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The Investigation of *MAPK7* Gene Variations in Colorectal Cancer Risk

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Abstract. *Background/Aim: Mitogen-activated protein kinases (MAPKs) are important regulatory molecules, which have essential roles in physiology and pathology. In the present study, we examined the possible correlation between the MAPK7 gene and colorectal cancer risk in the Turkish population. Materials and Methods: A total of 100 human DNA samples (50 colorectal cancer patients and 50 healthy individuals) were sequenced using next-generation sequencing to define the potential genetic variations in the MAPK7 gene. Results: Five genetic variations (MAPK7; rs2233072, rs2233076, rs181138364, rs34984998, rs148989290) were detected in our study group. The G (variant) allele of the MAPK7; rs2233072 (T>G) gene polymorphism was found in 76% of colorectal cancer cases, and 66% of controls. The prevalence of rs2233076, rs181138364, rs34984998, and rs148989290 gene variations was quite rare in the subjects and no significant association in terms of genotype and allele frequencies was observed between the cases and controls. Conclusion: No statistically significant correlation between the MAP7 kinase gene variations and colorectal cancer risk was observed. This is the first investigation in the Turkish population that may initiate additional studies in larger populations to analyze the effect of MAPK7 gene on the colorectal cancer risk.*

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Colorectal cancer (CRC) is a widespread malignancy worldwide (1). CRC development is a multi-stage process, which induces colorectal epithelial cell transformation through several alterations such as microsatellite instability (MIN), chromosomal and genomic instability, DNA repair defects, and abnormal DNA methylation (2). Moreover, the accumulation of various genetic mutations induces cell growth and proliferation while inhibiting apoptosis, thus promoting cancer development (3).

Protein kinases are crucial molecules that regulate the development of unicellular and multicellular organisms; they are major components of signaling cascades, which are associated with cellular functions (4, 5). The mitogen-activated protein kinase (MAPK) family has key roles in the signal-transduction from the cell membrane to the nucleus; this process is induced by various stimuli and participates in diverse intracellular processes involved in cell growth, differentiation, and stress responses. Furthermore, it has been known to affect the process of carcinogenesis (6). The mammalian MAPK cascade involves the sequential activation of MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK pathways by extracellular signals such as growth factors and hormones, which are regulated through tyrosine kinases, serine/threonine kinases, and G protein-coupled receptor groups, and also, inflammatory cytokines, and environmental stimuli (6-8). Furthermore, extracellular-signal-regulated kinase 1/2 (ERK1/2), c-Jun-amino-terminal kinase (JNK), p38, and ERK5 are four members of the MAPK family (5, 9).

The scientific advances have led to the investigation of novel biomarkers to clarify the possible risks of diseases and enhance our understanding of the disease process and predict therapeutic outcomes. The genome-wide association studies (GWAS) enable the identification of many genomic polymorphisms, and mutations, which are potentially associated with cancer risk and development. It has been shown that the members of the MAPK-signaling family are one of the strongest genomic markers associated with colorectal cancer (CRC) (10, 11). The

MAPK7 [also known as *ERK5* and big MAP kinase 1 (*BMK1*)] belongs to the MAPK family and has important effects on critical physiological processes. Further studies, which were designed to investigate *MAPK7* structure and gene function have concluded that it plays a role in the regulation of prominent cellular pathways, including cell differentiation, cell proliferation, and gene expression (12, 13). The human *ERK5* gene (*MAPK7*) encodes a protein of 816 amino acids with a molecular weight of 110 kDa, which is nearly double the size of other members of the family (14).

Although, several studies have shown that *MAPK7* may be involved in the clinical progression and treatment of many types of cancers (15), no study has directly focused on the association of *MAPK7* gene polymorphisms with colorectal cancer risk. Therefore, the aim of the present study was to analyze the possible link between the *MAPK7* variants and colorectal cancer risk in the Turkish population.

Materials and Methods

Ethics statement. A total of 50 colorectal cancer cases who were diagnosed at the Istanbul Education and Research Hospital General Surgery Clinic, and 50 symptom-free individuals were enrolled in the present study. This study was approved by the Ethical Committee of Istanbul University Medical Faculty and conducted according to the principles of the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). Peripheral blood samples were collected into EDTA tubes. All participants were informed about the study and gave their written consent. The patient samples were obtained before the chemotherapy or radiation therapy. The clinical data were obtained from the subjects using a questionnaire and classified as cases and healthy control groups. Furthermore, the pathological data were received to evaluate the cancer status of the patients.

DNA isolation. The DNA extraction kit (MasterPure™ Epicentre DNA Purification Kit, Madison, WI, USA) was used to extract genomic DNA from human leukocyte nuclei isolated from whole blood. The quality of the extracted DNA was then assessed using a Qubit fluorometer (Invitrogen, CA, USA) and 1% agarose gel electrophoresis.

MAPK7 next-generation sequencing (NGS). The *MAPK7* gene was sequenced in 50 control subjects and 50 patients. The long polymerase chain reaction (PCR) was used for the amplification of the *MAPK7* gene regions. PCR products were purified and quantified using a Qubit fluorometer (Invitrogen). Each sample of DNA was adjusted to 2 nmol and pooled to create the DNA library, before initiating the reaction of the next-generation sequencing (NGS) process. The DNA samples were equalized to 50 ng/μl and NGS was performed using the Miseq sequencing system (Illumina, San Diego, CA, USA). With an average of 221,000 units of 2×250 bp readings per sample, an average of 35 Mb of data was obtained per sample following standardized protocol. Then, the readings of the *MAPK7* gene regions were filtered from the sequence data. The raw sequence data were cleaved appropriately to the quality scores (Trimmomatic v0.27). The corrected raw sequence data were aligned to the human genome reference sequence (GRCh37, GRCh38) using Burrows-Wheeler Aligner (v.0.7.12) (16). The re-alignments around insertions and deletions (indels) were detected with the Genome Analysis Toolkit v3.3.0 (GATK) Indel Realigner. After combining and aligning sequences, GATK (v3.3.0) was used to filter the repetitive readings to determine the number of readings and re-calibrate base quality. In the final step, single nucleotide variations and insertions/deletions were determined with GATK (Unified Genotyper) (17). The locations of whole gene variants were mapped using the National Center for Biotechnology Information (NCBI) and Ensembl databases.

Table I. *Characteristic of patients and controls*.

	Colorectal cancer (n=50) (%)	Control (n=50) (%)	*p*-Value[a]
Age (Years)	51.93±10.8	53.84±8.9	*p*>0.05
Alcohol consumption			*p*>0.05
Yes	-	-	
No	69	31	
Family history			*p*>0.05
Yes	3.8	11.1	
No	96.2	88.9	
Smoke status			*p*>0.05
Yes	6.3	11.1	
No	93.8	88.9	
T stage			
T1+T2	28.6		
T3+T4	71.4		
Lymph node status			
Yes	57.1		
No	42.9		
Metastasis at diagnosis			
Yes	23.8		
No	76.2		

[a]*p*-value <0.05 denotes statistical significance. The clinicopathological characteristics of patients are given as percentages of the values.

Statistical analysis. The data were sorted and filtered for bioinformatic analysis. The significant variant classification was assessed according to the population frequency of the exome and whole-genome sequence data. All variants with a frequency of <5% in the Exome Aggregation Consortium (ExAC) and 1000 Genome databases were considered and compared in the study groups.

The results were analyzed with the SPSS statistical program (IBM Corporation version 20.0 SPSS Inc., Chicago, IL, USA). Numerical values were defined as mean, standard deviation, and percentage. The Student's *t*-test was applied to evaluate the distribution of variables between the cases and controls. A *p*-value of less than 0.05 was set as statistically significant. The significance was adjusted using Bonferroni correction.

Results

In the present study, we did not observe any statistically significant difference regarding age, family history, or smoking status among the studied groups. The mean age of colorectal cancer patients was 51.93±10.8 years and that of healthy individuals was 53.84±8.9 years. Characteristics of the colorectal cancer cases are presented in Table I. TNM

Table II. *MAPK7 gene variants in control and patient samples.*

Variant	Alleles	Reference (Ancestral) Allele	Control samples total	Control samples variant positive (%)	Patient samples total	Patient samples variant positive (%)	*p*-Value[a]
MAPK7 rs2233072	(T/G)	T	50	66	50	76	0.378
MAPK7 rs2233076	(A/G)	A	50	-	50	2	1.00
MAPK7 rs181138364	(C>A)	C	50	-	50	2	1.00
MAPK7 rs34984998	(A/AA)	A	50	2	50	-	1.00
MAPK7 rs148989290	(A/G)	A	50	2	50	-	1.00

[a]*p*-value <0.05 denotes statistical significance. The distribution of allele frequency in study groups are given as percentages of the values.

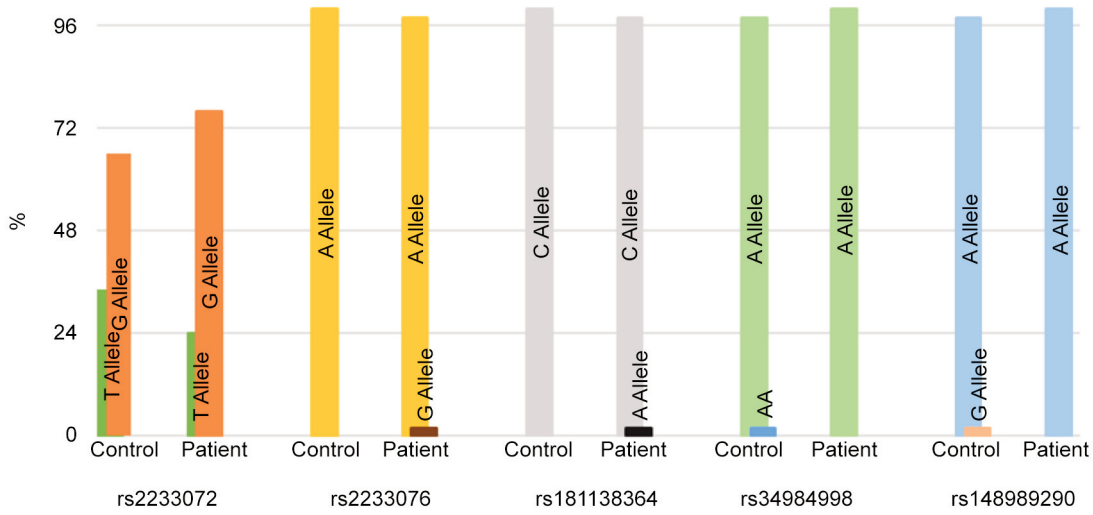

Figure 1. *MAPK7 gene variants in control and colorectal cancer patient samples.*

staging based on clinicopathological variables stratified the cohort as follows: a total of 28.6% of patients were T stage I and II (T1+T2) and 71.4% T stage III and IV (T3+T4). The incidence of CRC patients with distant metastasis was 23.8% and 71.2% of patients did not present distant metastasis.

Sequencing of genomic DNA samples was carried out to evaluate if there was any SNP, mutation, deletion, or insertion in the *MAPK7* gene. The five variations, which were previously described in the databases (rs2233072, rs2233076, rs181138364, rs34984998, and rs148989290) (location on chromosome 17) were found in our samples. The frequencies of these gene variations are shown in Table II and Figure 1. We did not observe any significant difference between patients and healthy controls in terms of allele frequency. In the present study, the variant allele (G) of the *MAPK7;* rs(rs2233072) polymorphism was observed in 76% of colorectal cancer cases and 66% of controls (Table II and Table III), but there was no significant difference between the study groups in terms of allele frequencies (p=0.378). Analysis of rs2233076, rs181138364, rs34984998, and rs148989290 variations in colorectal cancer patients and healthy individuals indicated that these gene variations were quite rare in cases and controls but there was no statistically significant difference among the groups (p>0.05). Thus, we did not perform analysis of the association between the genotype and allele frequencies according to the clinicopathological characteristics of the colorectal cancer patients such as tumor grade, lymph node status, and distant metastasis.

Discussion

CRC has become a highly prevalent malignancy in recent decades and its effective management depends on early detection (18). Although human genome sequencing has shed light on the identification of genetic alterations in cancer types, it has been reported that colorectal cancer has a high genetic heterogeneity, which makes it difficult to reveal the clinical consequences of individual mutations. It has been shown that rare variations in

Table III. *MAPK7 gene rs2233072 (T>G) variant analysis.*

Chr	Region	Type	rs	Reference	Allele	Bonferroni
Chr17	19281828	SNP	2233072	T	G	0.6650632

Bonferroni's correction was used for the MAPK7 gene rs2233072 (T>G) variant analysis.

colorectal cancer are quite common, and are involved in the risk and pathogenesis of many types of tumors (19-21).

There were several studies mentioning the importance of the MAPK family and its effects in the colorectal cancer development and prognosis. For instance, a genome-wide association study, which was carried out by Lascorz *et al.,* reported seven *MAPK* genes were important for CRC (10). In another study, Barault *et al*. observed that somatic mutations in *MAPK* were related to poor survival after diagnosis with CRC (22). Although, many articles have shown the impact of MAPKs in the physiological processes, cellular functions, disease development, and prognosis, there were limited studies on the association of *MAPK7* (ERK5) gene variants in cancer risk. However, the overexpression of *ERK5* has been indicated as a potential prognostic biomarker in both breast (8) and prostate cancer (23). Furthermore, it has been observed that *ERK5* amplification is present in almost half of the primary hepatocellular carcinoma (HCC) (24). was Another study carried out by Ivana *et al*. concluded that *MAPK7* is a potential gene marker for breast cancer, during the carcinogenesis process (25). Also, recent evidence has shown that *MAPK7* is related to poor survival of patients with breast cancer through the MEK5-ERK5 pathway after a systemic therapeutic approach (8, 26). Similarly, several studies have reported that the amplification of the *MAPK7* is genetic marker in high-grade osteosarcoma and has an important effect on prognosis (27, 28).

There were limited studies about the *MAPK7;* rs2233072 gene variant, which was relatively common in our study group. Recently, it was investigated whether changes in the *MAPK7* gene expression in osteosarcoma (OS) patient tissues taken before and after chemotherapy are associated with any genomic alterations. The findings revealed that there may be a significant correlation between the rs2233072 G allele variant and nonmetastatic disease at diagnosis and absence of relapse in OS (28).

In present study we did not observe any significant differences between the subjects in terms of genotype and allele frequencies ($p>0.05$). When we analyzed MAPK7; (rs2233072, rs2233076, rs181138364, rs34984998, rs148989290) variants interestingly, we observed that the genomic substitution of the three MAPK7 gene variations; (rs181138364, rs34984998, rs148989290) were described in the genomic databases, but to the best of our knowledge, there were no research articles, which were analyzed them in the pathogenesis of the diseases. According to their incidence, these rare variants could not be classified clinically and associated with histopathological characteristics of patients in our study.

In conclusion, this is the first study investigating the association between the genomic variants of *MAPK7* gene and colorectal cancer risk in the Turkish population. We did not find any significant correlation between *MAPK7* kinase gene variations and colorectal cancer development. However, our study has a limitation that needs to be mentioned. This is a preliminary report and the sample size is relatively small. Therefore, larger population data are needed to determine the role of *MAPK7* in colorectal cancer risk and prognosis.

Conflicts of Interest

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

Authors' Contributions

Canan Cacina designed the study and took the lead in writing the article. Soykan Arıkan contributed the sample collection and all authors discussed the results and commented and helped the investigation, analysis and writing the manuscript.

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