Fractalkine receptor polymorphism may not be associated with the development and clinical course of ulcerative colitis

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INTRODUCTION

The migration of leukocytes from the vascular compartment into the inflammation area requires a series of complex interactions between leukocytes and endothelium. These intercellular interactions depend on the presence of the chemotactic gradient created by a large family of molecules called chemokines (chemotactic cytokines), along with the cell adhesion molecules expressed on the surfaces of endothelial cells and leukocytes [1]. Under the normal physiological conditions, chemokines selectively divert leukocyte subtypes to all tissues and organs [2]. Ulcerative colitis (UC) and Crohn’s disease (CD) are two chronic inflammatory bowel diseases (IBD), characterized by the altered levels and types of chemokines resulting in improper leukocyte aggregation in the target tissue.

To date, more than 40 chemokines have been discovered. So far, the only member of the identified CX³C chemokine family is fractalkine (FKN-CX³C1L1). FKN shows dual characteristics, acting both as a chemokine and as an adhesion molecule [3,4].

FKN is expressed during an inflammatory process and therefore takes place in the pathogenesis of numerous inflammatory conditions including cardiovascular, renal, rheumatologic and allergic diseases [3,5-7]. FKN expression on the endothelial and epithelial cells of the human bowel mucosa questioned the role of FKN regulation in mucosal immune response in IBD [8,9].

CX³CR1 is the specific receptor of FKN. CX³CR1 is expressed on the surface of CD4⁺ and CD8⁺ T cells, CD14⁺ monocytes and macrophages, and CD16⁺ NK cells [4,10]. CX³CR1 is highly expressed on the cytotoxic T-lymphocytes. CX³CR1-expressing cells are bound to FKN with high affinity regardless of the presence of endothelial adhesion molecules such as selectin and integrin. To date, several gene variations have been identified on the CX³CR1 encoding gene.

Among these, V249I (rs3732379) and T280M (rs3732378) polymorphisms are more common than the other genetic variations. These polymorphisms are implicated in atherosclerosis, coronary artery disease, and susceptibility to HIV
infection. They also influence CD phenotype and localization [11-13].

In this study, we aimed to determine the CX3CR1 polymorphisms and their correlation with clinical findings in patients with UC.

MATERIALS AND METHODS

Study population

A total of 51 UC patients attending the Department of Gastroenterology and Hepatology, Baskent University Ankara Hospital, were enrolled in the study. The diagnosis of UC was made on the basis of previously defined clinical guidelines, according to endoscopic, radiologic and histopathological criteria. These criteria were also used as a tool for patient selection [14-17]. Patients with indeterminate colitis were excluded from the study. Control group was composed of 80 healthy subjects attending the gastroenterology outpatient clinic with dyspeptic complaints. Informed consent was obtained from all study participants.

Demographic data and medical history of patients (gender, age, age at diagnosis, follow-up duration of the disease, localization of the colonic involvement and extraintestinal involvement (musculoskeletal system, skin, eye, hepatobiliary system)) were recorded.

Genotyping

Venous blood sample was obtained from each participant. Genomic DNA was extracted using commercially available kit (High Pure PCR Template Kit, Roche Diagnostics GmbH, Mannheim, Germany). Genotypes were determined by polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method. Two regions, each of which contained a single nucleotide polymorphism (SNP) site of the fractalkine receptor gene, were amplified. Primers for the PCR amplification were forward: 5’AGAATCATCCAGACGCTGTTTTCC3’ and reverse: 5’CAGAGGACAGCCAGGCATTCC3’. The size of amplified fragment length polymorphism (RFLP) method. Two regions, each of which contained a single nucleotide polymorphism (SNP) site of the fractalkine receptor gene, were amplified. Primers for the PCR amplification were forward: 5’AGAATCATCCAGACGCTGTTTTCC3’ and reverse: 5’CAGAGGACAGCCAGGCATTCC3’. The size of amplified restriction enzyme digestion genotypes were evaluated.

CX3CR1 polymorphism genotyping and clinical correlations

The association between V249I and T280M polymorphisms with the clinical findings (gender, age, age at diagnosis and follow-up duration of the disease, location of the intestinal involvement, intestinal involvement type, perianal involvement and extraintestinal involvement) in patients was examined.

Statistical methods

Independent two-sample t test was used to compare two groups and one-way analysis of variance was used for comparison of more than two groups. All analyses had a confidence interval of 95%. Variants in the analyses were grouped among themselves according to their characteristics. Difference in allele frequencies of CX3CR1 polymorphisms between IBD patients and the control group was determined using universe sample size, restriction endonuclease and predicted fragment lengths of amplicon and genotyping

TABLE 1. Amplicon size, restriction endonuclease and fragment lengths

<table>
<thead>
<tr>
<th>Site</th>
<th>Amplicon size</th>
<th>Restriction endonuclease</th>
<th>Fragment lengths (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V249I</td>
<td>311</td>
<td>ACLI</td>
<td>107-204 V/V</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>107-204-311 V/V</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>311/I</td>
</tr>
<tr>
<td>T280M</td>
<td>311</td>
<td>BSMBI</td>
<td>75-118 T/T</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>75-118-193 T/M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>118-193 M/M</td>
</tr>
</tbody>
</table>

RESULTS

Study group consisted of 30 males (58.8 %) and 21 females. Mean age of patients was 45.9±13.4. The mean age was 39.4±12.7 years at the time of diagnosis and patients were followed-up during the period from 1 to 37 years (mean 6.5±6.7). Twenty-four patients had proctitis or proctosigmoid involvement, while nine (17.6%) patients had extraintestinal involvement. The most common extraintestinal involvement was the musculoskeletal system (arthritis, ankylosing spondylitis, other spondyloarthropathies). Other extraintestinal involvements were as follows: one patient with aphthous stomatitis, one patient with dry eye, while hepatobiliary tract was affected in two patients (primary sclerosing cholangitis). Clinical and demographic characteristics of patients are shown in Table 2.

The distribution of the c.745G>A (V249I) and c.839C>T (T280M) genotypes and the allele frequencies were not different between the patients and controls. For V249I polymorphism 22 (32.8%) patients were heterozygous, while 3 (4.5%) patients were homozygous. A total of 12 (17.9%) patients were heterozygous for T280M polymorphism.

For both polymorphisms (V249I and T280M), no statistically significant difference was observed for gender (p=0.16 and p=0.5 respectively), age (p=0.8 and p=0.1 respectively), age at diagnosis (p=0.8 and p=0.07 respectively), follow-up duration of the disease (p=0.9 and p=0.8 respectively), localization of colonic involvement (p=0.9 and p=0.2 respectively), and localization of extraintestinal involvement (p=0.9).
extraintestinal involvement ($p=0.7$ and $p=0.2$, respectively).

Genotype distributions and allele frequencies of FKN receptor polymorphisms were shown in Table 3 and Table 4, respectively.

**DISCUSSION**

Both chemokines and their receptors participate in the pathogenesis of inflammatory disease by navigating circulating leukocytes and T cells to inflammatory sites. At the molecular level, they orchestrate tissue- and cell type-specific trafficking as well as retention of leukocytes. Previous studies showed the role of FKN and its receptor system in the development of inflammatory diseases. Rapid recruitment and inappropriate retention of leukocytes, particularly T-cells at the site of inflammation is a sign of chronic inflammatory disorders such as CD and UC [18,19].

Due to an increased release of FKN from intraepithelial cells, there is an increased number of CX3CR1+ T cells both in peripheral blood and intestinal lamina propria of IBD patients. Increased FKN production in the mucosa causes migration of a large number of CX3CR1+ leukocytes to the inflammation site [8,11,20,21]. It was also demonstrated that the level of expression of FKN receptors is much higher on Th1 cells in comparison to Th2 cells as a response to FKN. [22]. Recently, two common SNPs, V249I and T280M, were identified in the FKN receptor encoding gene. Both polymorphisms are located in the transmembrane domains of the receptor, causing a reduction in cell adhesion and possibly leading to the decreased signaling and chemotaxis [23-24].

Although various data about the genotype-phenotype relationship between FKN receptor polymorphisms and CD have been reported, there are no reports about this relationship for UC. In a study conducted on the sample of CD patients, Brand et al. determined that 33% of participants were heterozygous, while 8.9% were homozygous for V249I polymorphism. On the other hand, these percentages for T280M polymorphism were 23.3% and 4.4%, respectively. Authors observed that intestinal stenosis and ileocolonic involvement occurred more frequently in patients with T280M and V249I homozygous polymorphism than in heterozygous patients and wild type. Ileal involvement (89% ileocolonic, 11% ileal) was also observed in T280M homozygous patients [11].

In another study that was conducted on CD patients, Sabate et al found that heterozygosity and homozygosity for V249I polymorphism were 37.4% and 8.8%, while the frequency for T280M polymorphism was 8.1% and 1.3%, respectively. In this study, T280M homozygous genotype was observed in three patients, with two of them having been diagnosed with stenosis. V249I polymorphism was detected in patients with fibrostenosis [13].

In contrast to previous studies exploring the role of FKN in CD, in our study we aimed to determine FKN receptor polymorphism frequency and its correlation with clinical presentation in UC patients. We found 5.9% homozygous and 43.1% heterozygous patients for the V249I polymorphism and 23.5% heterozygous patients for the T280M polymorphism. Frequency distributions of both polymorphisms were similar to those in the control group (Table 3 and Table 4).

Our sub-group analyses revealed that neither V249I nor T280M polymorphisms were associated with clinical signs of UC.

So far, several hundreds of genes residing within the 16p genetic risk loci have been identified for IBD [25]. Although both CD and UC are inflammatory bowel diseases that share some genetic susceptibility loci, there are actually some differences [26]. Among these loci, 30 are CD-specific and 23 are UC-specific, whereas 110 are associated with both disease phenotypes [27]. According to this genetic background, regulation of mucosal immune cells is different in UC from CD. The molecular mechanism of CD depends on the Th1/Th17 balance [28,29]. FKN receptor is particularly expressed on

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**Table 2.** Demographic and clinical characteristics of the study population

| Female n (%) | 21 (41.2) |
| Male         | 30 (58.8) |
| Age (year) - mean±SD | 45.9±13.4 |
| Age at diagnosis (year) - mean±SD | 39.4±12.7 |
| Disease duration (year) - mean±SD | 6.5±6.7 (min-max: 1-37 years) |

**Table 3.** The CX3CR1 polymorphism distribution in UC patients

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>UC (n=51)</th>
<th>Control (n=80)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>V249I (n %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VV</td>
<td>26 (51.0)</td>
<td>49 (61.25)</td>
<td>0.491</td>
</tr>
<tr>
<td>VI</td>
<td>22 (43.1)</td>
<td>28 (35.0)</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>3 (5.9)</td>
<td>3 (3.75)</td>
<td>-</td>
</tr>
<tr>
<td>T280M (n %)</td>
<td></td>
<td></td>
<td>0.179</td>
</tr>
<tr>
<td>TT</td>
<td>39 (76.5)</td>
<td>52 (65.0)</td>
<td>-</td>
</tr>
<tr>
<td>TM</td>
<td>12 (23.5)</td>
<td>28 (35.0)</td>
<td>-</td>
</tr>
<tr>
<td>MM</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 4.** Allele frequencies of V249I and T280M polymorphisms in patient and control subjects

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>UC (n=51)</th>
<th>Control (n=80)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>V249I (n %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>74 (72.55)</td>
<td>126 (78.75)</td>
<td>0.76</td>
</tr>
<tr>
<td>I</td>
<td>28 (27.45)</td>
<td>34 (21.25)</td>
<td>0.23</td>
</tr>
<tr>
<td>T280M (n %)</td>
<td></td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>T</td>
<td>90 (88.24)</td>
<td>132 (82.50)</td>
<td>-</td>
</tr>
<tr>
<td>M</td>
<td>12 (11.76)</td>
<td>28 (17.50)</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Th1 cells. However, it has been shown that molecular mechanisms of UC mainly depend on Th2 cells [22,30]. As indicated by Thomson et al, genetic factors seem to be somewhat less significant for UC than they are for CD [28]. Our results are consistent with the results of the study published by Thomson et al. According to these data, clinical signs of UC may not be related to FKN receptor polymorphism.

CONCLUSION

In conclusion, in this study we tried to determine the possible involvement of FKN receptor polymorphism in UC pathogenesis and its relation with clinical outcomes. So far, no studies on the relationship between FKN receptor polymorphisms and clinical signs of UC have been published. In our study, we found that FKN receptor polymorphism and genotype-phenotype relation is not statistically significant in UC patients. Therefore, these polymorphisms of FKN may not contribute to the molecular pathogenesis of UC. However, limited number of patients enrolled to this study may be the major limitation of this study. Therefore, further studies with larger groups are required in order to determine the precise role of the FKN receptor polymorphisms in disease pathogenesis and its relation to clinical outcomes in patients with UC.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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REFERENCES


