98	
	GG	41	24,01	
Patient	**CC**	49	31,92	<0,001
	GC	15	49,16	
	GG	36	18,92	

When the genotype frequencies of pre-miR-608 rs4919510 (C/G) polymorphism were compared, no statistically significant difference was found between the control group and CRC patients (p=0.395). When the genotype frequencies of CRC patients and individuals in the control group were analyzed,

The frequency of CC genotype was 49% in CRC patients and 43% in the control group;
The frequency of GC genotype was 15 % in CRC patients and 16 % in the control group;
The frequency of GG genotype
Control Group:
C allele 51%, G allele 49
Patients with CRC
C allele was found at 56.5% and G allele at 43.5%.
No statistically significant difference was observed between the patient and control groups regarding allele distributions for pre-miR-608 (p=0.316).

Table 6. *Distribution of pre-miR-608 (rs4919510) Polymorphism Genotype and Allele Ratios between Patient and Control Group*

Genotype	Control N (%)	Patient N (%)	P Value	OR	Confidence Interval (%95)	P Value
CC	43 (%43,0)	49 (%49,0)	0,395	Reference		
GC	16 (%16,0)	15 (%15,0)		0,823	0,364-1,858	0,639
GG	41 (%41,0)	36 (%36,0)		0,770	0,420-1,413	0,399
Allele						
C	102 (%51,0)	113 (%56,5)	0,316	1,248	0,842-1,850	0,270
G	98 (%49,0)	87 (%43,5)				

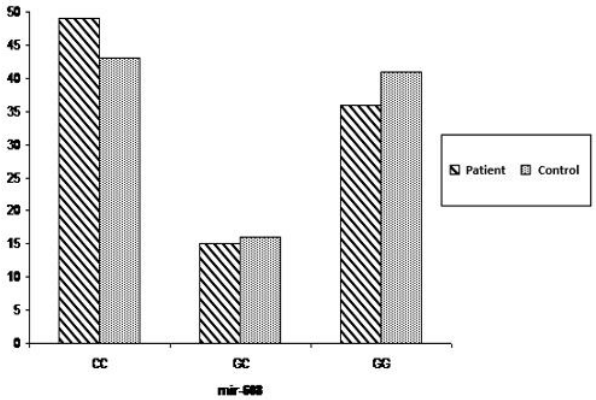

Fig 3. *Distribution of Genotype Ratios of pre-miR-608 C/G (rs4919510) Polymorphism between Control Group and CRC Patient Group*

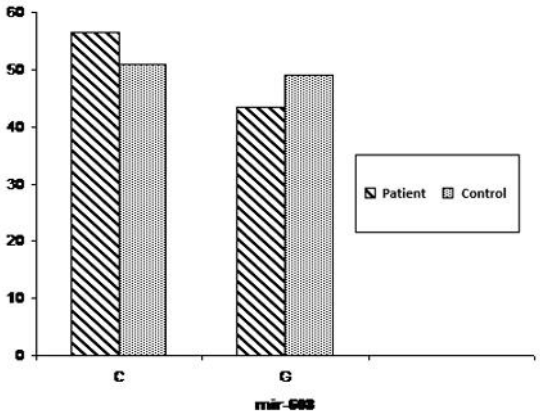

Fig 4. *Distribution of Genotype Ratios of pre-miR-608 C/G (rs4919510) Polymorphism between Control Group and CRC Patient Group*

Joint effect of pre-miR-423 and pre-miR-608 genes in CRC development

To determine the possibility of CRC development due to the joint effect of pre-miR-423 and pre-miR-608 genotypes, the relationship between the genotype combinations of both genes in the patient and control groups and the occurrence of CRC was compared. The combination of wild types in both groups was accepted as the reference group, and pre-miR-423 AA, CA, CC, and pre-miR-608 CC, CG, and GG genotypes were combined in homozygous and heterozygous individuals. Based on these results, no significant difference was observed in the genotype combinations of SNPs in both pre-miR genes. The study concluded that the combined effect of these two genes does not confer a risk of developing CRC.

Table 7. *Association between pre-miR genotype frequencies and risk of developing colorectal cancer*

Pre-miR 423	**Pre-miR 608**	**Control N (%)**	**Colorectal cancer N (%)**	**OR (Confidence Interval)**	p
AA	CC	14%	17%	1 (Reference)	-
AA	CG	4%	5%	1,029(0,231-4,581)	0,969
AA	GG	15%	23%	1,263(0,483-3,301)	0,634
CA	CC	10%	6%	0,494(0,144-1,698)	0,263
CA	CG	3%	4%	1,098(0,209-5,750)	0,912
CA	GG	7%	9%	1,059(0,314-3,568)	0,926
CC	CC	19%	13%	0,563(0,207-1,530)	0,260
CC	CG	9%	6%	0,549(0,157-1,920)	0,348
CC	GG	19%	17%	0,737(0,281-1,931)	0,534

DISCUSSION

In recent years, the methods used to diagnose CRC have improved and screening programs have begun to be widely implemented. In addition, new surgical techniques, radiotherapy, and systemic treatment methods have allowed colorectal cancer to be detected at earlier stages and improved survival rates [19]. In elucidating the molecular pathogenesis of CRC, miRNA molecules, which do not encode proteins and negatively regulate gene expression, have been identified. In pre-miRNAs, SNPs are essential molecules involved in many biological processes, such as cell proliferation and apoptosis, because they affect the processing and maturation of miRNAs. miRNAs involved in CRC pathogenesis regulate many oncogenic and tumor suppressor pathways [18]. A study has shown that pre-miRNAs can be exceptionally well protected and functional regarding mutations. Another study based on this study screened approximately 400 human pre-miRNAs, including Pre-miR-423, and genes in their surrounding regions. It found that pre-miRNA genes had fewer SNPs than the surrounding region [12].

SNPs in pre-miRNAs have been observed to be associated with breast, prostate, lung, esophageal, and liver cancer [20]. In addition, several recent studies have shown that SNPs in pri-miRNA and miRNA binding regions are associated with CRC development and progression risk. A study identified pri and pre-miRNA regions in mutations effective in chronic lymphocytic leukemia cell transformation and cancer development [21]. Another study identified approximately 40 miRNAs, including pre-miR-423 and pre-miR-608, in 426 Korean CRC patients. In addition, the SNP region was investigated, and reported that polymorphisms were not associated with CRC development [22]. In a study conducted in the Chinese population, 7 different pre-miRNA SNP regions were investigated in 408 patients diagnosed with colorectal cancer. It was reported that pre-miR-423 and pre-miR-608 were effective in developing colorectal cancer [17]. The results of these two investigated SNPs differ from other studies. For example, it has been reported that pre-miR-423 rs6505162 may increase the risk of ovarian cancer.

According to the literature review results, no study could be found that investigated pre-miR-423 (rs6505162) and pre-miR-608 (rs4919510) polymorphisms in patients with CRC in the Turkish population. This study investigated the association between pre-miR-423 and pre-miR-608 gene polymorphisms, which are thought to be associated with CRC and the disease. Based on the effects of SNPs in pre-miRNAs on mature miRNA, converting A to C in pre-miR423 may affect the processing and expression miR-423. According to many studies conducted today, changes in the expression of mature forms of miR-423 have been reported in many cell growth, differentiation, and cancer processes. miR-423 was first shown to influence the differentiation processes of mature miRNAs during differentiation experiments with 2-O-tetradecanoylporbol-13-acetate in untreatable HL-60 leukaemia cells [23]. In 2008, miR423 was identified as a mature miRNA. It was found through RNA cloning and Northern blotting techniques in neuroblastoma tissues and cell lines. Variations in the expression of mature miRNAs originating from miR423 have been observed in various types of cancer [13-14]. In our study, when the genotype rates for the pre-miR-423 rs6505162 (A/C) polymorphism, which is thought to be associated with CRC development, were evaluated, it was found that there was a significant difference between the CRC patient and control groups ($p = 0.039$). It was observed that the rate of patients with the mutant CRC genotype was 1.935 times higher than in the control group. According to this result, the risk of developing CRC increases in individuals with the CC genotype. In a study conducted in the Chinese population, different pre-miRNA SNP regions were screened in 408 patients diagnosed with colorectal cancer and it was observed that rs6505162 (A/C) polymorphism was effective in colorectal cancer development ($p=0.001$) [16]. They found the patients' AA, CA, and CC genotype rates to be 59.3%, 34.5%, and 6.1%, respectively, and 50%, 42.5%, and 7.4% in the control group. Our study determined AA, CA, and CC genotype rates as 36%, 19%, and 45% in the patients and 48%, 21%, and 31% in the control group, respectively. Consistent with the results of studies in the literature, rs6505162 SNP was found to increase the risk of CRC development ($p = 0.040$) [17]. In another study, the association between 40 miRNA polymorphisms, including 23 SNPs in the miRNA biogenesis pathway, 7 pre-miRNA and 10 pri-miRNA SNPs, and CRC, was investigated. They found that all SNPs studied, including rs6505162 A/C and rs4919510 C/G polymorphisms, were not effective in the development of CRC [22]. We could find two studies examining the relationship between CRC and rs6505162 polymorphism. Different results were obtained in two studies. While one study supported our study results, the other study obtained findings that did not support our results. Although the ethnic origins and study groups are the same in both studies, they do not support each other. Many studies in different populations are needed to resolve the contradictions on this issue.

In studies on different cancer types, especially ovarian, breast, and oesophageal cancer, rs6505162 was effective in cancer susceptibility. In 346 patients diagnosed with oesophageal cancer in a Caucasian population, the C allele significantly reduced the risk of disease [24]. The function of miR608 is not fully known, but it is thought to be a tumor suppressor miR. The C-G polymorphism in the pre-miR-608 rs4919510 SNP region is widely found in many populations [17]. pre-miR-608 has been reported in many human cancers such as colorectal, prostate, pancreatic, and brain, resulting from loss of heterozygosity at the chromosome 10q24 locus. Studies have reported that miR608 also influences TP53 (tumor protein p53) target sites, which play a role in the progression of colorectal cancer. Furthermore, it has been observed that CRC patients with TP53 mutations in stage 3 can benefit from 5-Fu-based chemotherapy [25].

In our study, when the genotype rates for pre-miR-608 rs4919510 (C/G) polymorphism, which is thought to be associated with colorectal cancer formation, were evaluated, no significant difference was found between CRC patient and control group ($p=0.395$). Allele frequencies were determined as 56.5% in patients and 51% in the control group for the C allele

and 43.5% in patients and 49% in the control group for the G allele, and no significant relationship was found (p=0.316).

In a study conducted on 245 patients with CRC and 446 controls consisting of African-Americans and Caucasians, it was determined that rs4919510 polymorphism did not pose a risk for colorectal cancer. The CC, CG, and GG genotype rates were 52%, 40%, and 8% in patients and 53%, 38%, and 8% in the control group, respectively. This study supports our findings. However, when they looked at the survival rates in patients, they found that the mutant GG genotype shortened (p=0.059) and increased survival in African Americans [18].

In the Chinese population study, rs4919510 (C/G) SNP was associated with colorectal cancer in 408 patients (p=0.027). The rates of CC, CG, and GG genotypes were 30%, 52.2%, and 17.6% in patients and 35.3%, 47.3%, and 17.2% in the control group, respectively. In our study, CC, CG, and CG genotypes were found to be 49%, 15%, and 36% in patients and 43%, 16%, and 41% in the control group, respectively, and were not found to be associated with the disease (p=0.39). They also found that the C allele variant decreased the overall survival of the cell and the risk of recurrence of the disease, whereas the G allele was not associated with the disease [17]. A study investigating 41 SNP regions in 26 miRNAs in 1097 colorectal adenocarcinoma patients reported that CG and GG genotypes in the miR608 rs4919510 SNP region increased the risk of CRC recurrence and death. The smaller sample size in our study may have caused us to obtain different results from this study [26]. As a result of the literature search, we could not find any other study investigating the relationship between rs4919510 SNP region and colorectal cancer. As a result of the literature search, we could not find any other study investigating the relationship between the rs4919510 SNP region and colorectal cancer; in a study conducted in a Caucasian population, 40 different SNPs belonging to 11 gene regions and 15 miRNAs involved in miRNA biogenesis were examined in 278 controls and 279 patients with renal carcinoma. It was reported that the Pre-miR-608 rs4919510 (C/G) region was not a risk factor in the development of renal carcinoma [27]. In a study with 346 patients diagnosed with oesophageal cancer and 346 controls in a Caucasian population, 41 SNP regions in 26 miRNAs were investigated. rs4919510 (C/G) SNP was found to be insignificant in developing oesophageal cancer [24]. A study observed that rs4919510 C/G polymorphism in miRNA608 increases the risk of breast cancer and tumor formation by affecting HER2 (Human Epidermal Growth Factor Receptor-2). In addition, HER2-induced tumor formation was found more frequently in patients carrying CG and GG genotype variants [28]. Based on this study, another study conducted in the Chinese population investigated the effect of miRNAs in breast cancer developing in the absence of HER2 (Human Epidermal Growth Factor Receptor), progesterone receptor (PR), and estrogen receptors (ER). 22 miRNA SNP regions were analyzed in 191 breast cancer patients and 192 control subjects and it was reported that all other miRNA SNPs, including pre-miR-423 rs6505162 and pre-miR-608 rs4919510, were not associated with disease [29]. In our study, combination analysis was performed to determine the joint effects of rs6505162 and rs4919510 SNPs in CRC formation. The analysis combined CC, CG, GG genotypes and AA, CA, and CC genotypes for pre-miR-423 and pre-miR-608 polymorphisms in homozygous and heterozygous individuals, respectively. Homozygous wild-type genotypes were determined as the reference group, and determined that the combined effect of both genes did not pose a risk in CRC development.

CONCLUSION

The literature on the association of pre-miR-423 rs6505162 and pre-miR-608 rs4919510 SNPs with CRC is limited, but many studies have different cancers. In most of these studies, conflicting results are observed. Population differences, sample sizes, and biological functions specific to other cancer types may be responsible for these contradictory results. Since CRCs

are multifactorial cancers involving genetic and environmental factors, whether these factors are effective between patient and control groups is unknown. In addition, more meaningful results can be obtained if a more comprehensive study is conducted with pre-miR-423 and pre-miR-608 polymorphisms and other genes involved in the etiology of CRC. Since our study's sample size was insignificant, the relationship between allele frequency, genotype distributions, and the disease may not reflect the population. Therefore, studies to be conducted in a larger sample group independent of this study to confirm the associations we found significant will make an essential contribution to the literature.

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