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Gastrointestinal Tract

Extracellular matrix protein 1 gene rs3737240 single nucleotide polymorphism is associated with ulcerative colitis in Turkish patients

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ABSTRACT

Background/Aims: Ulcerative colitis (UC) and Crohn's disease are chronic inflammatory diseases. Genetic, immunologic, and microbial factors play an important role in their pathogenesis. Extracellular matrix protein 1 *(ECM1)*, a gene related to mucosal barrier function, has been shown to be associated with UC. This study aims to determine the relationship between *ECM1* gene rs3737240 single nucleotide polymorphism (SNP) and UC in a group of Turkish patients.

Materials and Methods: Ninety-four UC patients and 120 healthy controls were enrolled in the study. ECM1 gene rs3737240 SNP genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism method.

Results: TT genotype was significantly more common in UC patients than in the healthy control group [p=0.034; odds ratio (OR) 2.34; 95% confidence interval (CI) 1.04-5.25]. The presence of C allele significantly lowered the UC risk (p=0.034; OR 0.42; 95% CI 0.19-0.95). TT genotype was significantly associated with azathioprine use in UC patients (p=0.037; OR 3.0; 95% CI 1.04-8.65). The C allele significantly reduced the probability of azathioprine use in UC patients (p=0.037; OR 0.33 CI 95% 0.11-0.96). No relation was found between rs3737240 SNP genotype and the phenotypical characteristics of UC patients.

Conclusion: The TT genotype of *ECM1* gene rs3737240 SNP significantly increased susceptibility for UC and azathioprine use in UC patients in a Turkish population.

Keywords: Ulcerative colitis, extracellular matrix protein 1 gene, single nucleotide polymorphism

INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic, chronic, relapsing inflammatory disease of the gastrointestinal tract and has two major types: Crohn's disease (CD) and ulcerative colitis (UC). The etiology of IBD remains unclear, but the currently accepted hypothesis is that genetically susceptible individuals have an impaired mucosal inflammatory response against the intestinal microbiota. A UC concordance of 10% between monozygotic twins and the relative risk of 8%-15% for the sibling of a UC patient to develop UC are evidence that genetic factors influence disease development (1). Recently, 163 IBD-associated susceptibility genes/loci were identified by means of genome-wide association (GWA) studies (2). Inflammation is limited to the mucosal surface in UC, and a defective mucosal barrier has gained importance in the pathogenesis of the disease; numerous studies on IBD-associated genes that regulate the intestinal barrier have been conducted in recent years (3). Among these genes, the extracellular matrix

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protein-1 gene (*ECM1*) is an important candidate as its mucosal barrier role is mainly characterized in the skin; however, its role in the intestine has not been fully understood (4). The *ECM1* gene is found on chromosome 1q21.2, and the rs3737240 and rs13294 single nucleotide polymorphisms (SNPs) have been found to be associated only with UC (5,6). *ECM1* is expressed throughout the intestine, displays an interaction with the basal membrane, inhibits matrix metalloproteinase 9 (MMP-9), and strongly activates NF-kappaB. MMP-9 is a tissue-degrading enzyme and is increased during active IBD (7). It is a fundamental immune regulator in IBD pathogenesis. Mutations that lead to tissue loss in patients with *ECM1* SNPs probably increase the tissue injury caused by MMP-9, resulting in an increase in intestinal ulcers and scars in IBD (8). In addition, *ECM1* is also associated with cell proliferation, angiogenesis, and differentiation; *ECM1* expression has been demonstrated to be increased in metastatic epithelial tumors, such as those in gastric and colorectal cancers (9). Moreover, rs3737240 and rs13294, which are the strongest *ECM1* markers, are also found to be associated with ankylosing spondylitis (10). The relation between the *ECM1* locus and UC has been demonstrated in subsequent studies as well (11,12). One study suggests that there is no relation between *ECM1* and UC (13). As indicated by this study, *ECM1* is a critical locus that is only associated with UC; however, recent studies have reported different outcomes. It has been demonstrated that including genetic markers rather than using inflammatory markers alone is quite useful in diagnosing IBD and in discriminating between UC and CD (14). Studies that identify the genetic risk factors of IBD would improve both our understanding of IBD pathophysiology and diagnostic and therapeutic methods.

The present study aims to evaluate the association between *ECM1* gene rs3737240 SNP in Turkish UC patients and to determine whether it may serve as a guide for diagnostic and therapeutic options.

MATERIALS AND METHODS

Study Population

A total of 94 patients diagnosed with UC who were admitted to the İstanbul Medeniyet University Göztepe Training and Research Hospital Gastroenterology Clinic between January 2011 and January 2013 were enrolled in the study. For all patients, the diagnosis of UC was made using standard clinical, endoscopic, histological, and radiological criteria. Patients who were diagnosed with IBD-unclassified were excluded. Age, sex, age at diagnosis, disease duration, disease localization, disease behavior, extraintestinal involvement, medications, history of colectomy, and family history of IBD (IBD in 1st degree relatives) were recorded for all patients. Steroid-refractory and steroid-dependent disease were noted according to previously defined criteria (15). The localization of the disease was defined in agreement with the Montreal classification (16). Clinical characteristics of the study population are outlined in Table 1.

One hundred and twenty age- and sex-matched healthy volunteers (68 females, 52 males; mean age: 39.8±9.1 years) were enrolled in the healthy control group. In the healthy control group, those with symptoms and family history of IBD were not included in the study. All patients and healthy controls have given informed and written consent. The study was approved by the İstanbul Medeniyet University Göztepe Training and Research Hospital Local Ethics Committee (Approval no/date: 19/T-14.02.2011).

DNA Extraction and Genotyping

A total of 2 mL of venous blood was collected from all patients into EDTA-containing tubes and stored at -80°C. Afterward, genomic DNA extraction was performed with the salting-out method (17). Buccal mucosa samples were collected from the healthy control group and genomic DNA extraction was performed with the standard phenol/chloroform method (18).

Genotyping was performed with polymerase chain reaction-restriction fragment length polymorphism analysis. In total, 50-100 ng of genomic DNA and 1×PCR buffer (Roche, Germany), 0.2 mM of each dNTP (Fermentas, Lithuania), 1 U of Taq DNA

Table 1. Clinical characteristics of ulcerative colitis patients

Number of patients	**94**
Sex-number of patients (%)	
Female	41 (43.6)
Male	53 (56.4)
Age (years), mean±SD	41±11.2
Age at diagnosis (years), median [interquartile range]	35.9 (16.7-62)
Disease duration (years), median [interquartile range]	3 (0.4-28)
Localization-number of patients (%)	
Proctitis	19 (8.9)
Left sided colitis	26 (12.1)
Extensive colitis	49 (22.9)
Medications-number of patients (%)	
Corticosteroid	29 (13.6)
Azathioprine	28 (13.1)
Anti-TNF-alpha	3 (1.4)
Extraintestinal manifestation-number of patients (%)	9 (4.2)
Colectomy-number of patients (%)	7 (3.3)
Family history of IBD	10 (4.7)

SD: standard deviation; Anti-TNF-alpha: anti-tumor necrosis factor-alpha; IBD: inflammatory bowel disease

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polymerase (Fermentas, Lithuania), 2.0 mM $MgCl_2$ (Fermentas, Lithuania), and 12.5 pmol of primers (ECM-OT-F: 5′ AGCCTTGAGAAGCAGGAGGA3′ and ECM-OT-R: 5′ AGTGAACGGGACCTGAGGTT 3′) were added to an overall volume of 15 µL PCR solution. PCR thermocycling conditions were in this manner: 5 min for initial denaturation at 94°C; for 30 s 30 cycles of denaturation at 94°C, for 30 s annealing at 58°C, and elongation at 72°C for 45 s; and a final elongation for 5 min at 72°C. The PCR product (100 bp) was digested with the enzyme BsmI (Roche, Germany) for 2 h, then it was separated on a 2% agarose gel, and after staining with ethidium bromide it was visualized under UV light. The CC homozygote was cleaved by BsmI to yield 378 and 293 bp bands. The TT homozygote was cleaved by BsmI to yield 378, 244, and 49 bp bands. The CT heterozygote contained all four bands (378, 293, 244, and 49 bp) after restriction digestion.

Statistical Analysis

All comparisons between the groups were performed using the Statistical Package for Social Sciences version 21.0.0.0 (IBM Corp.; Armonk, NY, USA) program. Differences between the groups in terms of continuous variables were analyzed as follows: normally distributed tests with independent samples t-test, non-normally distributed variables with Mann-Whitney U test. *ECM1* gene rs3737240 SNP allele and genotype frequencies were compared between the groups using χ^2 or Fisher's exact test. Two-sided p values were calculated along with odds ratios (OR) and 95% confidence intervals (CI). The level of significance was accepted at p<0.05.

RESULTS

Genotype Analysis

The Hardy-Weinberg equilibrium has been met in the healthy control group genotype distribution (p>0.05). The genotype distribution of the UC group was not in Hardy-Weinberg equilibrium (p<0.05). Allele and genotype frequencies for the *ECM1* rs3737240 SNP are presented in Table 2. The C allele frequency was significantly higher in the healthy control group than in the UC group (90.8% vs. 80.9%; p=0.034; OR 0.42 CI 95% 0.19-0.95). There was no significant difference between the UC and healthy control groups in terms of the frequency of the T allele (55.3% vs. 49.2%, p=0.371). The TT genotype frequency was significantly higher in the UC group than in the healthy control group (19.1% vs. 9.2%; p=0.034; OR 2.34; CI 95% 1.04-5.25). There was no significant difference between the groups in terms of the frequency of the CC genotype (UC 44.7% vs. control 50.8%; p=0.371) or the CT genotype (UC 36.2% vs. control 40%; p=0.567) (Table 2).

The relation between the rs3737240 SNP genotypes and allele frequencies and clinical parameters was also assessed. In the Ulcerative colitis patients, no significant association was found between the rs3737240 SNP genotypes and alleles and sex, age, localization of disease, duration of disease, age at diagnosis, history of colectomy, corticosteroid use, anti-TNF-alpha use, family history of IBD, or extraintestinal involvement (Table 3). The TT genotype was significantly associated with azathioprine use in UC patients (p=0.037; OR 3.0; CI 95% 1.68-8.65). In addition, there was a significant association between the C allele and azathioprine use in UC patients (p=0.037; OR 0.33 CI 95% 0.11-0.96) (Table 3). There was not a significant relationship

Table 2. *ECM1* rs3737240 SNP allele and genotype frequencies in ulcerative colitis and control group

	Controls (n=120)	UC (n=94)	p	OR (95% CI)
Allele frequencies (%)				
Allele C	109 (90.8)	76 (80.9)	0.034*	0.42 (0.19-0.95)
Allele T	59 (49.2)	52 (55.3)	0.371	1.28 (0.74-2.20)
Genotype frequencies (%)				
CC	61 (50.8)	42 (44.7)	0.371	0.78 (0.45-1.34)
CT	48 (40)	34 (36.2)	0.567	0.85 (0.48-1.48)
TT	11 (9.2)	18 (19.1)	0.034*	2.34 (1.04-5.25)

ECM 1: extracellular matrix protein-1; SNP: single nucleotide polymorphism; UC: ulcerative colitis; OR: odds ratio; CI: confidence interval
*p value is significant if <0.05

Table 3. Distribution of *ECM1* rs3737240 genotype and allele frequencies according to demographic and clinical characteristics of ulcerative colitis patients

	Genotype (n, %)			Allele (n, %)	
Clinical features	**CC**	**CT**	**TT**	**C**	**T**
Sex					
Female	21 (22.3)	12 (12.8)	8 (8.5)	33 (35.1)	20 (21.3)
Male	21 (22.3)	22 (23.4)	10 (10.6)	43 (45.7)	32 (34)
Localization					
Proctitis	7 (7.4)	9 (9.6)	3 (3.2)	16 (17)	12 (12.8)
Left sided colitis	10 (10.6)	11 (11.7)	5 (5.3)	21 (22.3)	16 (17)
Extensive colitis	25 (26.6)	14 (14.9)	10 (10.6)	39 (41.5)	24 (25.5)
Medications					
Corticosteroid	14 (14.9)	11 (11.7)	4 (4.3)	25 (26.6)	15 (16)
Azathioprine	11 (11.7)	8 (8.5)	9 (9.6)	19 (20.2)*	17 (18.1)
Anti-TNF-alpha	1 (1.1)	1 (1.1)	1 (1.1)	2 (2.1)	2 (2.1)
Extraintestinal manifestation	4 (4.3)	3 (3.2)	2 (2.1)	7 (7.4)	5 (5.3)
Colectomy	5 (5.3)	1 (1.1)	1 (1.1)	6 (6.4)	2 (2.1)
Family history of IBD	7 (7.4)	2 (2.1)	1 (1.1)	9 (9.6)	3 (3.2)

Anti-TNF-alpha: anti-tumor necrosis factor-alpha; IBD: inflammatory bowel disease
*p<0.05

Table 4. *ECM1* rs3737240 genotype and allele frequencies in patients with steroid-refractory and steroid-dependent disease

Response to steroid therapy	Genotype (n, %)			Allele (n, %)	
	CC	CT	TT	C	T
Steroid-refractory	6 (6.4)	5 (5.3)	6 (6.4)	11 (11.7)	11 (11.7)
Steroid-dependent	7 (7.4)	5 (5.7)	4 (4.3)	12 (12.8)	9 (9.6)

All p values were >0.05

between the rs3737240 SNP genotypes and allele frequencies and steroid-refractory or steroid-dependent patients (Table 4).

DISCUSSION

Both genetic and environmental factors play a role in the pathogenesis of UC. In recent years, more than 160 susceptibility loci for IBD have been identified in GWA studies (19). Of these loci, 23 have been identified as unique for UC (20). The identification of the epithelial barrier genes has been the most exciting development in the genetics of UC (21-24). Many studies show that the disruption of intestinal barrier function leads to IBD (25-27). The intestinal epithelial cells and intercellular junctions between these cells have the important task of gate keeping. *ECM1*, an intestinal barrier function gene, is a quite reasonable candidate in terms of predisposition to UC. The *ECM1* gene is encoding extracellular matrix protein 1, which is a glycoprotein expressed in epithelial organs. *ECM1* is expressed overall the intestine and interacts with the basal membrane. It has been shown that *ECM1*, inhibits MMP-9, and strongly activates NF-kappaB. NF-kappaB is the key immune regulator in the pathogenesis of IBD (28-30). It has been also shown that *ECM1* is overexpressed in metastatic epithelial tumors, including gastric and colorectal cancers, and its expression in hepatocellular carcinoma (HCC) is correlated with the metastatic potential of HCC (9,31).

In 2008, Fisher et al. (5) identified a previously unknown susceptibility locus at *ECM1* (rs3737240; $p=1.3\times10^{-4}$ and rs13294; $p=2.6\times10^{-4}$) as indicative of UC. This finding was replicated later by independent UC association studies (11,32). Additionally, the Wellcome Trust Case Control Consortium 2 (WTCCC 2) study of 15 complex disorders and traits reported a GWA scan in UC This study replicated the number of loci previously reported by Fisher et al. (5) to be associated *ECM1* with UC (33). Ankylosing spondylitis, like UC, is a chronic inflammatory disease, and there is a frequent clinical overlap between them. WTCCC 2 study reported a modest association between *ECM1* SNPs (rs3737240 and rs13294; p=0.0041 and 0.0044) and ankylosing spondylitis.

The present study examines the genotype and allele distribution of *ECM1* rs3737240 SNP and displays that there is an association between *ECM1* rs3737240 SNP and UC in a Turkish population. In the present study, there was a significantly higher frequency of the rs3737240 SNP TT genotype in the UC group than in healthy controls. The TT genotype increases the risk of UC by 2.34-fold (p=0.034, OR=2.34, 95% CI 1.04-5.25). Additionally, C allele frequency was significantly lower in UC patients than in healthy controls, demonstrating that the C allele is protective against UC. These results confirm that the rs3737240 SNP in the *ECM1* gene is associated with UC risk. Shi et al. (13) conducted a study in UC patients of Han Chinese descent and evaluated possible associations with 27 SNPs, including the *ECM1* rs3737240. This study, which included a total of 245 UC patients and 300 healthy controls, failed to demonstrate a relation between the rs3737240 SNP and UC. Gearry et al. (12) found no association between rs3737240 and UC or CD within the combined New Zealand Caucasian and Australian Caucasian cohorts. However, meta-analysis of rs3737240 SNP in all four cohorts has revealed a significant association with UC (p=0.0001, OR=1.14, 95% CI 1.08-1.20). Plevy et al. (14) showed in a North American multi-center study, which included 900 IBD patients (572 CD and 328 UC) that the *ECM1* rs3737240 SNP and the STAT3 rs744166 SNPs were not able to significantly differentiate between CD and UC, but these SNPs had a significant contribution to the random forest models. Festen et al. (11) confirmed in a large Dutch study sample the previously reported UC risk loci including *ECM1*, and a significant relation has been shown between the rs13294 SNP in the *ECM1* gene, but not with the rs3737240 SNP and UC. Fisher et al. (5) enrolled only European patients in their GWAS and found a significant relation between rs3737240 and UC. Genetic relationships between IBD and other autoimmune disorders usually give different results in European and Asian populations. The relation between NOD2/CARD15 gene mutations and Crohn's disease (CD) may be an example. While the relation is very strong in European patients with CD, the studies conducted in the Far East failed to find such a relation. In this study, *ECM1* rs3737240 SNP is associated with UC risk in Turkish patients, as in European Caucasians; and it indicates that Turkish UC patients might have a similar genotypic distribution as European Caucasians. In contrast, Meggyesi et al. (34) were not able to establish an association between the *ECM1* rs13294 SNP and UC in Eastern European patients, similarly, *ECM1* risk loci were not replicated in a Lithuanian-Latvian UC study sample (35). These results may have been because of the differences between various European populations.

In the present study, the UC patients with TT genotype had a significantly higher risk for the need of azathioprine (p=0.037; OR 3.0; CI 95% 1.68-8.65). Additionally, the C allele was protective against the need for azathioprine therapy (p=0.037; OR 0.33 CI 95% 0.11-0.96). However, no relationship was found between the genotypes and other disease phenotypes (e.g., localization of the disease, steroid use, disease course, age at diagnosis, and family history of IBD). This phenotypic association

is novel, but the functional implications of the polymorphism have not been defined. We did not find a novel association between the rs3737240 SNP in the *ECM1* gene and increased risk for severe forms of the disease, i.e., the necessity for colectomy or biological therapy. Therefore, further studies are required to reveal the association between the *ECM1* gene SNPs genotypes and phenotypes of the disease.

In conclusion, the present study demonstrates for the first time that there is a significant relation between the *ECM1* rs3737240 SNP and UC in a Turkish population. Small sample size of the polymorphism studies, as in the present study, is a limitation when detecting disease-polymorphism associations. In relation to that, population structure and admixture effects may increase the type I error rate of association. These can be overcome by using large healthy and patient populations with well-documented demographic characteristics. In this respect, the results presented here should be regarded as preliminary and might be considered as a first step of future research. Further studies with larger populations are required in order to confirm the clinical significance. *ECM1* gene polymorphisms in UC patients and to use these polymorphisms to predict disease progression and develop treatment strategies.

Ethics Committee Approval: Ethics committee approval was received for this study from Local Ethics Committee of İstanbul Medeniyet University, Göztepe Training and Research Hospital (Decision No: 19/T-14.02.2011).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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