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The role of polymorphisms of genes *CXCL12/CXCR4* and *MIF* in the risk development IBD the Polish population

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Abstract Inflammatory bowel disease (IBD) are characterized recurrent inflammation of gastrointestinal tract. The etiology and pathogenesis this disease is currently unclear, but it has become evident that immune and genetic factors are involved in this process. The aim of this study was to determine whether gene polymorphisms: *MIF*-173 G/C; *CXCL12*-801 G/A and *CXCR4* C/T exon 2 position of rs2228014 is associated with susceptibility to IBD. A total of 286 patients were examined with IBD, including 152 patients with ulcerative colitis and 134 with Crohn's disease (CD) and 220 healthy subjects were recruited from the Polish population. Genotyping for polymorphisms in *CXCL12/CXCR4* and *MIF* was performed by RFLP-PCR. Statistical significance was found for polymorphisms *CXCR4*, a receptor gene for *CXCL12* genotypes and alleles in CD and for genotype C/T and T allele in ulcerative colitis with respect to control. This confirms the effect of *CXCL12* gene. The interplay between *CXCL12* and its receptor *CXCR4* affects homeostasis and inflammation in the intestinal mucosa. Three-gene analysis in CD confirmed the association of genotype GGGGCT. Statistical analysis of clinical data of patients with ulcerative colitis showed significant differences in the distribution of genotype C/T and T allele for *CXCR4* in the left-side colitis. Having *CXCR4/CXCL12* chemokine axis polymorphisms may predispose to the development of IBD. Activation can also be their defensive reaction to the long-lasting inflammation.

Keywords *CXCL12* · *CXCR4* · Gene polymorphism · IBD · *MIF*

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Abbreviations

5-ASA	5-amino salicylic acid
AIDS	Acquired immune deficiency syndrome
AZP	Azothioprine
CD	Crohn's disease
CRP	C reactive protein
CXCL12/	Chemokine (C-X-C motif) Ligand
SDF-1	12/Stromal cell-Derived Factor 1 beta
CXCR-4	C-X-C chemokine receptor type 4
DSS	Dextran sulfate sodium
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV-1	Human immunodeficiency virus-1
IBD	Inflammatory bowel disease
IECS	Intestinal epithelial cells

MS	Multiple sclerosis
MTX	Methotrexate
OR	Odds ratio
RA	Rheumatoid arthritis
RFLP-PCR	Restriction fragment length polymorphism-polymerase chain reaction
SNP	Single nucleotide polymorphism
UC	Ulcerative colitis

Introduction

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are chronic intestinal, running with periods of exacerbation and remission, disorders associated with excessive immune system activity. Induction of pro-inflammatory cytokines contribute to excessive inflammation. The etiology and pathogenesis this disease currently unclear, but it has become evident that immune and genetic factors are involved in the process of IBD [1]. The frequency of occurrence in people with sick relatives as well as determining the racial-ethnic differences suggest a genetic etiology of these diseases [2]. Effect of environmental factors in people with genetic conditions for IBD leads to the overproduction of proinflammatory cytokines and cytokine abnormalities in the synthesis of intestinal mucosal homeostasis [3]. Etiology of IBD is associated with immune factors intestinal mucosa, intestinal microflora, and inter-individual predisposition [4]. In the mucosa of IBD patients had an increased concentration of chemokines released by the action of suitable cytokines, which may contribute to the fixation of tissue destructive inflammatory processes [5]. The *CXCL12* gene promoter region at position-801(rs1801157) showed the presence of genotype G/A. The change results in the formation sequence recognized by the transcription factor, which is associated with a significant increase in expression in the presence of the A allele. Having genotype A/A, G/A gene *CXCL12* increased risk of lung cancer [6] and has been associated with breast carcinoma [7]. CXCL12 is a constitutive chemokine involved in lung, brain, and joint inflammation [8]. Significantly higher levels of MIF has been found in the mucosa of the colon in mice with experimentally induced IBD as well as in the serum of patients with UC [9, 10]. There was a positive relationship between serum MIF levels with the CRP levels and activities of colitis [10]. It is also observed in serum C allele carriers with rheumatoid arthritis, acute respiratory distress syndrome and glomerulonephritis [11–13]. Since the change in the expression of these genes have been associated with changes in the gut, therefore, the possibility exists that the analyzed promoter region polymorphisms may predispose to the development of IBD. Due to the obtained in our previous studies of *MIF*-173 G/C, *CXCL12*-801 G/A result showing the importance of *MIF* gene polymorphism in the progression of colitis, and the lack of significance in the *CXCL12* gene, we tried to continue research on an extended group of patients. The results of the analysis of genotypes is likened to the patients clinical data indicating association between the polymorphism and the age of the patient, location of the disease, form its course, as well as to treatment. We studied the interaction between genetic polymorphisms. There is a need for genetic determinants of these diseases because of their unique inconvenient for patients. In patients resistant to drugs is performed to remove the affected segment or the entire colon. The operation brings effects mainly in the case of UC when changes are located exclusively in the colon. In the CD when the changes affect the entire gastrointestinal tract surgery usually does not produce the desired result. The search for genetic risk factor for IBD is early diagnosis of patients at risk. They can then take the program to prevent activation of IBD, of avoiding the known environmental risk factors and early treatment, reducing complications associated with a later injury.

Materials and methods

CXCL12, CXCR4 and MIF polymorphism was analyzed in 286 patients with IBD, including 152 patients with UC and 134 patients with CD. Control group consisted of 220 potential healthy subjects who showed no symptoms of the intestinal mucosa. Subjects enrolled to the study were from 18 to 60 years old, were hospitalized in Department of Gastroenterology and Internal Medicine, Medical University in Lodz and in Department of General and Colorectal Surgery, Medical University in Lodz. CU and CD were diagnosed on the basis of radiological, pathological and clinical criteria. Both the study group and control group were recruited from the Polish population. Patients constituting the control group were hospitalized for reasons not in connection with IBD. In this group were diagnosed; hernia, cholelithiasis, appendicitis, bowel ischemia, haemorrhoids, traumatic perforation of the intestine. In the test group are excluded chronic inflammatory and autoimmune diseases, cancer, and diabetes. Statistically, gender and age of patients in the control group did not let stand the test group. Bioethics Committee of Medical University in Lodz approved the study on 15.12.2009 (approval number RNN/835/09/KB).

Five milliliter of peripheral blood was collected from the study subjects to EDTA tubes; lymphocytes were obtained from these samples and were used to isolate DNA using DNA Gdansk kit, according to manufacturer's instructions. Analysis of polymorphic variants for *MIF and*

CXCL12, CXCR4 was performed using RFLP-PCR method [14–16].

Genotyping

The PCR oligonucleotide pairs were used restrictive test of polymorphic gene having the following sequence:

MIF-173:F; 5′ACTAAGAAAGACCCGAGGC-3′,
MIF-173:R; 5′GGGGCACGTTGGTGTTTA-3′,
CXCL-12:F; 5′-CTGGGCAAAGCTAGTGAAG-3′,
CXCL-12:R; 5′-AGAACGTGGAGGATGTGGAG-3′,
CXCR-4:F; 5′-AACTTCCTATGCAAGGCAGT-3′,
CXCR-4:R; 5′-TATCTGTCATCTGCCTCACT-3′.

PCR reactions for all tested gene polymorphisms was performed in a thermocycler MultiGene, manual Labnet International Inc., in a total volume of 20 µl containing 100 ng genomic DNA, 1.45 nmol of each oligonucleotide (Eurogentec, Seraing, Belgium), 200 mM of each deoxynucleotide dATP, dCTP, dGTP and dTTP (Qiagen, Germany), 20 mM TrisHCl (pH 8.4), 50 mM KCl, 1.5 µl MgCl2, and 1 U Taq polymerase (Qiagen, Germany). *MIF* gene reactions performed under the following temperature conditions: at 94 °C for predenaturation step 5 min, then 35 cycles of: denaturation at 94 °C for 30 s, attachment of oligonucleotides: 51 °C for 30 s, PCR amplification products at 72 °C for 30 s, and a final incubation at 72 °C for 5 min. The reaction products were subjected to PCR at 37 °C digested with the restriction enzyme *Alu*I (1.5 U). After 16 h in the restriction enzyme digestion, the samples were separated by electrophoresis on 2 % agarose gel, and the resulting restriction fragments were visualized by staining with ethidium bromide. In the case of homozygote G/G in the image electrophoretic bands are visible at a height of 2; 268, 98 bp; heterozygous G/C four bands are visible at a height of 268, 206, 98, 62 bp. Homozygous C/C illustrate three bands in the gel at a height of 206, 98, 62 bp. *CXCL12* gene PCR was performed under the following temperature conditions: the first step at 95 °C-5 min, then 35 cycles of 95 °C-30 s, 56 °C-30 s, 72 °C-30 s and a final step of 72 °C-5 min. PCR products were at 37 °C digested with the restriction enzyme *Msp*I (1.5 U). After 16 h of enzyme digestion, the samples were separated by electrophoresis on 8 % polyacrylamide gel, and the resulting fragments were stained with ethidium bromide. Homozygous G/G illustrate two stripes of 117 and 92 bp; heterozygous G/A-three bands on the amount of 209, 117, 92 bp. Homozygous A/A shows one band in the gel of 209 bp. PCR conditions *CXCR4* gene: predenaturation 94 °C—5 min, then 35 cycles of 94 °C—1 min, 60 °C—1 min, 72 °C—2 min, and a final incubation of 72 °C—20 min. The reaction products were subjected to PCR at 37 °C *BCC*I digestion with the restriction enzyme (1.5 U). After a 16-hour digestion with the enzyme, the samples were electrophoresed in 2 % agarose gel, and the resulting fragments were stained with ethidium bromide. Homozygous C/C gives an amount of two bands at 133, 103 bp, the heterozygote T/C three bands on the amount of 236, 133, 103 bp. Homozygous T/T one shows the band of the gel at a height of 236 bp.

Table 1 The *CXCL12*-801 G/A promoter region gene polymorphism in patients with IBD and control group

Genotype	IBD patients ($n = 286$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
G/G	199 (0.70)	146 (0.66)	Ref.
G/A	76 (0.27)	74 (0.34)	0.75 (0.51−1.12) $p = 0.149$
A/A	11 (0.04)	0 (0.0)	–
	$p = 0.278$	$p = 0.003$	
G	474 (0.83)	366 (0.83)	Ref.
A	98 (0.17)	74 (0.17)	1.02 (0.73–1.42) $p = 0.887$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy–Weinberg equilibrium

Statistical analysis

The odds ratio and the significance of differences in allele frequencies and genotype distributions between the two groups for *MIF, CXCL12, CXCR4* was assessed χ^2 test. The group performed the test and balance test Hardy–Weinberg's the corresponding 95 % confidence intervals (ORs) were used to assess the relationship between the genotypes and alleles of test and control groups. Statistical analysis was also conducted for these genetic polymorphisms in relation to clinical data of patients with CU and CD in order to determine the relationship between, age at onset, disease location, form its course, surgical and used prescribed drugs. The reference group in a particular location, the course of the disease and surgical treatment used were those in whom it did not exist. Patients were divided into three age ranges: up to 16, 16–40 and above 40 years. The control group for single age group accounted for the other person. IBD patients who have completed treatment applied only to acid and 5-ASA for the control group were treated with steroids and immunosuppressive drugs AZP; MTX.

Results

Genotype distributions of patients and relevant reference groups are given in Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. Tables 1, 2, and 3 shows the distribution of

Table 2 The *CXCL12*-801 G/A promoter region gene polymorphism in patients with UC and control group

Genotype	UC patients ($n = 152$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
G/G	104 (0.68)	146 (0.66)	Ref.
G/A	41 (0.27)	74 (0.34)	0.78 (0.49−1.23) $p = 0.280$
A/A	7 (0.05)	0 (0.00)	–
	$p = 0.268$	$p = 0.003$	
G	249 (0.82)	366 (0.83)	Ref.
A	55 (0.18)	74 (0.17)	1.09 (0.74−1.60) $p = 0.650$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy–Weinberg equilibrium

Table 3 The *CXCL12*-801 G/A promoter region gene polymorphism in patients with CD and control group

Genotype	CD patients ($n = 134$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
G/G	95 (0.71)	146 (0.66)	Ref.
G/A	35 (0.26)	74 (0.34)	0.73 (0.45−1.17) $p = 0.190$
A/A	4 (0.03)	0 (0.00)	–
	$p = 0.724$	$p = 0.003$	
G	225 (0.84)	366 (0.83)	Ref.
A	43 (0.16)	74 (0.17)	0.94 (0.63−1.42) $p = 0.790$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy–Weinberg equilibrium

Table 4 The *MIF*-173 G/C promoter region gene polymorphism in patients with IBD and control group

Genotype	IBD patients ($n = 286$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
G/G	200 (0.70)	139 (0.63)	Ref.
G/C	81 (0.28)	78 (0.35)	1.38 (0.95−2.02) $p = 0.090$
C/C	5 (0.02)	3 (0.02)	0.86 (0.20−3.67) $p = 0.570$
	$p = 0.322$	$p = 0.028$	
G	481 (0.84)	356 (0.81)	Ref.
C	91 (0.16)	84 (0.09)	0.80 (0.58−1.11) $p = 0.180$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy−Weinberg equilibrium

genotypes and allele frequencies for *CXCL12* polymorphism-801 G/A promoter region of the gene, respectively, in patients with IBD, UC, CD with respect to the control group. There were no statistically significant differences between the groups. Sample genotypes obtained by electrophoresis for polymorphisms of gene-801 G/A *CXCL12,* are shown in Fig. 1.

Table 5 The *MIF*-173 G/C promoter region gene polymorphism in patients with UC and control group

Genotype	UC patients ($n = 152$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
G/G	105 (0.69)	139 (0.63)	Ref.
G/C	45 (0.30)	78 (0.35)	0.76 (0.49−1.19) $p = 0.230$
C/C	2 (0.01)	3 (0.02)	0.88 (0.14−5.38) $p = 0.630$
	$p = 0.242$	$p = 0.028$	
G	255 (0.84)	356 (0.81)	Ref.
C	49 (0.16)	84 (0.09)	0.81 (0.55−1.20) $p = 0.300$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy–Weinberg equilibrium

Table 6 The *MIF*-173 G/C promoter region gene polymorphism in patients with CD and control group

Genotype	CD patients ($n = 134$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
G/G	95 (0.71)	139 (0.63)	Ref.
G/C	36 (0.27)	78 (0.35)	0.67 (0.42−1.08) $p = 0.100$
C/C	3 (0.02)	3 (0.02)	1.46 (0.29−7.40) $p = 0.470$
	$p = 0.849$	$p = 0.028$	
G	226 (0.84)	356 (0.81)	Ref.
C	42 (0.16)	84 (0.09)	0.79 (0.52−1.18) $p = 0.250$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy–Weinberg equilibrium

Table 7 *CXCR4* gene polymorphism C/T exon 2 position rs2228014 in patients with IBD and control group

Genotype	IBD patients ($n = 286$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
C/C	210 (0.73)	195 (0.89)	Ref.
C/T	62 (0.22)	23 (0.10)	2.50 (1.49−4.20) $p = 0.0004$
T/T	14 (0.05)	2 (0.01)	6.50 (1.46−28.97) $p = 0.005$
	$p = 0.002$	$p = 0.758$	
C	482 (0.84)	413 (0.94)	Ref.
T	90 (0.16)	27 (0.06)	2.86 (1.82−4.48) $p < 0.0001$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy–Weinberg equilibrium

Table 8 *CXCR4* gene polymorphism C/T exon 2 position rs2228014 in patients with UC and control group

Genotype	UC patients ($n = 152$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
C/C	120 (0.79)	195 (0.89)	Ref.
C/T	28 (0.18)	23 (0.10)	1.98 (1.09−3.59) $p = 0.023$
T/T	4 (0.03)	2 (0.01)	3.25 (0.59−18.01) $p = 0.159$
	$p = 0.146$	$p = 0.758$	
C	268 (0.88)	413 (0.94)	Ref.
T	36 (0.12)	27 (0.06)	2.05 (1.22−3.64) $p = 0.006$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy–Weinberg equilibrium

Table 9 *CXCR4* gene polymorphism C/T exon 2 position rs2228014 in patients with CD and control group

Genotype	CD patients ($n = 134$)[a]	Control ($n = 220$)[b]	OR (PU 95 %)
C/C	90 (0.67)	195 (0.89)	Ref.
C/T	34 (0.25)	23 (0.10)	3.20 (1.78−5.75) $p < 0.0001$
T/T	10 (0.07)	2 (0.01)	10.83 (2.32−50.46) $p = 0.0005$
	$p = 0.14$	$p = 0.758$	
C	214 (0.80)	413 (0.94)	Ref.
T	54 (0.20)	27 (0.06)	3.86 (2.36−6.30) $p < 0.0001$

[a] $p > 0.05$

[b] $p > 0.05$, Hardy–Weinberg equilibrium

Tables 4, 5, and 6 show the distributions for *MIF* polymorphism-173 G/C promoter region of the gene in patients with IBD, UC, CD compared to the control group. There were no statistically significant differences in the distribution of genotypes and allele frequencies between analyzed groups. There has been a tendency to higher incidence of disease in the group of IBD patients with genotype G/C *MIF* gene [OR = 1.38 (0.95−2.02) $p = 0.09$] (Table 4). Sample genotypes obtained by electrophoresis for polymorphisms of gene-173 G/C *MIF*, are shown in Fig. 2.

Tables 7, 8, and 9 shows the distribution of the polymorphism genotype C/T polymorphism in exon 2 of *CXCR4* rs2228014 position. In the case of IBD statistical significance concerned the distribution of genotypes C/T [OR = 2.50 (1.49−4.20) $p = 0.0004$], T/T [OR = 6.50 (1.46−28.97) $p = 0.005$] and T allele [OR = 2.86 (1.82−4.48) $p < 0.0001$] (Table 7). For UC statistically significant differences in the distribution of genotype C/T [OR = 1.98 (1.09−3.59) $p = 0.023$], and in the frequency of T allele [OR = 2.05 (1.22−3.64) $p = 0.006$] (Table 8). The CD significant differences related to the distribution of genotypes C/T [OR = 3.20 (1.78−5.75) $p < 0.0001$], T/T [OR = 10.83 (2.32−50.46) $p = 0.0005$] and T allele [OR = 3.86 (2.36−6.30) $p < 0.0001$] (Table 9). Sample genotypes obtained by electrophoresis for polymorphisms of gene C/T *CXCR4*, are shown in Fig. 3.

Tables 10, 11, and 12 show the three-gene according to the individual genotypes *CXCL12, CXCR4, MIF* in patients with IBD, UC, CD with respect to the control. Demonstrated statistical significance in the distribution of genotype GGGGCT for patients with IBD [OR = 4.49 (1.80−11.2) $p = 0.0006$] (Table 10), and the CD [OR = 7.08 (2.69−18.6) $p < 0.0001$] (Table 12). Tables 13, 14, and 15 show the relationship between the values of OR genotypes and alleles and the clinical data for each of the analyzed polymorphisms *CXCL12, MIF, CXCR4* in the CD. Not according to statistics found in the distribution of genotypes and alleles between the test and control group. Having a C allele polymorphism-173 G/C *MIF* reduced the risk of CD, an inflammatory form. Tables 16, 17, and 18 show the relationship between the distributions of individual genotypes and alleles for the studied polymorphisms *CXCL12, MIF, CXCR4* in UC. There was a statistical correlation for genotype C/T [OR = 2.44 (1.03−5.75) $p = 0.038$] and T allele [OR = 2.23 (1.09−4.56) $p = 0.025$] gene *CXCR4* (Table 18) in the left-side colitis. There was also a tendency for that gene in UC to a higher frequency of T allele [OR = 1.99 (0.95−4.17) $p = 0.064$] in patients 17–40 years of age (Table 18). Trend to genotype G/A [OR = 1.95 (0.93−4.09) $p = 0.076$], and A allele [OR = 1.73 (0.95−3.15) $p = 0.069$] in UC (Table 16) gene *CXCL12* was observed in patients over 40 years.

Discussion

MIF is an important pro-inflammatory cytokine and plays a role in immune reactions and exacerbation of inflammatory diseases such as asthma [17], chronic hepatitis B [18], allergic neuritis [19] and rheumatoid arthritis [20]. There are also reports of increased expression of MIF in gastric inflammation caused by Helicobacter pylori infection [21, 22] acute lung diseases [12] and glomerulonephritis [13]. Found higher levels of MIF in the serum of mice suffering from experimentally induced CD colitis [23], compared to healthy subjects. MIF plays important roles in the pathogenesis of gastrointestinal, hepatic, and pancreatic disorders [24]. Having the C allele of the gene *MIF*-173 G/C also increased risk of susceptibility to juvenile idiopathic arthritis [25]. MIF synthesis in human intestinal mucosa may indicate a role of this cytokine in regulating the

Table 10 Distributions of *CXCL12, CXCR4* and *MIF* SNPs combinations in patients with IBD and control group

Genotype	IBD patients ($n = 286$)	Control ($n = 220$)	OR (95 % PU)
GGGGCC	104 (0.36)	85 (0.39)	Ref.
GGGGCT	33 (0.12)	6 (0.03)	4.49 (1.80−11.2) $p = 0.0006$
GGGGTT	4 (0.01)	1 (0.00)	3.27 (0.36−29.8) $p = 0.263$
GGGCCC	42 (0.15)	47 (0.21)	0.73 (0.44−1.21) $p = 0.222$
GGGCCT	8 (0.03)	4 (0.02)	1.63 (0.47−5.61) $p = 0.431$
GGGCTT	5 (0.02)	0 (0.00)	–
GGCCCC	3 (0.01)	3 (0.01)	0.82 (0.16−4.15) $p = 0.562$
GGCCCT	0 (0.00)	0 (0.00)	–
GGCCTT	0 (0.00)	0 (0.00)	–
GAGGCC	36 (0.13)	34 (0.15)	0.86 (0.50−1.50) $p = 0.603$
GAGGCT	11 (0.04)	12 (0.05)	0.75 (0.31−1.78) $p = 0.512$
GAGGTT	3 (0.01)	1 (0.00)	2.45 (0.25−24.0) $p = 0.397$
GAGCCC	19 (0.07)	26 (0.12)	0.60 (0.31−1.15) $p = 0.122$
GAGCCT	5 (0.02)	1 (0.00)	4.09 (0.47−35.65) $p = 0.171$
GAGCTT	1 (0.00)	0 (0.00)	–
GACCCC	0 (0.00)	0 (0.00)	–
GACCCT	1 (0.00)	0 (0.00)	–
GACCTT	0 (0.00)	0 (0.00)	–
AAGGCC	5 (0.02)	0 (0.00)	–
AAGGCT	3 (0.01)	0 (0.00)	–
AAGGTT	1 (0.00)	0 (0.00)	–
AAGCCC	0 (0.00)	0 (0.00)	–
AAGCCT	1 (0.00)	0 (0.00)	–
AAGCTT	0 (0.00)	0 (0.00)	–
AACCCC	1 (0.00)	0 (0.00)	–
AACCCT	0 (0.00)	0 (0.00)	–
AACCTT	0 (0.00)	0 (0.00)	–

immune response in CD mucosa, especially that MIF is known to play an important role in many inflammatory diseases. These results demonstrate the important role of MIF in the course of inflammation in the colon. By examining a group of patients with IBD, we want to confirm whether the Polish population allele C at position-173 associated with increased expression of MIF gene can cause excessive activation and proliferation of T cells and increased production of pro-inflammatory cytokines and thus lead to chronic inflammation in the intestinal mucosa. Carried out by us earlier on a smaller group of patients with IBD study of polymorphism in the promoter region of the gene MIF showed significant differences in the distribution and allele frequencies between the study groups. The risk of IBD was about twice as high as for the G/C genotype [OR = 2.02 (1.08−1.93)] and the C allele [OR = 1.89 (1.09−3.29)]. The statistical significance was found in genotype distributions of patients with UC and controls. The results showed that a high proportion of C allele is associated with UC genotype G/C [OR = 2.15 (1.05−4.39)] C allele [OR = 1.95 (1.04−3.66)], were also not statistically different parts in the distribution of genotypes and allele frequencies of polymorphism G/C *MIF* gene of patients with CD and the control group [26]. It is known that the effect of each polymorphism on risk of developing a particular disease entity varies between populations, and studies in different populations are inconclusive results. After the test group increased tendency in the group with IBD remains, however, there is no statistical dependence. MIF gene polymorphisms tested-173 G/C, although it does not increase the risk of these diseases in the course can affect their progression. Gene polymorphism-173G/C *MIF* shows its different roles depending on the population. The population of Spanish possession-173G/C MIF significantly increased the risk of GC and CC genotypes (95 % confidence interval CI 1.41 (1.10−1.81); $p = 0.005$). The results indicate an increase of predisposition to CD in the vectors of the polymorphism [27]. MIF is a proinflammatory cytokine with increased expression in IBD. MIF was expressed in the intestinal mucosa of patients with CD and

Table 11 Distributions of *CXCL12, CXCR4* and *MIF* SNPs combinations in patients with UC and control group

Genotype	UC patients ($n = 152$)	Control ($n = 220$)	OR (95 % PU)
GGGGCC	58 (0.38)	85 (0.39)	Ref.
GGGGCT	10 (0.07)	6 (0.03)	2.44 (0.84−7.09) $p = 0.092$
GGGGTT	0 (0.00)	1 (0.00)	–
GGGCCC	27 (0.18)	47 (0.21)	0.84 (0.47−1.50) $p = 0.560$
GGGCCT	5 (0.03)	4 (0.02)	1.83 (0.47−7.11) $p = 0.292$
GGGCTT	2 (0.01)	0 (0.00)	–
GGCCCC	2 (0.01)	3 (0.01)	0.98 (0.16−6.03) $p = 0.675$
GGCCCT	0 (0.00)	0 (0.00)	–
GGCCTT	0 (0.00)	0 (0.00)	–
GAGGCC	20 (0.13)	34 (0.15)	0.86 (0.45−1.64) $p = 0.655$
GAGGCT	9 (0.06)	12 (0.05)	1.10 (0.43−2.78) $p = 0.841$
GAGGTT	1 (0.01)	1 (0.00)	1.46 (0.09−23.9) $p = 0.650$
GAGCCC	9 (0.06)	26 (0.12)	0.51 (0.22−1.16) $p = 0.074$
GAGCCT	2 (0.01)	1 (0.00)	2.93 (0.26−33.08) $p = 0.367$
GAGCTT	0 (0.00)	0 (0.00)	–
GACCCC	0 (0.00)	0 (0.00)	–
GACCCT	0 (0.00)	0 (0.00)	–
GACCTT	0 (0.00)	0 (0.00)	–
AAGGCC	4 (0.03)	0 (0.00)	–
AAGGCT	2 (0.01)	0 (0.00)	–
AAGGTT	1 (0.01)	0 (0.00)	–
AAGCCC	0 (0.00)	0 (0.00)	–
AAGCCT	0 (0.00)	0 (0.00)	–
AAGCTT	0 (0.00)	0 (0.00)	–
AACCCC	0 (0.00)	0 (0.00)	–
AACCCT	0 (0.00)	0 (0.00)	–
AACCTT	0 (0.00)	0 (0.00)	–

intestinal epithelial cells, but its expression correlated with the degree of inflammation and was not up regulated by pro-inflammatory cytokines. Polymorphism-173 G/C did not affect the level of *MIF* in serum. The present polymorphism CD showed a protective effect for the upper digestive tract prior to its engagement in severe disease activity [28]. This confirms we have obtained depending on the protective effect of clinical forms of inflammatory gene *MIF*.

Another cytokine affecting the increased infiltration of lymphocytes and macrophages and their steady stream to the site of inflammation was CXCL12 with its receptor CXCR4. CXCL12 is involved in stem cell proliferation and migration [8]. The CXCL12 and its receptor, CXCR4, are essential for life during development, and this ligand-receptor pair has been shown to have a fundamental role in neuron migration during cerebellar formation. Expression of these cytokines may be increased by inflammatory mediators, causing maintaining chronic inflammation [29]. Increased levels of expression CXCR4/CXCL12 found in inflammatory changes in MS, trauma, stroke, Alzheimer's disease, tumor progression, and acquired immunodeficiency syndrome-associated dementia which indicates the role of chemokines in inflammation of the central nervous system [29, 30]. Activation of chemokine axis found in a number of inflammatory diseases such as RA, IBD, uveitis, nephritis, and lupus erythematosus [31]. The human intestinal mucosa CXCR4/CXCL12 involved in the mechanism of regulation of angiogenesis [32]. CXCL12 through CXCR4 regulates the migration of intestinal epithelial cells, is involved in innate immunity [33]. CXCL12/CXCR4 controls the important contribution of neutrophils to atherogenesis in mice [34], played an important role in the formation and maintenance of immune cells in the liver during chronic HCV and HBV [35]. CXCR4 is also a major receptor for strains of HIV-1 that arise during progression to immunodeficiency and AIDS dementia [36]. CXCL12 is involved in cell neoplasia [29]. Axis of these chemokines play a major role in carcinogenesis, chronic inflammation increases the risk of cancer of the esophagus and stomach,

Table 12 Distributions of *CXCL12, CXCR4* and *MIF* SNPs combinations in patients with CD and control group

Genotype	CD patients ($n = 134$)	Control ($n = 220$)	OR (95 % PU)
GGGGCC	46 (0.34)	85 (0.39)	Ref.
GGGGCT	23 (0.17)	6 (0.03)	7.08 (2.69−18.6) $p < 0.0001$
GGGGTT	4 (0.03)	1 (000)	7.39 (0.80−68.09) $p = 0.610$
GGGCCC	15 (0.11)	47 (0.21)	0.59 (0.30−1.17) $p = 0.128$
GGGCCT	3 (0.02)	4 (0.02)	1.38 (0.30−6.46) $p = 0.480$
GGGCTT	3 (0.02)	0 (0.00)	–
GGCCCC	1 (0.01)	3 (0.01)	0.61 (0.06−6.09) $p = 0.566$
GGCCCT	0 (0.00)	0 (0.00)	–
GGCCTT	0 (0.00)	0 (0.00)	–
GAGGCC	16 (0.12)	34 (0.15)	0.87 (0.43−1.74) $p = 0.689$
GAGGCT	2 (0.01)	12 (0.05)	0.31 (0.07−1.44) $p = 0.097$
GAGGTT	2 (0.01)	1 (0.00)	3.69 (0.33−41.86) $p = 0.291$
GAGCCC	10 (0.07)	26 (0.12)	0.71 (0.31−1.60) $p = 0.409$
GAGCCT	3 (0.02)	1 (0.00)	5.54 (0.56−54.82) $p = 0.136$
GAGCTT	1 (0.01)	0 (0.00)	–
GACCCC	0 (0.00)	0 (0.00)	–
GACCCT	1 (0.01)	0 (0.00)	–
GACCTT	0 (0.00)	0 (0.00)	–
AAGGCC	1 (0.01)	0 (0.00)	–
AAGGCT	1 (0.01)	0 (0.00)	–
AAGGTT	0 (0.00)	0 (0.00)	–
AAGCCC	0 (0.00)	0 (0.00)	–
AAGCCT	1 (0.01)	0 (0.00)	–
AAGCTT	0 (0.00)	0 (0.00)	–
AACCCC	1 (0.01)	0 (0.00)	–
AACCCT	0 (0.00)	0 (0.00)	–
AACCTT	0 (0.00)	0 (0.00)	–

promotes tumor progression and metastasis to distant organs of high CXCL12 production such as lungs, liver, bone marrow [37, 38]. CXCL12/CXCR4 axis by blocking antagonist of CXCR4 involving eased artificially induced colitis in mice with DSS [39]. Adhesion, migration and proliferation of cells grown in response to chemokine CXCL12 in samples derived from patients with colon cancer. These effects were abrogated by the addition of anti-CXCR4 antibodies [40]. CXCL12 may be a factor in many inflammatory diseases of different surfaces, but it can also be anti-inflammatory, which challenges the idea of a strictly pathogenic role of this chemokine in chronic inflammatory and autoimmune [29]. CXCL12/CXCR4 chemokine axis is involved in the angiogenic response, which may play an important role in intestinal homeostasis and inflammation in the repair of physiological human intestinal mucosa [32]. CXCR4 plays an important role in regulating constitutive functions intestinal epithelial cells, such as epithelial cells, the secretion of electrolytes. The expression of CXCR4 by IECS, and its cognate ligand SDF-1/CXCL12, by cells within the intestinal mucosa suggests that CXCR4 plays a role in intestinal mucosal function [41]. CXCL12 plays a role in the regeneration of the colonic epithelium [42]. Increased expression of CXCL12/CXCL4 by the IECS IBD may be a defense mechanism aimed at the reconstruction of the damaged epithelium. CXCL12 and its receptor, CXCR4, would have a dual, constitutive, and inflammatory role in the intestinal immune system. The pair CXCL12–CXCR4 may have a significant dual role in intestinal homeostasis and inflammation [8]. Due to reports that high levels of CXCL12 was associated with extensive changes in the intestine, conducted research towards finding the relationship between the presence of this polymorphism, and increased incidence of IBD in the Polish population. Preliminary results of our analysis of *CXCL12* gene polymorphism-801 G/A in the group of 188 patients with IBD, 103 CU, 72 CD were compared to 184 healthy subjects of reference. There were

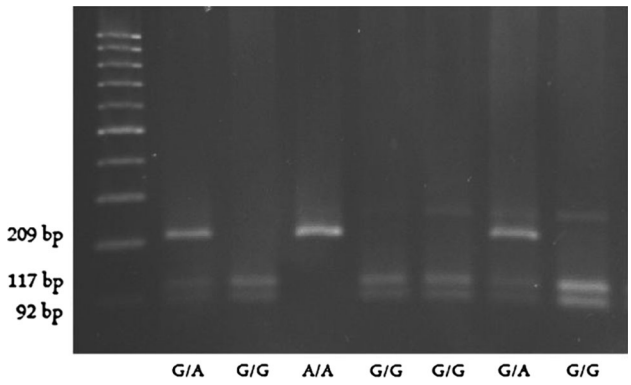

Fig. 1 *CXCL12* after digestion: G/G 117,92 bp; G/A 209,117,92 bp; A/A 209 bp

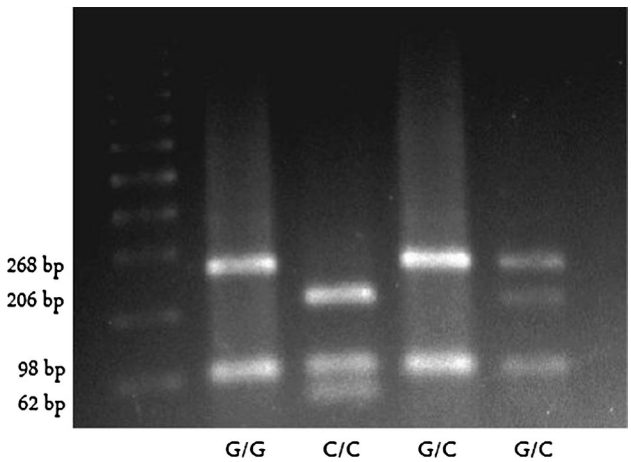

Fig. 2 *MIF* after digestion: G/G 98,268 bp; G/C 62,98,206,268 bp; C/C 62,98,206 bp

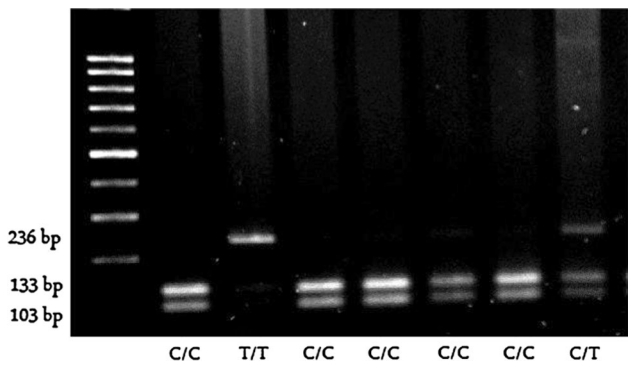

Fig. 3 *CXCR4* after digestion C/C 133,103 bp; C/T 236,133,103 bp; T/T 236 bp

no statistically significant differences in the distribution of genotypes and allele frequencies between the two groups [43]. The resulting statistical relationships *CXCL12* receptor *CXCR4* gene confirm the effect of this gene. The results indicate that the possession of *CXCL12* gene polymorphism at position-801 G/A, despite not achieving statistical significance in the Polish population by the active form can CXCR4 receptor to increase the risk of UC and CD. The presence of genotype C/T (T allele have) receptor *CXCR4* in patients with UC was associated with an increased incidence of left-sided colitis, confirming the association of the disease with the occurrence location *CXCR4* gene polymorphism of *CXCL12*. Left-sided colitis is inflammation of the rectum, sigmoid and descending colon up to the splenic bend and the initial part of the transverse colon. The left side of the rectum is much narrower than the right, sigmoid portion is exposed to the formation of colonic diverticulitis, in the rectum and the sigmoid colon polyps appear frequently hyperplastic whose frequency increases with age. Also in the end portion (rectum) and the sigmoid usually develop malignant tumors often not removed polyps, which can lead to total or partial obstruction of the bowel. Since CXCR4 plays an important role in regulating intestinal function of epithelial cells, and its expression can be increased by inflammatory mediators maintaining the state of chronic inflammation, found a statistical correlation between having genotype C/T and T allele *CXCR4*, left-side and the occurrence of inflammation of the colon may be associated with the susceptibility of the intestine due to its construction and the function of the formation of inflammation. In the literature, there were no more similar ones. The left side of the colon is also more vulnerable to ischemia in the form of ischemic colitis [44].

Having *CXCR4/CXCL12* gene polymorphisms with age also gave predisposition to developing UC. The resulting three-gene depending on the statistical analysis of genotype GGGGCT confirm the participation of polymorphism C/T *CXCR4* gene in IBD, as well as CD. The results obtained for *CXCR4/CXCL12* suggest that polymorphisms studied indicate the involvement of these proteins in the progression of IBD, and having a genotype C/T and G/A may be associated with disease severity.

The clinical manifestations of CD and UC are highly variable, with significant diversity in phenotypes of the diseases. This diversity may manifest as a difference in age of onset. The primary difference in disease phenotype is extent of disease. Patients are more likely to have severe disease and become steroid-dependent [45]. The studies on the prevalence of IBD in the Spanish population showed that CD patients diagnosed at age younger than 35 years, while in patients with UC diagnosed between the ages of 25 and 64 years old. CD affects mainly young people, while UC prevalent in middle-aged patients [46]. Our research in the Polish population showed a similar upward trend with age predisposition to UC. The literature lacks

Table 13 Analysis of the ORs (and 95 % CI) genotypes and allele of *CXCL12* gene polymorphism-801G/A with respect to clinical data of CD

CD disease	*CXCL12* OR (CI 95 %)				
Genotype/allele	G/G	G/A	A/A	G	A
Age at first diagnosis	Ref.			Ref.	
0–16 years		0.46 (0.12−1.68) $p = 0.228$	4.56 (0.60−34.85) $p = 0.167$		1.03 (0.42−2.52) $p = 0.920$
17–40 years		1.04 (0.46−2.36) $p = 0.92$	0.68 (0.09−5.04) $p = 0.542$		0.95 (0.48−1.88) $p = 0.887$
>40 years		1.5 (0.61−3.67) $p = 0.371$	−		1.04 (0.48−2.27) $p = 0.920$
CD location					
Colonic		0.76 (0.34−1.72) $p = 0.51$	1.17 (0.16−8.68) $p = 0$		0.86 (0.44−1.70) $p = 0.670$
Ileal		1.26 (0.49−3.26) $p = 0$	1.31 (0.13−13.4) $p = 0$		1.22 (0.56−2.7) $p = 0.610$
Ileocolonic		1.12 (0.49−2.59) $p = 0$	0.65 (0.06−6.57) $p = 0$		1.0 (0.49−2.03) $p = 1.000$
Upper Gi		0.66 (0.07−6.17) $p = 0.589$	−		0.56 (0.07−4.55) $p = 0.495$
CD behaviour					
Inflammatory		1.05 (0.46−2.38) $p = 0.920$	0.54 (0.05−5.39) $p = 0.515$		0.92 (0.46−1.85) $p = 0.823$
Penetrating		0.69 (0.26−1.79) $p = 0.442$	0.85 (0.08−8.59) $p = 0.689$		0.76 (0.34−1.69) $p = 0.497$
Stricturing		0.87 (0.34−2.18) $p = 0.764$	0.90 (0.09−9.10) $p = 0.708$		0.89 (0.41−1.94)
Perianal disease		1.21 (0.51−2.85) $p = 0.663$	2.42 (0.32−18.1) $p = 0.349$		1.35 (0.67−2.73) $p = 0.393$
Bowel resection		1.16 (0.52−2.60) $p = 0.718$	−		1.61 (0.80−3.24) $p = 0.179$
Other surgical treatment		0.61 (0.26−1.43) $p = 0.256$	0.47 (0.05−4.68) $p = 0.458$		0.62 (0.30−1.29) $p = 0.203$
CD therapy					
Steroid		1.96 (0.73−5.29) $p = 0.179$	1.31 (0.13−13.13) $p = 0.651$		1.64 (0.72−3.75) $p = 0.238$
AMP i MTX		0.58 (0.25−1.35) $p = 0.206$	1.17 (0.16−8.68) $p = 0.633$		0.73 (0.37−1.46) $p = 0.374$

Table 14 Analysis of the ORs (and 95 % CI) genotypes and allele of *MIF* gene polymorphism-173 G/C with respect to clinical data of CD

CD disease	*MIF* OR (CI 95 %)				
Genotype/allele	G/G	G/C	C/C	G	C
Age at first diagnosis	Ref.			Ref.	
0–16 years		0.68 (0.21−2.20) $p = 0.517$	4.75 (0.28−79.99) $p = 0.33$		1.0 (0.39−2.58) $p = 1.000$
17–40 years		1.69 (0.72−3.97) $p = 0.224$	0.77 (0.05−12.68) $p = 0.685$		1.43 (0.68−3.00) $p = 0.345$
>40 years		0.65 (0.24−1.78) $p = 0$	_		0.60 (0.24−1.52) $p = 0.277$
CD location					
Colonic		1.20 (0.53−2.69) $p = 0.663$	–		1.48 (0.73−3.00) $p = 0.277$
Ileal		0.43 (0.14−1.35) $p = 0.140$	–		0.41 (0.14−1.23) $p = 0.103$
Ileocolonic		1.41 (0.62−3.24) $p = 0.413$	–		1.11 (0.53−2.31) $p = 0.791$
Upper Gi		0.71 (0.08−6.60) $p = 615$	–		0.66 (0.08−5.35) $p = 0.569$
CD behaviour					
Inflammatory		0.43 (0.18−1.06) $p = 0.064$	–		0.41 (0.18−0.95) $p = 0.034$
Penetrating		1.17 (0.47−2.90) $p = 0.729$	3 (0.18−49.92) $p = 0.447$		1.29 (0.59−2.79) $p = 0.522$
Stricturing		0.94 (0.37−2.38) $p = 0.920$	_		1.1 (0.50−2.42) $p = 0.823$
Perianal disease		1.74 (0.75−4.02) $p = 0.195$	–		1.31 (0.63−2.75) $p = 0.471$
Bowel resection		1.46 (0.64−3.34) $p = 0.365$	–		1.66 (0.79−3.49) $p = 0.177$
Other surgical treatment		0.93 (0.41−2.14) $p = 0.862$	–		0.78 (0.37−1.65) $p = 0.527$
CD therapy					
Steroid		1.89 (0.70−5.11) $p = 0.202$	–		2.01 (0.80−5.07) $p = 0.132$
AMP i MTX		1.06 (0.47−2.38) $p = 0.887$	1.36 (0.08−22.40) $p = 0.673$		0.93 (0.45−1.89) $p = 0.841$

Table 15 Analysis of the ORs (and 95 % CI) genotypes and allele *CXCR4* gene polymorphism C/T in exon 2 position rs2228014 with respect to clinical data of CD

CD disease	*CXCR4* OR (CI 95 %)				
Genotype/allele	C/C	C/T	T/T	C	T
Age at first diagnosis	Ref.			Ref.	
0–16 years		2.0 (0.73−5.47) $p = 0.173$	0.67 (0.08−5.75) $p = 0.583$		1.25 (0.57−2.74) $p = 0.578$
17–40 years		0.45 (0.20−1.04) $p = 0.059$	2.34 (0.47−11.72) $p = 0.245$		0.91 (0.49−1.69) $p = 0.764$
>40 years		1.55 (0.63−3.85) $p = 0.337$	0.38 (0.04−3.19) $p = 0.324$		0.95 (0.46−1.96) $p = 0.887$
CD location					
Colonic		1.47 (0.65−3.33) $p = 0.354$	2.20 (0.58−8.41) $p = 0.199$		1.61 (0.87−2.97) $p = 0.125$
Ileal		0.96 (0.36−2.56) $p = 0.920$	0.38 (0.04−3.19) $p = 0.324$		0.72 (0.33−1.59) $p = 0.416$
Ileocolonic		0.67 (0.27−1.63) $p = 0.374$	0.73 (0.18−3.04) $p = 0.478$		0.74 (0.38−1.43) $p = 0.368$
Upper Gi		0.64 (0.07−6.00) $p = 0.577$	–		0.42 (0.05−3.36) $p = 0.350$
CD behaviour					
Inflammatory		1.4 (0.61−3.21) $p = 0.424$	1.2 (0.31−4.59) $p = 0.522$		1.25 (0.67−2.32) $p = 0.484$
Penetrating		0.88 (0.35−2.25) $p = 0.791$	0.66 (0.13−3.36) $p = 0.472$		0.81 (0.40−1.66) $p = 0.566$
Stricturing		0.66 (0.25−1.72) $p = 0.396$	0.26 (0.03−2.18) $p = 0.174$		0.52 (0.24−1.15) $p = 0.102$
Perianal disease		0.83 (0.34−2.02) $p = 0.680$	0.90 (0.22−3.77) $p = 0.600$		0.88 (0.45−1.72) $p = 0.708$
Bowel resection		0.68 (0.30−1.54) $p = 0.354$	0.68 (0.18−2.53) $p = 0.401$		0.72 (0.39−1.33) $p = 0.301$
Other surgical treatment		1.17 (0.51−2.69) $p = 0.708$	1.14 (0.30−4.35) $p = 0.551$		1.13 (0.60−2.11) $p = 0.698$
CD therapy					
Steroid		0.60 (0.25−1.45) $p = 0.250$	0.73 (0.17−3.09) $p = 0.462$		0.70 (0.36−1.36) $p = 0.299$
AMP i MTX		0.60 (0.26−1.41) $p = 0.242$	0.77 (0.20−2.92) $p = 0.483$		1.39 (0.74−2.61) $p = 0.301$

Table 16 Analysis of the ORs (and 95 % CI) genotypes and allele of *CXCL12* gene polymorphism-801 G/A with respect to clinical data of UC

CU disease	*CXCL12* OR (CI 95 %)				
Genotype/allele	G/G	G/A	A/A	G	A
Age at first diagnosis	Ref.			Ref.	
0–16 years		–	–		–
17–40 years		0.60 (0.29−1.27) $p = 0.181$	0.61 (0.13−2.88) $p = 0.406$		0.66 (0.36−1.20) $p = 0.170$
>40 years		1.95 (0.93−4.09) $p = 0.076$	1.92 (0.41−9.03) $p = 0.326$		1.73 (0.95−3.15) $p = 0.069$
CU location					
Proctitis		0.47 (0.15−1.49) $p = 0.193$	1.70 (0.31−9.47) $p = 0.415$		0.82 (0.36−1.86) $p = 0.631$
Left side colitis		1.47 (0.68−3.18) $p = 0.327$	1.84 (0.39−8.72) $p = 0.348$		1.45 (0.79−2.68) $p = 0.228$
Extended colitis		1.17 (0.56−2.45) $p = 0.671$	0.38 (0.07−2.07) $p = 0.226$		0.87 (0.48−1.57) $p = 0.639$
Bowel resection		1.55 (0.72−3.34) $p = 0.254$	1.75 (0.37−8.30) $p = 0.370$		1.48 (0.81−2.72) $p = 0.202$
CU therapy					
Steroid		1.12 (0.51−2.44) $p = 0.777$	0.72 (0.15−3.39) $p = 0.480$		0.97 (0.52−1.81) $p = 0.920$
AMP i MTX		1.08 (0.47−2.46) $p = 0.862$	0.47 (0.05−4.13) $p = 0.433$		0.89 (0.45−1.77) $p = 0.740$

Table 17 Analysis of the ORs (and 95 % CI) genotypes and allele of *MIF* gene polymorphism-173 G/C with respect to clinical data of UC

CU disease	*MIF* OR (CI 95 %)				
Genotype/allele	G/G	G/C	C/C	G	C
Age at first diagnosis	Ref.			Ref.	
0–16 years		2.36 (0.32−17.3) $p = 0.354$	–		1.74 (0.34−8.89) $p = 0.385$
17–40 years		0.75 (0.37−1.54) $p = 0.438$	–		0.95 (0.51−1.77) $p = 0.887$
>40 years		1.19 (0.59−2.43) $p = 0.624$	–		0.98 (0.53−1.82)
CU location					
Proctitis		1.37 (0.55−3.38) $p = 0.498$	–		1.15 (0.52−2.56) $p = 0.729$
Left side colitis		1.35 (0.64−2.86) $p = 0.427$	2.37 (0.14−39.1) $p = 0.513$		1.34 (0.70−2.55) $p = 0.368$
Extended colitis		0.72 (0.36−1.48) $p = 0.374$	0.87 (0.05−14.3) $p = 0.717$		0.78 (0.42−1.45) $p = 0.431$
Bowel resection		–	–		–
CU therapy					
Steroid		1.07 (0.51−2.25)	–		1.2 (0.62−2.34) $p = 0.584$
AMP i MTX		0.81 (0.35−1.85) $p = 0.610$	2.74 (0.16−45.4) $p = 0.472$		0.95 (0.46−1.93) $p = 0.887$

Table 18 Analysis of the ORs (and 95 % CI) genotypes and allele of *CXCR4* gene polymorphism C/T in exon 2 position rs2228014 with respect to clinical data of UC

CU disease	*CXCR4* OR (CI 95 %)				
Genotype/allele	C/C	C/T	T/T	C	T
Age at first diagnosis	Ref.			Ref.	
0–16 years		1.45 (0.14−14.5) $p = 0.571$	–		1.06 (0.13−8.87) $p = 0.642$
17–40 years		1.88 (0.80−4.46) $p = 0.145$	3.33 (0.34−32.9) $p = 0.284$		1.99 (0.95−4.17) $p = 0.064$
>40 years		0.50 (0.21−1.20) $p = 0.118$	0.33 (0.03−3.30) $p = 0.322$		0.49 (0.23−1.04) $p = 0.061$
CU location					
Proctitis		0.83 (0.26−2.68) $p = 0.509$	1.60 (0.16−16.2) $p = 0.542$		1.01 (0.40−2.58)
Left side colitis		2.44 (1.03−5.75) $p = 0.038$	2.62 (0.35−19.4) $p = 0.318$		2.23 (1.09−4.56) $p = 0.025$
Extended colitis		–	–		–
Bowel resection		0.52 (0.19−1.40) $p = 0.190$	1.83 (0.25−13.5) $p = 0.452$		0.78 (0.36−1.69) $p = 0.522$
CU therapy					
Steroid		0.76 (0.32−1.80) $p = 0.543$	–		1.38 (0.66−2.90) $p = 0.396$
AMP i MTX		0.65 (0.23−1.87) $p = 0.424$	8.60 (0.86−85.9) $p = 0.063$		1.37 (0.63−2.94) $p = 0.424$

reports on the relationship between the age of diagnosis of UC, and polymorphism CXCL12/CXCR4 chemokine axis.

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