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

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ABSTRACT

Fractalkine (CX3C), a chemokine expressed by epithelial cells within normal and inflamed colorectal mucosa, induces leukocyte adhesion and migration via fractalkine receptor. The aim of this study was to investigate two single nucleotide polymorphisms of the fractalkine receptor gene as a risk factor both for the development and clinical findings of ulcerative colitis. In this study, 51 patients with ulcerative colitis (UC) and 80 controls were recruited. Genotypes of fractalkine receptorc.745G>A (V249I) and c.839C>T (T280M) polymorphisms were identified by restriction fragment length polymorphism analyses after polymerase chain reaction.Genotype distribution and allele frequencies of V249I and T280M were not statistically significantly different between UC and control groups (p>0.05). No statistically significant relationship was found between fractalkine receptor polymorphisms and clinical findings of UC. We observed no significant difference in fractalkine receptor polymorphism between patients and control group and no genotype-phenotype relation. Therefore, we concluded that fractalkine receptor polymorphisms may not contribute to the molecular pathogenesis of UC.

KEY WORDS: Fractalkine; CX3CR1 polymorphism; 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 chemoattractant 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 CX_3C chemokine family is fractalkine (FKN-CX_3CL1). 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

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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'CACAGGACAGCCAGGCATTTCC3.' The size of amplicon, restriction endonuclease, and predicted fragment lengths are shown in Table 1. After 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.

TABLE 1. Amplicon size, restriction endonuclease and fragment lengths

Site	Amplicon size	Restriction endonuclease	Fragment lengths (bp)
V249I	311	ACLI	107-204 V/V 107-204-311 V/I 311I/I
T280M	311	BSMBI	75-118 T/T 75-118-193 T/M 118-193 M/M

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 ratio significance test. The value of $p<0.05$ was considered statistically significant. Statistical analyses were performed in SPSS 17.0 and MINITAB 13.0 statistical software programs (SPSS Inc., Chicago, IL).

RESULTS

Study group consisted of 30 males (58.8 %) and 21 females. Mean age of patients was 45.9±13.4.The mean age was39.4±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

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 was18.1% 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 163 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

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)
Localization n (%)	
Proctitis/proctosigmoiditis	24 (47%)
Left sided colitis	4 (7.8%)
Extensive colitis	17 (33.4%)
Pancolitis	6 (11.8%)
Extraintestinal manifestations n (%)	9 (17.6)

TABLE 3. The CX3CR1 polymorphism distribution in UC patients

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

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

Genotypes	*UC* (n=51)	*Control* (n=80)	*p*
V249I (n (%))			
V	74 (72.55)	126 (78.75)	0.76
I	28 (27.45)	34 (21.25)	0.23
T280M (n (%))			
T	90 (88.24)	132 (82.50)	0.84
M	12 (11.76)	28 (17.50)	0.11

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

[1] Proudfoot AE, Power CA, Rommel C, Wells TN. Strategies for chemokine antagonists as therapeutics. Semin Immunol 2003;15(1):57-65. DOI: 10.1016/S1044-5323(02)00128-8.

[2] Van Buul JD, Hordijk PL. Signaling in leukocyte transendotelial migration. Arterioscler Thromb Vasc Biol 2004; 24:824-833. DOI: 10.1161/01.ATV.0000122854.76267.5c.

[3] Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, et al. A new class of membrane-bound chemokine with a CX3C motif. Nature 1997;385(6617):640-644. DOI: 10.1038/385640a0.

[4] Imai T, Hieshima K, Haskell C, Baba M, Nagira M, Nishimura M, et al. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell 1997;91(4):521-530. DOI: 10.1016/S0092-8674(00)80438-9.

[5] Muehlhoefer A, Saubermann LJ, Gu X, Luedtke-Heckenkamp K, Xavier R, Blumberg RS, et al. Fractalkine is an epithelial and endothelial cell-derived chemoattractant for intraepithelial lymphocytes in the small intestinal mucosa. J Immunol 2000;164(6):3368-3376. DOI: 10.4049/jimmunol.164.6.3368.

[6] Furuichi K, Wada T, Iwata Y, Sakai N, Yoshimoto K, Shimizu M, et al. Upregulation of fractalkine in human crescentic glomerulonephritis. Nephron 2001; 87(4):314-320. DOI: 10.1159/000045936.

[7] Robinson LA, Nataraj C, Thomas DW, Howell DN, Griffiths R, Bautch V, et al. A role for fractalkine and its receptor (CX3CR1) in cardiac allograft rejection. J Immunol 2000;165(11):6067-6072. DOI: 10.4049/jimmunol.165.11.6067.

[8] Imaizumi T, Yoshida H, Satoh K. Regulation of CX3CL1/fractalkine expression in endothelial cells. J Atheroscler Thromb 2004; 11(1):15-21. DOI: 10.5551/jat.11.15.

[9] Chapman GA, Moores KE, Gohil J, Berkhout TA, Patel L, Green P, et al. The role of fractalkine in the recruitment of monocytes to the endothelium.Eur J Pharmacol 2000;392(3):189-195. DOI: 10.1016/S0014-2999(00)00117-5.

[10] Combadiere C, Salzwedel K, Smith ED, Tiffany HL, Berger EA, Murphy PM. Identification of CX3CR1. A chemotactic receptor for the human CX3C chemokine fractalkine and a fusion coreceptor for HIV-1. J Biol Chem 1998; 273(37):23799-23804.DOI: 10.1074/jbc.273.37.23799.

[11] Brand S, Haufbauer K, Dambacher J, Schnitzler F, Staudinger T, Pfennig S, et al. Increased expression of the chemokine fractalkine in Crohn's disease and association of the fractalkine receptor T280M polymorphism with a fibrostenosing disease phenotype. Am J Gastroenterol 2006; 101(1):99-106. DOI: 10.1111/j.1572-0241.2005.00361.x.

[12] McDermott DH, Fong AM, Yang Q, Sechler JM, Cupples LA, Merrell MN, et al. Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. J Clin Invest 2003; 111(8):1241-1250. DOI: 10.1172/JCI16790.

[13] Sabate JM, Ameziane N, Lamoril J, Jouet P, Farmachidi JP, Soulé JC, et al. The V249I polymorphism of the CX3CR1 gene is associated with fibrostenotic disease behavior in patients with Crohn's disease. Eur J Gastroenterol Hepatol 2008; 20(8):748-755. DOI: 10.1097/MEG.0b013e3282f824c9.

[14] Dignass A, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. J Crohns Colitis 2012;6(10):965-990. DOI: 10.1016/j.crohns.2012.09.003.

[15] Annese V, Daperno M, Rutter MD, Amiot A, Bossuyt P, East J, et al. European Crohn's and Colitis Organisation.European evidence based consensus for endoscopy in inflammatory bowel disease. J Crohns Colitis 2013;7(12):982-1018. DOI: 10.1016/j.crohns.2013.09.016.

[16] Magro F, Langner C, Driessen A, Ensari A, Geboes K, Mantzaris GJ, et al. European Society of Pathology (ESP); European Crohn's and Colitis Organisation (ECCO). European consensus on the histopathology of inflammatory bowel disease. J Crohns Colitis 2013;7(10):827-851. DOI: 10.1016/j.crohns.2013.06.001.

[17] Tontini GE, Vecchi M, Pastorelli L, Neurath MF, Neumann H. Differential diagnosis in inflammatory bowel disease colitis: state of the art and future perspectives. World J Gastroenterol 2015;21(1):21-46. DOI: 10.3748/wjg.v21.i1.21.

[18] Thomas S, Baumgart DC. Targeting leukocyte migration and adhesion in Crohn's disease and ulcerative colitis. Inflammopharmacology 2012; 20(1):1-18. DOI: 10.1007/s10787-011-0104-6.

[19] Nishimura M, Kuboi Y, Muramoto K, Kawano T, Imai T. Chemokines as novel therapeutic targets for inflammatory bowel disease. Ann N Y Acad Sci 2009; 1173:350-356. DOI: 10.1111/j.1749-6632.2009.04738.x.

[20] Sans M, Danese S, de la Motte C, de Souza HS, Rivera-Reyes BM, West GA, et al. Enhanced recruitment of CX3CR1+ T cells by mucosal endothelial cell-derived fractalkine in inflammatory bowel disease. Gastroenterology 2007; 132(1):139-153. DOI: 10.1053/j.gastro.2006.10.010.

[21] Kobayashi T, Okamoto S, Iwakami Y, Nakazawa A, Hisamatsu T, Chinen H, et al. Exclusive increase of CX3CR1+CD28-CD4+ T cells in inflammatory bowel disease and their recruitment as intraepithelial lymphocytes. Inflamm Bowel Dis 2007; 13(7):837-846.DOI: 10.1002/ibd.20113.

[22] Babakurban ST, Erbek SS, Terzi YK, Arslan F, Sahin FI.Fractalkine receptor polymorphism and chronic tonsillitis.Eur Arch Otorhinolaryngol 2014; 271(7):2045-2048. DOI: 10.1007/s00405-014-2908-7.

[23] Moatti D, Faure S, Fumeron F, Amara Mel-W, Seknadji P, McDermott DH, et al. Polymorphism in the fractalkine receptor CX3CR1 as a genetic risk factor for coronary artery disease. Blood 2001; 97(7):1925–1928. DOI: 10.1182/blood.V97.7.1925.

[24] Courivaud C, Bamoulid J, Loupy A, Deschamps M, Ferrand C, Simula-Faivre D, et al. Influence of fractalkine receptor gene polymorphisms V249I-T280M on cancer occurrence after renal transplantation. Transplantation 2013; 95(5):728–732. DOI: 10.1097/TP.0b013e31827d61cb.

[25] Fransen K, Mitrovic M, van Diemen CC, Weersma RK. The quest for genetic risk factors for Crohn's disease in the post-GWAS era. Genome Med 2011;3:13.

[26] Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science 2006; 314(5804):1461-1463. DOI: 10.1126/science.1135245.

[27] Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012;491(7422):119-124. DOI: 10.1038/nature11582.

[28] Thompson AI, Lees CW. Genetics of ulcerative colitis. Inflamm Bowel Dis 2011; 17(3):831-848. DOI: 10.1002/ibd.21375.

[29] Mannon PJ, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, et al. Anti-IL-12 Crohn's Disease Study Group: Anti-interleukin-12 antibody for active Crohn's disease. N Engl J Med 2004;351:2069–2079. DOI: 10.1056/NEJMoa033402.

[30] Gálvez J. Role of Th17 Cells in the Pathogenesis of Human IBD. ISRN Inflamm 2014;2014:928461. DOI: 10.1155/2014/928461.