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The association of MMP-13 rs2252070 with non-small cell lung cancer in the Turkish population

Khumara Vagif Qizi[1], Fatih Karatas[2], Yasemin Oyaci[1], Murat Acat[3], Fatih Inci[2], Sacide Pehlivan[4]

[1]*Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Türkiye*
[2]*Department of Medical Oncology, Faculty of Medicine, Karabuk University Karabuk, Türkiye*
[3]*Department of Chest Diseases, Private Cagsu Hospital, Duzce, Türkiye*
[4]*Department of Medical Biology, Istanbul Faculty of Medicine, Istanbul, Türkiye*

ABSTRACT

Aim: To evaluate the role of *MMP-13* rs2252070 in patients with non-small cell lung cancer (NSCLC) in the Turkish population.

Method: A total of 95 NSCLC patients and 94 healthy controls were included in this study. The *MMP-13* rs2252070 variant was genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The results of the analyses were evaluated for statistical significance.

Results: There was no G/G homozygous genotype in the patient or control groups. The prevalence of genotypes of A/A and A/G profiles for the *MMP-13* rs2252070 variant was 34.7% and 65.3%, respectively, in patients and 46.8% and 53.2%, respectively, in the control group. No significant difference was found between the patient and control groups in terms of *MMP-13* rs2252070 genotype distribution and allele frequency (*p= 0.091,* OR: 0.605, CI 95%:0.337-1.086; *p: 0.199,* OR: 1.337, CI 95%: 0.858-2.083, respectively).

Conclusions: Our results in this study showed no association between *MMP-13* rs2252070 and NSCLC. To fully comprehend the mechanisms underlying NSCLC development, more research is required.

Key words: Non-small cell lung cancer, matrix metalloproteinase, variant, MMP-13, PCR-RFLP.

✉ *Yasemin Oyaci*
Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Türkiye
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Introduction

Lung cancer is one of the most common cancers worldwide and is a leading cause of cancer-related deaths [1]. Non-small cell lung cancer (NSCLC) is the most common type, accounting for about 85% of all lung cancers [2]. Lung cancer patients have a five-year survival rate of less than 10% despite breakthroughs in treatment. The majority of patients are identified at a late stage [3]. The main cause of lung cancer is usually long-term exposure to substances that damage the lungs, primarily cigarette smoking. However, non-smokers can also develop lung cancer, and other risk factors include exposure to secondhand smoke, environmental pollutants, radon gas, asbestos, and genetic factors [4]. Many experimental and epidemiological studies have found a relationship between lung cancer and genetic polymorphisms [5].

Matrix metalloproteinases (MMPs) are a family of enzymes that play a crucial role in the breakdown and remodeling of the extracellular matrix (ECM), which is the complex network of

proteins and other molecules surrounding cells in tissues [6]. MMPs are involved in various physiological and pathological processes, including tissue repair, embryogenesis, and inflammation. However, they are also implicated in the progression of several diseases, particularly cancer and various connective tissue disorders. Matrix metalloproteinase 13 (MMP-13), also known as collagenase-3, is a specific member of the MMP family. Among the various MMPs, MMP-13 has a particular function in the degradation of collagen, which is the main structural protein in the ECM. The *MMP-13* gene is localized on the long arm of chromosome 11 at position 11q22.3. It consists of 11 exons and spans approximately 13 kilobases [7]. The *MMP-13* -77A/G variant, also known as rs2252070, refers to a genetic variation in the *MMP-13* gene at position -77, where either an adenine (A) or a guanine (G) nucleotide can be present. Variants in the promoter region can potentially affect gene expression and protein production, leading to variations in enzyme activity and potentially influencing disease susceptibility, including cancer [8]. The *MMP-13* rs2252070 has been of interest in research because it may influence the regulation of *MMP-13* gene expression and potentially affect the risk or severity of certain diseases. Several studies have investigated the association between *MMP-13* rs2252070 and cancer risk, with varying results depending on the specific cancer type and population studied. Therefore, we investigated whether the *MMP13* rs2252070 variant is a risk factor for NSCLC in Turkish patients.

Materials and methods

The study group comprised 95 NSCLC patients recruited prospectively from those treated and followed up in the Department of Chest Diseases, Karabuk University, Karabuk, Turkey. The diagnosis of NSCLC was made by pathological examination of the biopsy material. A total of 94 healthy individuals of similar age and gender, without any family or personal history of cancer or chronic diseases, were included in the control group. Each participant gave their written, informed consent, and they were all of Turkish descent. The Declaration of Helsinki's guiding principles were followed when carrying out the study protocols. The ethics committee evaluated and approved the experimental study process and protocol (77192459-050.99 /55053 Karabuk University).

Genotyping: Genomic DNA was obtained from peripheral blood using a commercial DNA isolation kit (GeneMark kit). The *MMP-13* rs2252070 variant was genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as previously described [9]. The *Bsr I* (Cat No: R0527S NEB) restriction enzyme was used to digest the PCR products. Two bands of 248 bp and 197 bp are produced by the G allele; three bands (445, 248, and 197 bp) are produced by A/G heterozygosity, and just one band of 445 bp is produced by the A allele. To verify the findings, 10% of the samples were randomly re-analyzed.

Statistical analysis: Using Statistical Package for the Social Sciences (SPSS) 22.0 for Windows (SPSS Inc., Chicago, IL, USA), all data was analyzed. Logistic regression analysis was used to determine whether the differences between the groups were statistically significant. Additionally calculated were the odds ratio (OR) and 95% confidence interval (CI). The chi-square test and Fisher's exact test, when necessary, were used to examine variations in *MMP-13* rs2252070 genotype and allele distribution between the groups. Hardy-Weinberg equilibrium (HWE) was calculated using this calculator (https://gene-calc.pl/). Differences

were judged statistically significant at *p* 0.05 in all two-tailed analyses.

Results

A total of 95 NSCLC patients and 94 healthy controls were genotyped for the *MMP-13* rs2252070 variant. Baseline clinical and demographic features of the patient groups are shown in Table 1.

Table 1. Baseline clinical and demographic features of the patients.

Characteristics	Patients N: 95 (%)
Age (mean, min-max, years)	66 (43-81)
Sex	
Female	6 (6.3)
Male	89 (93.7)
Stage	
I	1 (1.2)
I	5 (6.2)
III	38 (46.3)
IV	38 (46.3)
Metastasis	
Bone	23 (60.5)
Brain	4 (10.5)
Lung	8 (21)
Liver	2 (5.4)
Lymphadenopathy	1 (2.6)
Smoker **Non-smoker**	70 (85.4) 25 (14.6)
ALK test	
0	67 (82.7)
1	11 (13.6)
2	3 (3.7)
EGFR test	
0	68 (84)
1	12 (14.8)
2	1 (1.2)
PDL-1 test	
0	65 (80.2)
1	9 (11.1)
2	7 (8.7)
Haemoglobin (gr/dL)	12.5 (8.8-17.3)
Leukocyte (µL)	8100 (3340-19300)
Thrombocyte (10^3/µL)	244 (110-538)
Neutrophil (µL)	5600 (1470-1699)
Lymphocyte (µL)	1480 (1200-4230)
MPV (fl)	9.6 (7.3-12.6)

ALK: anaplastic lymphoma kinase; EGFR: epidermal growth factor receptor; PDL-1: programmed death ligand-1; MPV: mean platelet volume; fl: femtolitre; µL:microliter; dL: deciliter.

genotypes of A/A and A/G profiles for the *MMP-13* rs2252070 variant was 34.7% and 65.3%, respectively, in patients and 46.8% and 53.2%, respectively, in the control group. There was no statistical significance between the patient and control groups in terms of *MMP-13* rs2252070 genotype distribution (*p= 0.091,* OR: 0.605, CI 95%:0.337-1.086). *MMP-13* rs2252070 allele frequency was also similar between groups (*p: 0.199,* OR: 1.337, CI 95%: 0.858-2.083). There was a deviation from HWE in the control group (*p=0.002*). Table 2 represents the *MMP-13* rs2252070 genotype distribution and allelic frequency in the groups.

Discussion

Cancer, which is the most important cause of death worldwide, is an important cause of morbidity and mortality despite targeted therapies. The most recent data, released in 2022, show that the mortality rate from lung cancer is 21% globally, making it the most common cancer form in both sexes [10]. Lung cancers are mostly in the metastatic stage when diagnosed. In the development of metastasis, neoplastic cells must have certain characteristics in order to pass through the several stages that make up this process. Malignant features of lung cells such as tissue invasion, unregulated tumor growth, tissue remodeling, and inflammation are associated with proteolysis [11].

In cancer, MMPs are often upregulated and can contribute to tumor growth, invasion, angiogenesis, and metastasis [12]. MMPs degrade ECM components, such as collagen and fibronectin, creating pathways for cancer cells to migrate and invade adjacent tissues. MMPs can also break down the basement membrane, a specialized ECM structure that separates epithelial or endothelial cells from underlying tissues, allowing cancer cells to invade blood

Table 2. Genotype distribution and allele frequencies of the *MMP-13* rs2252070 variant in groups.

***MMP*-13 rs2252070**	**Patient group** n=95 (%)	**Control group** n=94 (%)	**OR Exp (B)**	**95% CI**	***p* ***
Genotypes					
A/A	33 (34.7)	44 (46.8)	0.605	0.337-1.086	0.091
A/G	62 (65.3)	50 (53.2)			
G/G	0 (0)	0 (0)			
Alleles					
A	128 (67.3)	138 (73.4)	1.337	0.858-2.083	0.199
G	62 (32.7)	50 (26.6)			
HWE		**0.002**			

*HWE: Hardy-Weinberg equilibrium *Pearson Chi-Square*

vessels and lymphatics, leading to metastasis [13]. MMPs can degrade the ECM surrounding blood vessels, allowing endothelial cells to migrate and form new blood vessels. In addition, MMPs can also promote the release of vascular endothelial growth factor (VEGF) from the extracellular matrix, making it available to stimulate angiogenesis [14]. This interplay between MMPs and VEGF contributes to the formation of a vascular network that supports tumor growth and facilitates the metastatic process. Also, MMPs can influence the immune response in the tumor microenvironment. They can cleave and inactivate immune signaling molecules, such as cytokines and chemokines, impairing immune cell recruitment and activation [15]. Studies have shown that MMPs, particularly MMP-2 and MMP-9, are often overexpressed in NSCLC tumors and are associated with increased invasiveness and metastatic potential [16, 17]. High levels of MMPs have been correlated with a worse prognosis and poorer survival outcomes in NSCLC patients.

MMP gene polymorphisms refer to genetic variations in the DNA sequence of the genes that code for MMPs. These polymorphisms can influence the expression, activity, and function of MMPs, potentially impacting their role in cancer development and progression, including lung cancer. *MMP-1* -1607 1G/2G is a variation in the promoter region of the *MMP-1* gene that may affect its expression levels. Some studies have suggested that the 2G allele of this polymorphism may be associated with an increased risk of lung cancer [18]. The *MMP-2* -1306 C/T polymorphism has been investigated in relation to lung cancer risk, but the results have been inconsistent across different studies [19, 20]. In one study, the *MMP-7* -181 A/G polymorphism was linked to an increased risk of lung cancer [21]. *MMP-9* -1562 C/T polymorphism has been studied in relation to lung cancer risk in Asians, and some evidence suggests an association between the T allele and increased risk [20].

MMP-13 expression in human tissue is regulated by various hormones, cytokines, and growth factors. In cancer, the control of MMP-13 production and activity is impaired by intrinsic and extrinsic factors. Intrinsic mechanisms include oncogenes (e.g., Ror2) or proto-oncogenes (e.g., c-fos) and tumor suppressor genes (e.g., p53) that directly activate MMP-13 expression [22]. Extrinsic mechanisms include hypoxia and inflammation. With increasing tumor size, the hypoxic microenvironment within the tumor induces cell necrosis [23]. This attracts leukocytes to the site, producing

cytokines such as IL-1, IL-6, and TNF-α. These cytokines increase MMP-13 expression. MMP-13 expression was detected in various types of cancers, including papillary thyroid carcinoma [24], colorectal cancer [25], and breast cancer [26]. MMP-13 expression has been detected in lung tissue, particularly in conditions associated with lung injury and inflammation [27].

There are several binding sites for transcription factors in the human *MMP-13* gene promoter. The *MMP-13* rs2252070, located in the promoter region, affects gene expression. The rs2252070 A allele has around twice the transcriptional activity of the G allele, according to Yoon et al. [28]. There are studies investigating the relationship between *MMP-13* rs2252070 and cancer. A study revealed that the *MMP-13* rs2252070 variant was associated with colorectal cancer in a Mexican population [29]. No association was detected between *MMP-13* rs2252070 and breast, lung, or colon cancer risk among Polish patients [30]. Similarly, it was found that the *MMP-13* rs2252070 variant was not related to esophageal squamous cell carcinoma or gastric cardiac adenocarcinoma [31]. González Arriaga et al. reported that there was no significant association between *MMP-13* rs2252070 and lung cancer risk in a Spanish study of 501 patients and 506 controls [32]. Li et al. showed that *MMP-13* rs2252070 was not associated with lung cancer risk in a meta-analysis [19]. However, one study showed that *MMP-13* rs2252070 reduced the risk of lung cancer [20]. In a study conducted in our country, it was found that *MMP-13* rs2252070 A/G and G/G genotypes increased in NSCLC tumor tissue [33].

This study aimed to elaborate on a possible genetic relationship between *MMP-13* rs2252070 and NSCLC in a Turkish population. There was no person with the G/G genotype in both the patient and control groups. We found no significant relationship between *MMP-13* rs2252070 genotype and allele distribution and NSCLC (Table 2). The HWE analysis showed that there may be selection for heterozygosity.

There were some limitations to this study. One of the limitations of our study was the small size of the study group. This may not reflect the general characteristics of the Turkish population. The absence of G/G homozygous individuals in the patient and control groups may affect statistical power. Another limitation of our study was that only one variant in the MMP pathway was analyzed. Other SNPs in the MMP family may also play a role in cancer formation. Finally, the other limitation was that MMP-13 expression levels were not evaluated.

Conclusions

MMPs are a large family that acts in many different ways in cancer. It should be noted that different MMPs may have different roles in cancer, and their expression may vary according to tumor type and stage. The dysregulation of MMPs in cancer highlights their potential as therapeutic targets. Therefore, studies revealing the role of MMPs in different cancers are required. Although our results suggest that *MMP-13* rs2252070 may not be a genetic risk for the development of NSCLC, research in larger populations and different ethnic groups is needed to support and extend these results.

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