Acta Clin Croat 2020; 59:67-80

doi: 10.20471/acc.2020.59.01.09

Original Scientific Paper

INTERACTIONS AMONG INTERLEUKIN-6, C-REACTIVE PROTEIN AND INTERLEUKIN-6 (-174) G/C POLYMORPHISM IN THE PATHOGENESIS OF CROHN'S DISEASE AND ULCERATIVE COLITIS

Boris Takač[1,3], Silvio Mihaljević[2,3], Ljubica Glavaš-Obrovac[3], Aleksandar Kibel[2,3], Mirjana Suver-Stević[4], Silvija Canecki-Varžić[3,5], Marko Samardžija[2], Ines Rajkovac[3,7], Damir Kovač[6] and Mario Štefanić[3]

[1]Department of Nuclear Medicine and Radiation Protection, Osijek University Hospital Centre, Osijek, Croatia;
[2]Department of Internal Medicine, Division of Gastroenterology, Osijek University Hospital Centre, Osijek, Croatia;
[3]Josip Juraj Strossmayer University of Osijek, Faculty of Medicine, Osijek, Croatia;
[4]Department of Transfusion Medicine, Osijek University Hospital Centre, Osijek, Croatia;
[5]Department of Internal Medicine, Division of Diabetes, Endocrinology and Metabolism Disorders, Osijek University Hospital Centre, Osijek, Croatia;
[6]Department of Surgery, Division of Vascular Surgery, Osijek University Hospital Centre, Osijek, Croatia;
[7]Department of Gastroenterology and Hepatology, Dr. Josip Benčević General Hospital, Slavonski Brod, Croatia

SUMMARY – Inflammatory bowel diseases are multifactorial disorders the clinical manifestation of which depends on the interaction among immune response, genetic and environmental factors. There is growing evidence that cytokines and gene polymorphisms have an important role in disease pathogenesis in various populations although molecular mechanism of their signaling and interactions is not fully understood yet. The present study aimed at exploring the effects of interleukin-6, C-reactive protein and interleukin-6 rs1800795 polymorphism on the development of Crohn's disease, ulcerative colitis and inflammatory bowel diseases overall and at determining differences between inflammatory bowel disease patients and healthy controls. A total of 132 inflammatory bowel disease patients and 71 healthy blood donors were investigated. In order to assess the clinical relevance of interleukin-6 and C-reactive protein serum concentration and interleukin-6 rs1800795 single nucleotide polymorphism in patients with Crohn's disease and ulcerative colitis, we performed a cross-sectional, case-control study. Quantitative assessment of serum interleukin-6 and C-reactive protein was performed with solid-phase, enzyme-labeled, chemiluminescent sequential immunometric and immunoturbidimetric assay, respectively. A real-time fluorescence resonance energy transfer-based method on a LightCyclerTM PCR 1.2 was used for genotyping of IL-6 rs1800795 polymorphism. Both interleukin-6 and C-reactive protein serum levels were elevated in Crohn's disease and ulcerative colitis patients. Positive correlations were observed between C-reactive protein and interleukin-6 serum concentration and ulcerative colitis activity index as measured by modified Truelove-Witt's severity index scale. C-reactive protein serum level was higher in Crohn's disease patients without intestinal resection than in Crohn's disease patients with prior intestinal resection. In ulcerative colitis patients, interleukin-6 and C-reactive protein serum levels were statistically significantly higher in CC interleukin-6 genotype in comparison to GG+GC genotype. Analysis of the promoter region of the interleu-

Correspondence to: *Boris Takač, mag biol. et chem.*, Department of Nuclear Medicine and Radiation Protection, Osijek University Hospital Centre, Josipa Huttlera 4, HR-31000 Osijek, Croatia
E-mail: btakac@gmx.net

Received March 18, 2019, accepted April 12, 2019

kin-6 rs1800795 gene polymorphism showed no statistically significant difference in allele frequency either between inflammatory bowel disease patients and healthy controls or between the two inflammatory bowel disease phenotypes and healthy controls. Associations presented in this study give a potentially important insight into the role of interleukin-6 and C-reactive protein signaling and interleukin-6 polymorphism in the pathogenesis of Crohn's disease and ulcerative colitis disease.

Key words: *Crohn's disease; Colitis, ulcerative; Interleukin-6; C-reactive protein; Polymorphism, single nucleotide; Inflammatory bowel diseases*

Introduction

Inflammatory bowel diseases (IBD) comprise a large spectrum of clinical presentations, the major phenotypes being Crohn's disease (CD) and ulcerative colitis (UC). These are chronic idiopathic inflammatory disorders of the gastrointestinal tract characterized by variable disease course and prognosis with episodes of clinical activity as a result of active inflammation[1,2]. IBDs are multifactorial, polygenic diseases characterized by an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host[3]. The underlying immunopathogenesis of IBD is not fully understood. Genetic studies highlighted the importance of host-microbe interactions with genetic factors such as nucleotide oligomerization domain 2 (NOD2), autophagy genes and components of the interleukin-23-type 17 helper T-cell (Th17) pathway playing major roles in perpetuating the abnormal inflammatory response in IBD[3,4]. We previously studied the effect of interleukin-23 receptor single nucleotide polymorphisms (SNP) on the development of CD and UC in patients from a Croatian tertiary clinical center and found that certain polymorphisms were associated with a protective role in the development of IBD, which was also previously described in other populations[5].

Interleukin 6 (IL-6), together with other cytokines such as interleukin 8 (IL-8), interleukin 1ß (IL-1ß) or tumor necrosis factor alpha (TNFα), was found to play a key role in the signaling mechanisms in the development of IBD[6-8]. Serum IL-6 level was elevated in CD and UC patients and correlated positively with disease activity and other inflammation markers in a Croatian patient population, as studied by our research group[7]. Emerging specific anti IL-6 therapies might contribute to better disease control in the future[9,10]. Disruption of IL-6 regulation has been related to several immune-mediated inflammatory diseases such as rheumatoid arthritis, systemic juvenile idiopathic arthritis, Castleman disease, various types of cancer, and IBD[11]. Baran *et al*. and Scheller *et al*. state that IL-6 cytokine family members have pro- and anti-inflammatory activities that are preceded by activation of target genes involved in differentiation, survival, apoptosis and proliferation[12,13]. In activated T lymphocytes at the site of inflammation, IL-6 activates transcription of anti-apoptotic genes Bcl-2 and Bcl-xl *via* signal transducer and activator of transcription 3 (STAT3), enabling survival and clonal expansion of T lymphocytes[14]. These processes result in intensive cytokine synthesis, as well as in matrix metalloproteinases, thus promoting chronic inflammation and tissue destruction, which is one of the clinical features of IBD.

C-reactive protein (CRP) is often considered as a nonspecific inflammatory marker but it is also an immunological synapse between innate and adoptive immunity, as well as in the interaction with IL-6, between pro- and anti-inflammatory activity in complex IBD mechanisms[15]. CRP expression is activated by IL-6, but also by IL-1β and TNFα signaling in hepatocytes[16], with significant interindividual variations in basal serum concentrations. By feedback mechanism, CRP positively and negatively regulates IL-6 synthesis, which is dependent on CRP concentration and FcγR macrophage isoforms[17]. In IBD, there are significant but yet insufficiently known interindividual differences in CRP response to disease activity, including the unexplained role of IL-6 in CRP serum concentrations. It is also unknown whether and to what extent gene variations in the IL-6 signaling pathway affect CRP dynamics in IBD.

Since increased secretion of the pro-inflammatory IL-6 seems to be important in the immunopathogenesis of IBD, the question arises if the functionally relevant polymorphism of the promoter region of IL-6 (G/C at position -174) is associated with IBD. The IL-6 G/C SNP at position -174 has been linked to changes in IL-6 production[18,19]. Baseline IL-6 release from macrophages is significantly lower in CC geno-

types compared to GG genotypes, with GC individuals having an intermediate phenotype[18]. Klein *et al*. did not find an association of this polymorphism with CD and UC patients[20], but Balding *et al*. found a significant difference in the frequency of IL-6-174 genotypes in the UC group (GG=40%, GC=41%, CC=19%) compared with the CD group (GG=22%, GC=64%, CC=14%), suggesting a potential difference in the pathophysiology of these two diseases[6]. Vickers *et al*., Wypasek *et al*. and Ferrari *et al*. state that IL-6 polymorphism may also be related to CRP values[21-23], but whether and how much gene variation in IL-6 signal pathway affects the dynamics of CRP in IBD is still unknown.

The aim of the present study was to determine if the IL-6 -174 G/C SNP was associated with CD and UC patients from a Croatian tertiary center and whether there was difference in SNP genotype frequency between CD and UC individuals. In addition, the study aimed at exploring the effect of IL-6 -174 G/C SNP on CRP and IL-6 serum concentration. The research is relevant for gaining more insight into the pathogenetic mechanisms of IBD in the Croatian patient population, as well as for optimization and efficient use of future individualized therapies specifically targeting pro-inflammatory signaling factors.

Materials and methods

Patients

A total of 50 patients with CD (26 males, median age 35 yrs, interquartile range 30-45 yrs), and 93 patients with UC (52 males, median age 36 yrs, interquartile range 26-47 yrs), and 71 ethnically and geographically matched healthy control subjects (median age 36 yrs, interquartile range 26-47 yrs) were included in the study. All were adults, Caucasian and living in eastern Croatia. Diagnosis of IBD (CD or UC) was established according to standard clinical criteria, including endoscopic, radiological and histopathologic analysis at the Osijek University Hospital Centre, Osijek, Croatia. Clinical activity of CD and UC was evaluated using the Crohn's disease activity index (CDAI) and modified Truelove-Witt's severity index (MTWSI), respectively. Subjects with infective and nonspecific colitis, multiple sclerosis, confirmed autoimmune or malignant diseases were excluded from the study. There were 29 patients under treatment with corticosteroids. They received either methylprednisolone (2 CD patients, median dose 16 mg/daily, range 8-40 mg; n=10) or prednisone (9 CD patients, median dose 17.5, range 5-40 mg, n=12). Median follow-up for the entire cohort of IBD patients was 6 (interquartile range 3-12) years. The institutional Ethics Committee approved the study (reference number: 602-04/17-08/12, reg. no.: 2158-61-07-17-217). A written informed consent was obtained from each patient.

SNP determination – real-time PCR assay for interleukin-6 promoter (-174G → C) genotyping

Genomic DNA was extracted from 200 μL EDTA blood with a DNA isolation kit (High Pure PCR Template Preparation Kit, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Primers were obtained from TIBMOLBIOL (Berlin, Germany) and kit for PCR (LightCycler FastStart DNA Master SYBR Green Kit) was purchased from Roche Diagnostics, Mannheim, Germany. Genotyping was carried out using primers and fluorescent labeled probes in a LightCycler System (Roche Diagnostics, Mannheim, Germany) with subsequent fluorescent probe melting point analysis[24]. A master mix contained 2.25 mM $MgCl_2$, 0.5 μM of the primers 5´- TTA CTC TTT GTC AAG ACA TGC CA - 3´ and 5´- ATG AGC CTC AGA CAT CTC CAG - 3´, and 0.2 μM of the probes: 5´- CTA AGC TGC ACT TTT CCC CCT AGT --FL and LC640-GTG TCT TGCGAT GCT AAA GGA --PH, and 2 μL of FastStart (Roche Diagnostics, Mannheim, Germany) mixture. The 175 bp PCR product obtained was analyzed using melting curve analysis (mutant homozygote IL6 -174C/C - 57.0 °C; heterozygote IL6 -174G/C - 57.0 °C and 64.0; wild type homozygote IL6 -174G/G - 64.0 °C).

IL-6 serum level determination

Blood samples were obtained by venipuncture in the morning when the subjects were fasting. Blood was drawn from cubital vein of each patient into a tube without clot promoting additives (Becton Dickinson Vacutainers Systems, Belliver Industrial Estate, Plymouth, UK). After resting for 30 minutes in upright position, samples were centrifuged for 10 minutes at 3,000 rpm (Hettich Rotina 380 R, Tuttlingen, Ger-

many). Sera were separated from cells and stored at -20 °C until analysis.

Quantitative assessment of IL-6 serum concentration was performed with solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay on a Siemens Immulite 1000 (Siemens Healthcare Diagnostics, Llanberis, Gwynedd, UK) in incubation cycles of 2x30 minutes according to the manufacturer's instructions.

Commercial Immulite IL-6 kit (Siemens Healthcare Diagnostics, Llanberis, Gwynedd, UK) contained test units coated with a monoclonal murine anti-IL-6 antibody, and two IL-6 reagent wedges containing 7.5 mL of a protein/buffer matrix and 7.5 mL of alkaline phosphatase conjugated to polyclonal sheep anti-IL-6 antibody in buffer, respectively, as well as IL-6 low and high adjustors of lyophilized IL-6 in a protein buffer matrix. IL-6 assay was calibrated with low and high adjustors, both reconstituted with 3 mL distilled water and run in tetraplicates. As an aid in monitoring performance of assays, two controls (Siemens Healthcare Diagnostics, Llanberis, Gwynedd, UK) containing different concentrations of IL-6 lyophilized cytokines in a human serum matrix were used. Both were reconstituted with 5 mL distilled water within 30 minutes prior to use and were assayed in duplicates in the same manner as patient samples.

CRP serum level determination

Blood samples were obtained by venipuncture in the morning when the subjects were fasting. Blood was drawn from cubital vein of each patient into a tube without clot promoting additives (Becton Dickinson, New Jersey, USA). After resting for 30 minutes in upright position, samples were centrifuged for 10 minutes at 3,000 rpm (Hettich Rotina 380 R, Tuttlingen, Germany). Sera were separated from cells and stored at 2-8 °C until analysis. To determine CRP serum concentration, a commercial CRP Latex reagent was used (Beckman Coulter, Brea, CA, USA) on a Beckman Coulter AU680 analyzer according to the manufacturer's instructions. To calibrate reagents, Beckman Coulter CRP Latex Calibrator Normal Set was used (cat. no. ODC0026). CRP calibrator values are traceable to CRM 470[25] of the International Federation of Clinical Chemistry (IFCC). For quality control, commercial control samples at two concentration levels were used, as follows: Seronorm Immunoprotein Lyo-L1 cat. no. 202805 and Seronorm Immunoprotein Lyo-L2 cat. No. 202905 (Sero, Bilinstad, Norway). The test principle is based on the turbidity measurement of the reaction solution (immune turbidimetry). After mixing the test sample with the reagent, CRP from the sample specifically binds to the anti-CRP antibodies on reagent latex particles, thereby producing insoluble aggregates and increasing turbidity of the reaction mixture. The increase in turbidity is proportional to CRP concentration in the sample.

Statistical methods and analysis

Differences between groups were tested using Mann-Whitney or Kruskal-Wallis test with Bonferroni-Dunn post hoc procedure. Fisher exact test and χ^2-test were used for categorical variables. To test deviations from Hardy-Weinberg equilibrium, Guo-Thompson exact test was used[26], and to describe allelic association D' coefficient was used (PLINK 1.07 program)[27-29].

For individual polymorphisms, statistical significance was simultaneously tested by Westfall-Young permutations (10^4 randomizations)[30] and empirical p-values corrected for multiple testing were obtained (maxT, PLINK 1.07). The statistical power of alleles and genotype associations was estimated using the Genetic Power Calculator program[31]. If not otherwise specified, statistical analysis was conducted using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Two-tailed p-values were considered statistically significant when lower than <0.05.

Results

Interleukin-6

Serum level of IL-6 (pg/mL) was assessed in 32 CD, 68 UC and 71 control group subjects. In both patient groups, IL-6 was significantly higher when compared to the control group, but there was no significant difference in IL-6 serum levels between CD and UC groups (Kruskal-Wallis test) (Table 1). There was no statistically significant difference between patients receiving corticosteroid therapy (n=21, confidence interval (CI)=3.5, interquartile range (IQR)=2.675-14.15) and those not receiving this therapy (n=79, CI=2.31, IQR=1.99 -6.38, U=604, p=0.50; Mann Whitney).

Table 1. IL-6 levels in CD and UC patients and healthy controls

	Kruskal-Wallis p	CD patients (n=32)		UC patients (n=68)		Controls (n=71)	
		Median	IQR	Median	IQR	Median	IQR
IL-6 (pg/mL)	<0.001‡	3.25	1.99-5.96	2.67	1.99-11.20	1.99	1.99-1.99

IL-6 = interleukin-6; CD = Crohn's disease; UC = ulcerative colitis; n = number of subjects, IQR = interquartile range, ‡ post hoc Dunn-Bonferroni: K<CD (p<0.001); K<UC (p<0.001)

Table 2. Comparison of allele frequency of interleukin-6 (IL-6) single nucleotide polymorphism (SNP) between patients with inflammatory bowel diseases (IBD) and healthy controls

SNP	Allele	Frequency (IBD)	Frequency (controls)	OR (95% CI)	p*	HWE† p (controls)	HW p
rs1800795	C	0.419	0.391	1.12-1.75	0.973	0.613	0.425

SNP = single nucleotide polymorphism; IBD = inflammatory bowel disease patients; CI = confidence interval, HWE = Hardy-Weinberg equilibrium; OR = odds ratio; *empirical p-value, 10^4 permutation, Westfall-Young correction, †Guo-Thompson exact test

The difference in IL-6 levels between CD patients with prior intestinal resection (n=8, median=2.61, IQR=2.02-3.75) and those with no previous resection (n=24, median=3.55, IQR=1.99-7.23) was not statistically significant (U=72, p=0.313, Mann Whitney).

The difference in IL-6 levels between patients with inactive CD (CDAI <150, n=17, CI=1.99, IQR=1.99-3.77) and healthy controls (n=71, CI=1.99, IQR=1.99-1.99) was statistically significant (U=362, p<0.001, Mann-Whitney).

In both CD and UC patients, there was a strong positive correlation between IL-6 and CRP levels (ρ=0.68, p<0.01 and r=0.78, p<0.01, respectively).

Serum IL-6 levels correlated statistically significantly with CDAI in CD patients (ρ=0.48, p<0.01) and with MTWSI in UC patients (ρ=0.66, p<0.01).

CRP

C-reactive protein levels were obtained in 132 IBD patients (84 UC) but not in healthy controls. CRP levels in both CD and UC patients were higher than reference intervals (<5.0 mg/L). There were no statistically significant differences in CRP levels in IBD patients between those receiving corticosteroid therapy and those not receiving it (p=0.777, Kruskal-Wallis test). In IBD patients, there was a strong correlation between CRP and IL-6 levels (ρ=0.756, p<0.001), as well as between CRP and MTWSI (ρ=0.586, p<0.001). The correlation between CRP and CDAI was not statistically significant (ρ=0.208, p=0.166).

Difference in CRP levels between CD (n=48, CI=9.35, IQR=2.2-39.02) and UC patients (n=84, CI=6.75, IQR=2.03-51.63) was not statistically significant (U=1984.5, p=0.882, Mann-Whitney).

There was a statistically significant difference in CRP levels between CD patients with prior intestinal resection (n=11, CI=2.2, IQR=0.9-4.5) and those with no previous resection (n=37, CI=10.3, IQR=6.05-46.75, U=88, p=0.005, Mann Whitney).

IL-6 rs1800795 polymorphism

Analysis of IL-6 rs1800795 polymorphism showed that the genotype distribution was in Hardy-Weinberg equilibrium (Table 2).

In order to investigate the role of IL-6 genotype in IL-6 serum levels and CRP acute phase protein levels, analysis of rs1800795 polymorphism in IL-6 gene was conducted. Patients were categorized by IBD type and IL-6 genotype. Genotyping was carried out in 32 CD and 65 UC patients. Genotype distributions are shown in Table 3. No statistically significant difference was observed in allele frequency either between IBD or two IBD phenotypes and healthy controls.

Association of rs1800795 and IL-6 serum level

A statistically significant difference in IL-6 levels was found among the three genotypes in UC patients (p=0.045) (Table 4). Post hoc Bonferroni-Dunn test showed statistically significant IL-6 levels in CC relative to GC genotype. No statistically significant differ-

Table 3. Comparison of IL-6 rs1800795 genotype and allele frequency between inflammatory bowel disease patients and healthy controls

Genotype association					
IL-6 (rs1800795)	Genotype	IBD n (%)	Controls n (%)	p*	OR (95% CI)
IBD	CC	16 (16.2)	9 (13)	0.976	1.33 (0.5-3.65)§
	GC	51 (51.5)	36 (52.2)		1.06 (0.53-2.1)
	GG	32 (32.3)	24 (34.8)		Ref
CD	CC	8 (25)	9 (13)	0.145	2.37 (0.67-8.24)
	GC	15 (46.9)	36 (52.2)		1.11 (0.42-3.05)
	GG	9 (28.1)	24 (34.8)		Ref
UC	CC	8 (11.9)	9 (13)	0.846	0.93 (0.3-2.89)
	GC	36 (53.7)	36 (52.2)		1.04 (0.5-2.19)
	GG	23 (34.4)	24 (34.8)		Ref
Allele associations					
Phenotype	Allele	Frequency (IBD)	Frequency (controls)	OR (95% CI)	p*
CD	C	0.484	0.391	1.46 (0.8-2.65)	0.651
UC	C	0.388	0.391	0.99 (0.59-1.66)	Ref

IL-6 = interleukin 6; IBD = inflammatory bowel disease patients; CD = Crohn's disease patients; CI = confidence interval; OR = odds ratio; Ref = reference genotype; UC = ulcerative colitis patients; *empirical p-value; 10^4 permutation, Westfall-Young correction

Table 4. Comparison of IL-6 among genotypes (GG, GC and CC) by disease (Kruskal-Wallis

Genotype IL-6 (pg/mL)	GG	n	GC	n	CC	n	p†
IBD	3.16 (1.99-7.91)	32	2.35 (1.99-5.27)	49	4.62 (2.79-9.36)	16	0.092
UC	3.49 (1.99-15.80)	23	2.05 (1.99-6.28)	34	7.17 (3.12-36.85)	8	0.045‡
CD	2.11 (1.99-5.16)	9	3.19 (1.99-4.69)	15	3.77 (2.24-8.03)	8	0.701

CD = Crohn's disease patients; UC = ulcerative colitis patients; IBD = inflammatory bowel disease patients; n = number of cases; †Kruskal-Wallis test; ‡post hoc Dunn-Bonferroni (z): GC<CC (p=0.032)

Table 5. Comparison of interleukin-6 (IL-6) serum levels between GG+GC and CC genotypes

SNP IL-6 (pg/mL)	GG+GC	n	CC	n	p
IBD	2.46 (1.99-6.44)	81	4.62 (2.79-9.36)	16	0.052
UC	2.31 (1.99-8.94)	57	7.17 (3,12-36.85)	8	0.02
CD	3.16 (1.99-4.68)	24	3.77 (2.24-8.03)	8	0.683

Data are presented as median with interquartile range, Mann-Whitney U test; n = number of cases; CD = Crohn's disease patients; UC = ulcerative colitis patients; IBD = inflammatory bowel disease patients; SNP = single nucleotide polymorphism

Table 6. Comparison of C-reactive protein levels (CRP) between IL-6 genotypes (GG, GC and CC) in IBD, UC and CD patients (Kruskal-Wallis test)

SNP CRP	GG	n	GC	n	CC	n	p
IBD	8.20 (2.00-21.60)	31	6.70 (1.85-37.30)	45	8.70 (4.30-167.40)	15	0.469
UC	6.00 (1.60-29.10)	23	6.20 (2.15-19.23)	30	90.20 (6.03-293.23)	8	0.068
CD	9.25 (3.30-18.30)	8	6.70 (1.10-70.10)	15	5.90 (1.70-16.30)	7	0.701

CD = Crohn's disease patients; UC = ulcerative colitis patients; IBD = inflammatory bowel disease patients; SNP = single nucleotide polymorphism; n = number of cases

Table 7. Comparison of C-reactive protein (CRP) levels between GG+GC and CC genotypes

SNP CRP	GG+GC	n	CC	n	p
IBD	7.40 (2.00-23.25)	76	8.70 (4.30-167.40)	15	0.963
UC	6.00 (2.00-22.70)	53	90.20 (6.03-293.23)	8	0.022
CB	8.20 (1.10-40.70)	23	5.90 (1.70-16.30)	7	0.624

Data are presented as median with interquartile range; Mann-Whitney U test; CD = Crohn's disease patients; UC = ulcerative colitis patients; IBD = inflammatory bowel disease patients; SNP = single nucleotide polymorphism; n= number of cases

ences in IL-6 levels were found among the three genotypes in CD patients and IBD patients overall.

Next, GG and GC alleles were joined into one category and its IL-6 level was compared with that of the CC genotype, by disease (UC and CD) and UBC (UC and CD combined) (Table 5). IL-6 level in the GG+GC allele (median=2.31, IQR=1.99-8.94) was statistically significantly lower than in the CC allele (median=7.17, IQR=3.12-36.85) in UC patients (U=341, p=0.02, Mann-Whitney), as well as in IBD patients. In CD patients and in IBD patients overall, there was no statistically significant difference in IL-6 levels between GG+GC and CC genotypes (U=114, p=0.454 and U=863, p=0.032, respectively).

Association between rs1800795 and CRP serum level

No statistically significant differences were found in CRP levels among the three IL-6 genotypes (Table 6).

In UC patients, CRP levels were statistically significantly lower in GG+GC than in CC genotype (U=323.5, p=0.017). In IBD (U=692.5, p=0.19) and CD patients (U=70.5, p=0.631), difference in CRP levels was not statistically significant between the genotypes (Table 7).

Discussion

Inflammatory bowel diseases are chronic inflammatory intestinal disorders often followed by unpredictable relapsing-remitting course[32,33]. The IBD pathophysiology is not completely understood yet and therefore therapeutic strategies are far from ideal[34]. The disease pathogenesis includes immune, genetic, environmental and microbial factors, which are the focus of intensive studies conducted with the aim to generate new insights and advances in diagnostics and treatment[35].

Our results pointed to a statistically significant difference in IL-6 serum level between IBD patients and healthy control subjects, with no significant difference between CD and UC patients in CRP and IL-6 serum levels. CRP level in IBD patients was significantly higher than the CRP reference interval (<5.0 mg/L), with a positive correlation with IL-6 serum levels. Solem *et al.*, Poullis *et al.* and Yang *et al.* also found higher CRP serum levels in CD and UC patients, with a positive correlation with IL-6 serum levels[36-38], whereas in the study by Fagan *et al.*, CRP was significantly higher in UC than in CD patients[39]. Similar to our results, Beck and Wallace, Seegert *et al.* and Yoshi-

moto *et al*. also report elevated IL-6 levels in both IBD phenotypes[40-42]. Rogler *et al*. and Feng *et al*. defined IBD as proinflammatory and anti-inflammatory cytokine imbalance with elevated IL-6, TNF, IL-8 and IL-12[43,44]. In contrast to our results, Korolkova *et al*. and Mahida *et al*. report elevated IL-6 serum levels in patients with active CD but not in patients with UC[45,46].

Interleukin-6 released from macrophages by TLR/NOD2 cascades is the main cytokine which induces acute phase CRP, serum amyloid A, haptoglobin and fibrinogen proteins[47-49]. Newly synthesized CRP induces shedding of neutrophils mIL-6R, which are the first cells to arrive at the site of intestinal epithelial barrier damage and mediate IL-6 trans-signaling[50,51]. The IL-6 - sIL-6Rα interactions stabilize the complex and therefore increase IL-6 half-life and bioavailability by 50%[52]. Consequently, IL-6 trans-signaling in activated T lymphocytes activates anti-apoptotic Bcl-2 and Bcl-xl genes, which enables accumulation, survival and antigen-specific T cell expansion[53,54]. Mitsuyama *et al*., Scheller *et al*. and Kishimoto demonstrated that, in addition to IL-6, serum level of soluble IL-6Rα (sIL-6Rα) was elevated too[55-57]. Jones *et al*. found elevated CRP and IL-6 serum levels in IBD patients and a positive correlation between endoscopic but not clinical disease activity[58]. Florin *et al*. report on elevated CRP levels in CD patients but also on active CD patients with persistently low CRP values[59]. This phenomenon could indicate lower BMI and stenosing *versus* penetrating CD. We found a positive relationship between IL-6 serum level and disease activity index according to MTWSI/CDAI scale in both IBD phenotypes, and a positive CRP correlation with MTWSI but not with CDAI. IL-6 serum levels were statistically significantly higher in patients with active CD (CDAI >150) and severe UC (MTWSI >6) than in inactive CD (CDAI <150) and mild UC (MTWSI <4) patients. Similar to our results, Hyams *et al*. and Reinisch *et al*. report on higher IL-6 serum level in IBD patients with positive correlation with disease inflammatory activity[60,61]. Brown *et al*. found that IL-6 serum levels correlated more significantly with UC than CD disease activity[62]. Contrary to our research, Gross *et al*. could not confirm the relationship between IL-6 serum levels and disease activity[63]. However, *in vivo* animal studies support the role of IL-6 in disease severity, i.e. adoptive transfer $CD4^{+}CD45RB^{high}$ T cells $IL\text{-}6^{-/-}$ donors in severe combined immunodeficiency (SCID) model resulted in milder inflammatory colon disease[64]. Next, statistically significantly higher IL-6 levels were observed in patients with inactive disease than in healthy controls. As the synthesis of many cytokines is coactivated by the same transcriptional factors such as NFκB and AP-1, which bind to *cis* regulatory elements within the gene promoter region, similar stimuli often lead to co-activation of primordial cytokine expression such as IL-6, TNFα and IL-1β[65-67]. In their study of IL-6 mRNA in IBD patient and healthy control colon samples, Stevens *et al*. found elevated IL-6 transcript only in active CD and UC[68].

Additional studies are needed to illuminate the contributions of different cellular and tissue sources of IL-6 to its serum level and dynamics. In this context, elevated IL-6 serum level in patients with inactive disease leads to many hypotheses about its origin, from residual subclinical inflammation to the role of intestinal flora and the process of tissue injury healing. Concerning the latter, in addition to inflammatory signaling, IL-6 has a role in the processes of intestinal epithelial regeneration, and thus inflammatory stimuli reduction. This is reinforced by cases of disease aggravation after tocilizumab administration, as first described by Atreya *et al*. and Shetty *et al*.[69,70].

Increased IL-6 and CRP levels in patients with inactive CD found in our study may indicate activation of the mucus immune system or increased intestinal permeability. All of these stimuli can activate IL-6 and CRP synthesis in patients with inactive CD but increased IL-6 production may also be attributed to the potential existence of subclinical but relevant residual inflammatory activity of the disease. Van Kemseke *et al*., Schreiber *et al*. and Wyatt *et al*. state that these biological abnormalities may be associated with an increased risk of relapse[71-73]. According to Reinisch *et al*., different acute phase protein serum levels may predict clinical relapse but only a combination of multiple laboratory tests can provide a more reliable predictive index[74]. On the other hand, CRP half-life is not affected by physiological or pathophysiological mechanisms, resulting in decrease in its concentration in the period of reduced inflammatory activity, usually 19 hours after CRP synthesis, which allows for CRP to be used as a therapeutic efficacy biomarker[75,76]. The potential pathobiological role of CRP is invariable and unclear, and probably outweighs its role as a mere bio-

chemical and therapeutic marker. Namely, Zouki *et al*. emphasize that CRP peptides inhibit L-selectin mediated neutrophil interaction with endothelial cells probably by binding to CD32, which is the first step to neutrophil extravasation[77]. Lower CRP concentrations favor CRP association to macrophage FcγRI, FcγRIIa and FcγRIIIa, which leads to additional synthesis and release of proinflammatory IL-1, IL-6 and TNFα cytokines[78,79]. Higher CRP concentrations, in contrast, favor binding to macrophage FcγRIIb, which results in proinflammatory cytokine suppression partially mediated by IL-10$^+$Treg cells.

Although the difference in IL-6 serum levels between CD patients with and without intestinal resection was not statistically significant, the existence of difference cannot be unreservedly excluded. Certain differences may be a consequence of therapeutic modalities but also of dual effects of IL-6 signaling. Finally, gender, smoking, fat tissue and comorbidities can also contribute to these differences[80,81]. These results indicate that IL-6 should be observed in a wider context; in addition to its proinflammatory signaling effect, IL-6 has an anti-inflammatory and regenerative effect, and as such is potentially actively involved in the mechanisms of intestinal epithelium healing[82,83].

Measurement of IL-6 serum concentration can therefore be useful for stratifying patients with a high relapse risk or stratifying disease severity. However, the study was partially limited by the lack of longitudinal IL-6 serum measurement, which, we believe, would give better insight into the dynamics and relapse predictive value of IL-6.

Our results indicate that IL-6 serum level reflects CD and UC inflammatory activity. Given the significance of signaling in IBD, IL-6 may be considered a potentially significant target for cytokine specific therapy. However, spatial and temporal context of inflammatory reaction and possible interference with anti-inflammatory or regenerative mechanisms of IL-6 signaling should be taken into account. In this way, the possible role of IL-6 in the individualized screening of patients for personalized biological or other therapy is also open. This would allow IL-6 to have a role in screening of patients for personalized biological or other therapy.

Gene variations such as SNPs can affect gene transcription efficiency, mRNA half-life and protein structure, as well as protein function[84]. Therefore, when genetic variations are related to inflammatory cytokines, and the inflammatory process is one of the risk factors, certain variations may result in a higher intensity of the inflammatory process and thereby increased risk of inflammatory tissue injuries[85].

The most widely studied functional polymorphism, transversion -174 G/C promoter region of IL-6 gene (rs1800795, 7_22766645_C_G, GRCh37.p13), was also a subject of the present study. IL-6 gene is located on chromosome 7 (7p15.3), and comprises 6 exons and 5 introns[86]. It was observed that the G/C transition at position 174 of the promoter region affects transcription of the gene activity, resulting in changes in IL-6 production[87,88]. G allele, which some authors consider highly productive (GG and GC genotypes), is associated with enhanced IL-6 expression[89], while C allele (CC) is considered as a low productive variant indicating a possible protective function[90]. Limited results of *in vitro* transfection studies support this distinction[91]. In this study, IBD patients and healthy controls did not differ significantly in the distribution of rs1800795 allelic and genotype frequencies. The observed frequency of mutated alleles in healthy controls corresponded to the expected values for European populations and populations of European origin[92]. There was no statistically significant difference in the distribution of allele frequencies between the two IBD phenotypes either, suggesting that the predisposition role of this polymorphism in IBD is neither strong nor differential, or that the expected effect size is smaller than the statistical power in the study. Although other functional or regulatory polymorphism in the linkage disequilibrium with the analyzed variant cannot be excluded, there are currently no such apparent candidates in the IL-6 locus. However, the investigation of the relationship between rs1800795 and CRP and IL-6 serum levels in UC patients indicated statistically significantly higher IL-6 and CRP levels in CC genotype carriers compared to GG+GC genotypes, especially in the C-allele recessive model.

A study conducted in Dublin by Balding *et al*. showed that rs1800795 was involved in inflammatory response and contributed to susceptibility to disease and phenotype in IBD patients[93]. A Spanish population study by Guerreiro *et al*. showed that GG homozygotes had a six times higher risk of CD[94]. However, Pawlik *et al*. report that there was no significant difference between the patients and the control group in the

distribution of the IL-6 genotype[95]. Banday *et al*. found that whereas CC variant rs1800795 did not correlate significantly with the prospects of the disease, it did correlate significantly with the prospects for long-term complications such as development of colorectal cancer as one of the consequences of long-lasting IBD[96]. At the level of gene transcription regulation, Sawczenko *et al*. report on higher IL-6 and CRP serum levels associated with GG genotype in children with CD[97].

After measuring IL-6 plasma levels in 102 healthy subjects from the London area, plasma IL-6 levels were also significantly lower in C allele than in G allele[98]. It remains unclear, however, whether this result is an independent effect of rs1800795, or the result of the synergy of more collocated, nonspecific gene variants within the same haplotype block. In addition to *cis* and *trans* effects on IL-6 expression[99], effects of distant allelic variants are also possible[100].

Results of research on the influence of genetic mutations on tissue expression and cytokine serum levels in IBD patients are still contradictory, although the latest studies indicate a possible role of gene polymorphisms in the disease development and progression[101]. Mutations are therefore potential therapeutic and diagnostic markers of many diseases[102]. The causes of controversial associations remain unclear. IBDs in different populations may have different immunogenic mechanisms. From the above discussion, it is clear that IL-6 expression regulation exceeds simple divisions such as that into universal low and highly productive IL-6 allelic variants.

Acknowledgment

This work was supported by research grant from the Croatian Ministry of Science, Education and Sports #219-2190372-3119 and #219-2190372-2068.

References

1. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet. 2007;369(9573):1641-57, http://dx.doi.org/10.1016/S0140-6736(07)60751-X
2. Hanauer SB. Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis. 2006;12 Suppl 1:S3-9, http://dx.doi.org/10.1097/01.MIB.0000195385.19268.68
3. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(21):2066-78, http://dx.doi.org/10.1056/NEJMra0804647
4. Abraham C, Cho J. Interleukin-23/Th17 pathways and inflammatory bowel disease. Inflamm Bowel Dis. 2009;15(7):1090-100, http://dx.doi.org/10.1002/ibd.20894
5. Mihaljevic S, Kibel A, Stefanic M, Glavas-Obrovac L, Takac B, Krznaric Z, *et al*. Polymorphisms of interleukin-23 receptor in patients with inflammatory bowel disease in a Croatian tertiary center. Coll Antropol. 2013;37(4):1171-7.
6. Balding J, Livingstone WJ, Conroy J, Mynett-Johnson L, Weir DG, Mahmud N, *et al*. Inflammatory bowel disease: the role of inflammatory cytokine gene polymorphisms. Mediators Inflamm. 2004;13(3):181-7, http://dx.doi.org/10.1080/09511920410000171352 9
7. Takac B, Mihaljevic S, Stefanic M, Glavas-Obrovac L, Kibel A, Samardzija M. Importance of interleukin 6 in pathogenesis of inflammatory bowel disease. Coll Antropol. 2014;38(2):659-64.
8. Shanahan F. Crohn's disease. Lancet. 2002;359(9300):62-9, http://dx.doi.org/10.1016/S0140-6736(02)07284-7
9. Mudter J, Neurath MF. Il-6 signaling in inflammatory bowel disease: pathophysiological role and clinical relevance. Inflamm Bowel Dis. 2007;13(8):1016-23, http://dx.doi.org/10.1002/ibd.20148
10. Allocca M, Jovani M, Fiorino G, Schreiber S, Danese S. Anti-IL-6 treatment for inflammatory bowel diseases: next cytokine, next target. Curr Drug Targets. 2013;14(12):1508-21, http://dx.doi.org/10.2174/13894501131466602 24
11. Macaubas C, Wong E, Zhang Y, Nguyen KD, Lee J, Milojevic D, *et al*. Altered signaling in systematic juvenile idiophatic arthritis monocytes. Clin Immunol. 2016;163:66-74, http://dx.doi.org/10.1016/j.clim2015.12.011
12. Baran P, Hansen S, Waetzig GH, Arkbarzadeh M, Lamertz L, Huber JH, *et al*. The balance of interleukin (IL)-6, IL-6-soluble IL-6 receptor (sIL-6R), and IL-6-sIL-6-sgp130 complexes allows simultaneous classic and trans-signaling. J Biol Chem. 2018;293(18):6762-75, http://dx.doi.org/10.1074/jbc.RA117.001163
13. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta. 2011;1813(5):878-88, http://dx.doi.org/10.1016/j.bbamcr.2011.01.034
14. Mudter J, Neurath MF. Apoptosis of T cells and the control of inflammatory bowel disease: therapeutic implications. Gut. 2007;56(2):293-303, http://dx.doi.org/10.1136/gut.2005.090464
15. Sllats J, Oever JT, Van de Veerdonk FL, Netea MG. IL-1β/IL-6/CRP and IL-18/ferritin: distinct inflammatory programs in infections. Plos Pathog. 2016;12(12):e1005973, http://dx.doi.org/10.1371/journal.ppat.1005973
16. Gabay C. Interleukin-6 and chronic inflammation. Arthritis Res Ther. 2006;8(2):1-6, http://dx.doi.org/10.1186/ar1917
17. Peisajovich A, Marnell L, Mold C, Du Clos TW. C-reactive protein at the interface between innate immunity and inflam-

mation. Expert Rev Clin Immunol. 2008;4(3):379-90, http://dx.doi.org/10.1586/1744666X.4.3.379

18. Castellucci L, Menezes E, Oliveira J, Magalhaes A, Guimaraes LH, Lessa M, *et al*. IL6 -174 G/C promoter polymorphism influences susceptibility to mucosal but not localized cutaneous leishmaniasis in Brazil. J Infect Dis. 2006;194(4):519-27, http://dx.doi.org/10.1086/505504
19. Doyle WJ, Casselbrant ML, Li-Korotky HS, Doyle AP, Lo CY, Turner R, *et al*. The interleukin 6 -174 C/C genotype predicts greater rhinovirus illness. J Infect Dis. 2010;201(2):199-206, http://dx.doi.org/10.1086/649559
20. Klein W, Tromm A, Griga T, Fricke H, Folwaczny C, Hocke M, *et al*. The polymorphism at position -174 of the IL-6 gene is not associated with inflammatory bowel disease. Eur J Gastroenterol Hepatol. 2001;13(1):45-7. PubMed PMID: 11204808
21. Vickers MA, Green FR, Terry C, *et al*. Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. Cardiovasc Res. 2002;53:1029-34, http://dx.doi.org/10.1016/S0008-6363(01)00534-X
22. Wypasek E, Potaczek DP, Lamplmayr M, Sadowski J, Undas A. Interleukin-6 receptor Asp358Ala gene polymorphism is associated with plasma C-reactive protein levels and severity of aortic valve stenosis. Clin Chem Lab Med. 2014;52(7):1049-56, http://dx.doi.org/10.1515/cclm-2013-0606
23. Ferrari SL, Ahn-Loung L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and marker of bone resorption in postmenopausal women. J Clin Endocrinol Metab. 2003;88:225-9, http://dx.doi.org/10.1210/jc.2002-020092
24. Bertsch T, Zimmer W, Casarin W, Denz C, Quintel M, Fassbender K. Real-time PCR assay with fluorescent hybridization probes for rapid interleukin-6 promoter (-174G-->C) genotyping. Clin Chem. 2001;47(10):1873-4. PubMed PMID: 11568111
25. Merlini G, Bilirup-Jensen S, Johnson AM, Sheldon J, Zegers I. Standardizing plasma protein measurements worldwide: a challenging enterprise. Clin Chem Lab Med. 2010;48(11):1567-75, http://dx.doi.org/10.1515/CCLM.2010.314
26. Guo SW, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics. 1992;48(2):361-72, http://dx.doi.org/10.2307/2532296
27. Lewontin RC. The interaction of selection and linkage. I. General considerations; heterotic models. Genetics. 1964;49(1):49-67. PubMed PMID: 17248194; PubMed Central PMCID: PMC1210557
28. Hedrick PW. Gametic disequilibrium measures: proceed with caution. Genetics. 1987;117(2):331-41. PubMed PMID: 3666445; PubMed Central PMCID: PMC1203208.
29. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81(3):559-75, http://dx.doi.org/10.1086/519795
30. Westfall PH, Young SS. Resampling-Based Multiple Testing: Examples and Methods for p-Value Adjustment. New York: John Wiley & Sons Inc.; 1993.
31. Purcell S, Cherny SS, Sham PC. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics. 2003;19(1):149-50, http://dx.doi.org/10.1093/bioinformatics/19.1.149
32. Gaiendran M, Loganathan P, Catinella AP, Hashas JG. A comprehensive review and update on Crohn's disease. Dis Mon. 2018;64(2):201-57, http://dx.doi.org/10.1016/j.disamonth.2017.07.001
33. Kim DH, Cheon JH. Pathogenesis of inflammatory bowel disease and recent advances in biologic therapies. Immune Net. 2017;17(1):25-40, http://dx.doi.org/10.4110/in.2017.17.1.25
34. Ahluwalia B, Moraes L, Magnusson MK, Ohman L. Immunopathogenesis of inflammatory bowel disease and mechanisms of biological therapies. Scand J Gastroenterol. 2018;53(4):379-89, http://dx.doi.org/10.1080/00365521.2018.1447597
35. Lee SH, Kwon JE, Cho ML. Immunological pathogenesis of inflammatory bowel disease. Intest Res. 2018;16(1):26-42, doi:105217/ir.2018.16.1.26
36. Yang DH, Yang SK, Park SH, Lee HS, Boo SJ, Park JH, *et al*. Usefulnes of C-reactive protein as a disease activity marker in Crohn's disease according to the location of disease. Gut Liver. 2015;9(1):80-6, http://dx.doi.org/10.5009/gnl13424
37. Solem CA, Loftus EVJ, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. Inflamm Bowel Dis. 2005;11(8):707-12, http://dx.doi.org/10.1097/01.MIB.0000173271.18319.53
38. Poullis AP, Zar S, Sundaram KK, Moodie SJ, Risley P, Theodossi A, Mendall MA. A new, highly sensitive assay for C-reactive protein can aid the differentiation of inflammatory bowel disorders from functional bowel disorders. Eur J Gastroenterol Hepatol. 2002;14(4):409-12, http://dx.doi.org/10.1097/00042737-200204000-00013
39. Fagan EA, Dyck RF, Maton PN, Hodgson HJ, Chadwick VS, Pepys MB. Serum levels of C-reactive protein in Crohn's disease and ulcerative colitis. Eur J Clin Invest. 1982;12(4):351-9 http://dx.doi.org/10.1111/j.1365-2362.1982.tb02244.x
40. Beck PL, Wallace JL. Cytokines in inflammatory bowel disease. Mediators Inflamm. 1997;6(2):95-103, http://dx.doi.org/10.1080/09629359791785
41. Seegert D, Rosenstiel P, Pfahler H, Verkorn PP, Nikolaus S, Schreiber S. Increased expression of IL-16 in inflammatory bowel disease. Gut. 2001;48(3):326-32, http://dx.doi.org/10.1136/gut.48.3.326
42. Yoshimoto T, Nakanishi K, Hirose S, Hiroishi K, Okamura H, Takemoto Y, *et al*. High IL-6 level reflects susceptible status of the host endotoxin and IL-1/tumor necrosis factor. J Immunol. 1992;148(11):3596-603. PubMed PMID: 1588048
43. Rogler G, Andus T. Cytokines in inflammatory bowel disease. World J Surg. 1998;22(4):382-9. PubMed PMID: 9523521
44. Feng JS, Yang T, Zhu YZ, Liu Z, Guo CC, Zheng XB. Serum IL-17 and IL-6 increased accompany with TGF-β and IL-13

respectively in ulcerative colitis patients. Int J Clin Med. 2014;7(12):5498-504. PubMed PMID: 25664061; PubMed Central PMCID: PMC4307508

45. Korolkova OY, Myers JN, Pellom ST, Wang L, M'Koma AE. Characterisation of serum cytokine profile in predominantly colonic inflammatory bowel disease to delineate ulcerative and Crohn's colitides. Clin Med Insights Gastroenterol. 2015;6(8):29-44, http://dx.doi.org/10.4137/CGast.S20612
46. Mahida YR, Kurlac L, Gallagher A, Hawkey CJ. High circulating concentrations of interleukin-6 in active Crohn's disease but not ulcerative colitis. Gut. 1991;32(12):1531-4, http://dx.doi.org/10.1136/gut.32.12.1531
47. Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: from physiopathology to therapy. J Hepatol. 2016;64:1403-15, http://dx.doi.org/10.1016/j.hep.2016.02.004
48. Zhou Z, Xu MJ, Gao B. Hepatocytes: a key cell type for innate immunity. Cell Mol Immunol. 2016;13:301-15, http://dx.doi.org/10.1038/cmi.2015.97
49. Song SNJ, Yoshizaki K. Mechanism and clinical significance of IL-6 combined with TNF-α or IL-1 for the induction of acute phase proteins SAA and CRP in chronic inflammatory diseases. J Alcohol Drug Depend. 2016;4(3):1-4, http://dx.doi.org/10.4172/2329-6488.1000239
50. Rose-John S. IL-6 trans-signaling *via* the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci. 2012;8(9):1237-47, http://dx.doi.org/10.7150/ijbs.4989
51. Vollmer P, Walev I, Rose-John S, Bhakdi S. Novel pathogenic mechanism of microbial metalloproteinases: liberation of membrane anchored molecules in biologically active form exemplified by studies with the human interleukin-6 receptor. Infect Immun. 1996;64(9):3646-51. PubMed PMID: 8751912; PubMed Central PMCID: PMC174276
52. Jones SA, Scheller J, Rose-John S. Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. J Clin Invest. 2011;121(9):3375-83, http://dx.doi.org/10.1172/JCI57158
53. Itoh J, de la Motte C, Strong SA, Levine AD, Fioch C. Decreased Bax expression by mucosal T cells favours resistance to apoptosis in Crohn's disease. Gut. 2001;49:35-41, http://dx.doi.org/10.1136/gut.49.1.35
54. Ponemone V, Keshavarzian A, Brand MI, Saclarides T, Abcarian H, Cabay RJ. Apoptosis and inflamation: role of adipokines in inflammatory bowel disease. Clin Transl Gastroenterol. 2010;1(10):e1, http://dx.doi.org/10.1038/ctg.2010.1
55. Mitsuyama K, Tomiyasu N, Suzuki A, Takaki K, Takedatsu H, Masudas J, *et al*. A form of circulating interleukin-6 receptor component soluble gp130 as a potential interleukin-6 inhibitor in inflammatory bowel disease. Clin Exp Immunol. 2006; 143(1):125-31, http://dx.doi.org/10.1111/j.1365-2249.2005.02960.x
56. Scheller J, Ohnesorge N, Rose-John S. Interleukin-6 transsignaling in chronic inflammation and cancer. Scand J Immunol. 2006;63(5):321-9, http://dx.doi.org/10.1111/j.1365-3083.2006.01750.x
57. Kishimoto T. Interleukin-6: from basic science to medicine – 40 years in immunology. Annu Rev Immunol. 2005;23:1-21, http://dx.doi.org/10.1146/annurev.immunol.23.021704.115806
58. Jones J, Loftus EVJ, Panaccione R, Chen LS, Peterson S, McConell J. Relationship between disease activity and serum and fecal biomarkers in patients with Crohn's disease. Clin Gastroenterol Hepatol. 2008;6(11):1218-24, http://dx.doi.org/10.1016/j.cgh.2008.06.010
59. Florin TH, Paterson EW, Fowler EV, Radford-Smith GL. Clinically active Crohn's disease in the presence of a low C-reactive protein. Scand J Gastroenterol. 2006;41(3):306-11, http://dx.doi.org/10.1080/00365520500217118
60. Reinisch W, Gasche C, Tillinger W, Wyatt J, Lichtenberg C, Willheim M, *et al*. Clinical relevance of serum interleukin-6 in Crohn's disease: single point measurements, therapy monitoring, and prediction of clinical relapse. Am J Gastroentrol. 1999;94(8):2156-64, http://dx.doi.org/10.1111/j.1572-0241.1999.01288.x
61. Hyams JS, Fitzgerald JE, Treem WR, Wyzga N, Kreutzer DL. Relationship of functional and antigenic interleukin-6 to disease activity in inflammatory bowel disease. Gastroenterology. 1993;104(5):1285-92, http://dx.doi.org/10.1016/0016-5085(93)90336-B
62. Brown KA, Back SJ, Ruchelli ED, Markowitz J, Mascarenhas M, Verma R, *et al*. Lamina propria and circulating interleukin-6 in newly diagnosed pediatric inflammatory bowel disease patients. Am J Gastroenterol. 2002;97(10):2603-8, http://dx.doi.org/10.1111/j.1572-0241.2002.06030.x
63. Gross V, Andus T, Caesar I, Roth M, Schölmerich J. Evidence for continuous stimulation of interleukin-6 production in Crohn's disease. Gastroenterology. 1992;102(2):514-9, http://dx.doi.org/10.1016/0016-5085(92)90098-J
64. Kuhn KA, Manieri NA, Liu TC, Stappenbeck TS. IL-6 stimulates epithelial proliferation and repair after injury. PLoS One. 2014;5(9):e114195, http://dx.doi.org/10.1371/journal.pone.0114195
65. Cahill CM, Rogers JT. Interleukin (IL) 1ß induction of IL-6 is mediated by a novel phosphatidylinositol 3-kinase-dependent AKT/IκB kinase α pathway targeting activator protein-1. J Biol Chem. 2008;2836(38):25900-12, http://dx.doi.org/10.1074/jbc.M707692200
66. Confalone E, D'Alesio G, Furia A. IL-6 Induction by TNFα and IL-1ß in an osteoblast-like cell line. Int J Biomed Sci. 2010;6(2):135-40. PubMed PMID: 23675187; PubMed Central PMCID: PMC3614745
67. Lawrence T. The nuclear factor NF-κB pathway in inflammation. Cold Spring Harb Perspect Biol. 2009;1(6):a001651, http://dx.doi.org/10.1101/cshperspect.a001651
68. Stevens C, Walz G, Singaram C, Lipman ML, Zanker B, Muggia A, *et al*. Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 expression in inflammatory bowel disease. Dig Dis Sci. 1992;37(6):818-26. PubMed PMID: 1587185
69. Atreya R, Billmeier U, Rath T, Mudter T, Vieth M, Neumann H, Neurath MN. First case of exacerbated ulcerative colitis

after anti-interleukin-6R salvage therapy. World J Gastroenterol. 2015;21(45):12963-9, http://dx.doi.org/10.3748/wjg.v21.i45.12963

70. Shetty A, Hanson R, Korsten P, Shawagfeh M, Arami S, Volkov S, *et al*. Tocilizumab in the treatment of rheumatoid arthritis and beyond. Drug Des Devel Ther. 2014;8:349-64, http://dx.doi.org/10.2147/DDDDT.S41437
71. Van Kemseke C, Belaiche J, Louis E. Frequently relapsing Crohn's disease is characterized by persistent elevation in interleukin-6 and soluble interleukin-2 receptor serum levels during remission. Int J Colorectal Dis. 2000;15(4):206-10, http://dx.doi.org/10.1007/s003840000226
72. Schreiber S, Nikolaus S, Hampe J, Hämling J, Koop I, Groessner B, Lochs H, Raedler A. Tumour necrosis factor alpha and interleukin 1 beta in relapse of Crohn's disease. Lancet. 1999;353(9151):459-61, http://dx.doi.org/10.1016/S0140-6736(98)03339-X
73. Wyatt J, Vogelsgang H, Hübl W, Waldohöer T. Intestinal permeability and the prediction of relapse in Crohn's disease. Lancet. 1993;341(8858):1437-9, http://dx.doi.org/10.1016/0140-6736(93)90882-H
74. Reinisch W, Gasche C, Tillinger W, Wyatt J, Lichtenberg C, Willheim M, *et al*. Clinical relevance of serum interleukin-6 in Crohn's disease: single point measurements, therapy monitoring, and prediction of clinical relapse. Am J Gastroentrol. 1999;94(8):2156-64, http://dx.doi.org/10.1111/j.1572-0241.1999.01288.x
75. Vermeire S, Van Assche G, Rutgeerts P. C-reactive protein as a marker for inflammatory bowel disease. Inflamm Bowel Dis. 2004;10(5):661-5, http://dx.doi.org/10.1097/00054725-200409000-00026
76. Nielsen OH, Vainer B, Madsen SM, Seidelin JB, Heegard NH. Established and emerging biological activity markers of inflammatory bowel disease. Am J Gastroenterol. 2000;95(2):359-67, http://dx.doi.org/10.1111/j.1572-0241.2000.t01-1-01790.x
77. Zouku C, Beauchamp M, Baron C, Fliep JG. Prevention of *in vitro* neutrophil adhesion to endothelial cells through shedding of L-selectin by C-reactive protein and peptides derived from C-reactive protein. J Clin Invest. 1997;100(3):522-9, http://dx.doi.org/10.1172/JCI119561
78. Bodman-Smith KB, Melendez AJ, Campbell I, Harrison PT, Allen JM, Raynes JG. C-reactive protein mediated phagocytosis and phospholipase D signalling through the high-affinity receptor for immunoglobulin G (FcgammaRI). Immunology. 2002;107:252-60, http://dx.doi.org/10.1046/j.1365-2567.2002.01481.x
79. Manolov DE, Rocker C, Hombach V, Nienhaus GU, Torzewski J. Ultrasensitive confocal fluorescence microscopy of C-reactive protein interacting with FcyRIIa. Arterioscler Thromb Vasc Biol. 2004;24(12):2372-7, http://dx.doi.org/10.1161/01ATV.0000147407.17137.02
80. Diehl S, Rincon M. The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol. 2002;39(9):531-6. PubMed PMID: 12431386
81. Yamada S, Gotoh T, Nakashima Y, Kayaba K, Ishikawa S, Nago N, *et al*. Distribution of serum C-reactive protein and its association with atherosclerotic risk factors in a Japanese population: Jichi Medical School Cohort Study. Am J Epidemiol. 2001;153(12):1183-90. PubMed PMID: 11415953
82. Tebbutt NC, Giraud AS, Inglese M, Jenkins B, Waring P, Clay FJ, *et al*. Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice. Nat Med. 2002;8(10):1089-97, http://dx.doi.org/10.1038/nm763
83. Jin X, Zimmers TA, Zhang Z, Pierce RH, Koniaris LG. Interleukin-6 is an important *in vivo* inhibitor of intestinal epithelial cell death in mice. Gut. 2010;59(2):186-96, http://dx.doi.org/10.1136/gut.2008.151175
84. Heubner C, Petermann I, Lam WJ, Shelling AN, Ferguson LR. Characterization of single-nucleotide polymorphism relevant to inflammatory bowel disease in commonly used gastrointestinal cell line. Inflamm Bowel Dis. 2010;16(2):285-95, http://dx.doi.org/10.1002/ibd.21043
85. Chen J, Ying XM, Huang XM, Huang P, Yan SC. Association between polymorphisms in selected inflammatory response genes and the risk of prostate cancer. Onco Targets Ther. 2016;9:223-9, http://dx.doi.org/10.2147/OTT.S91420
86. Genetic Testing Registry. IL 6 Interleukin 6. Available at: https://www.ncbi.nlm.nih.gov/gene/3569.
87. Wujcicka W, Gaj Z, Wilczyński J, Nowakowska D. Contribution of IL-6174 G>C and IL1B +3954 C>T polymorphisms to congenital infection with *Toxoplasma gondii*. Eur J Clin Microbiol Infect Dis. 2015;34(11):2287-94, http://dx.doi.org/10.1007/s10096-015-2481-z
88. Belluco C, Olivieri F, Bonafè M, Giovagnetti S, Mammano E, Scalerta R, *et al*. -174 G>C polymorphism of interleukin 6 gene promoter affects interleukin 6 serum level in patients with colorectal cancer. Clin Cancer Res. 2003;9(6):2173-6. PubMed PMID: 12796383
89. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem. 2000;275(24):18138-44, http://dx.doi.org/10.1074/jbc.M000379200
90. Mandić S, Sudarević B, Marczi S, Horvat V, Mihaljević S, *et al*. Interleukin-6 polymorphism and prostate cancer risk in population of eastern Croatia. Coll Antropol. 2013;37(3):907-11. PubMed PMID: 24308236
91. Kim Y, Hooten NN, Dluzen DF, Martindale JL, Gorospe M, Evans MK. Posttranscriptional regulation of the inflammatory marker C-reactive protein by the RNA-binding protein HuR and MicroRNA 637. Mol Cell Biol. 2015;35(24):4212-21, http://dx.doi.org/10.1128/MCB.00645-15
92. Cross DS, Ivacic LC, Stefanski EL, McCarty CA. Population based allele frequencies of disease associated polymorphisms in the Personalized Medicine Research Project. BMC Genet. 2010;11:51, http://dx.doi.org/10.1186/1471-2156-11-51
93. Balding J, Livingstone WJ, Conroy J, Mynett-Johnson, Weir DG. Inflammatory bowel disease: the role of inflammatory cy-

tokine gene polymorphisms. Mediators Inflamm. 2004;13 (3):181-7, http://dx.doi.org/10.1080/09511920410001713529

94. Guerreiro CS, Ferreira P, Tavares L, Santos PM, Neves M, Brito M, Cravo M. Fatty acids, IL6, and TNFalpha polymorphisms: an example of nutrigenetics in Crohn's disease. Am J Gastroenterol. 2009;104(9):2241-9, http://dx.doi.org/10.1038/ajg.2009.313
95. Pawlik A, Wrzesniewska J, Florezak M, Gawronska SB, Herezyiska M. IL-6 promoter polymorphism in patients with rheumatoid arthritis. Scand J Rheumatol. 2005;34(2):109-13. PubMed PMID: 16095005
96. Banday MZ, Balkhi HM, Sameer AS, Chowdri NA, Hag E. Strong association of interleukin-g-174 G/C promoter nucleotide polymorphism with decreased risk of colorectal cancer in ethnic Kashmiri population: a case control study. Tumour Biol. 2017;39(3):396-403, http://dx.doi.org/10.1177/1010428317695940
97. Sawczenko A, Azooz O, Paraszczuk, Idestrom M, Croft NM, Savage MO, *et al*. Intestinal inflammation-induced growth retardation acts through IL-6 in rats and depends on the -174 IL-6 G/C polymorphism in children. Proc Natl Acad Sci U S A. 2005;102(37):13260-5, http://dx.doi.org/10.1073/pnas.0503589102
98. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. The effect of novel polymorphism in the IL-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest. 1998;102(7):1369-76, http://dx.doi.org/10.1172/JCI2629
99. Reich D, Patterson N, Ramesh V, De Jager PL, McDonals GJ, Tandon A, *et al*. Admixture mapping of an allele affecting interleukin 6 soluble receptor and interleukin 6 levels. Am J Hum Genet. 2007;80(4):716-26, http://dx.doi.org/10.1086/513206
100. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem. 2000;275(24):18138-44, http://dx.doi.org/10.1074/jbc.M000379200
101. Senhaji N, Serrano A, Serbati N, Karkouri M, Zaid Y, Nadifi S, Martin J. Association of inflammatory cytokine gene polymorphisms with inflammatory bowel disease in a Moroccan cohort. Genes Immun. 2016;7(1):60-5, http://dx.doi.org/10.1038/gene.2015.52
102. Deng N, Zhou H, Fan H, Yan Y. Single nucleotide polymorphisms and cancer susceptibility. Oncotarget. 2017;8(66):110635-49, http://dx.doi.org/1018632/oncotarget.22372

Sažetak

INTERAKCIJA IZMEĐU INTERLEUKINA-6, C-REAKTIVNOG PROTEINA I INTERLEUKINA-6 (-174) G/C POLIMORFIZMA U PATOGENEZI CROHNOVE BOLESTI I ULCEROZNOG KOLITISA

B. Takač, S. Mihaljević, Lj. Glavaš-Obrovac, A. Kibel, M. Suver-Stević, S. Canecki-Varžić, M. Samardžija, I. Rajkovac, D. Kovač i *M. Štefanić*

Upalne bolesti crijeva predstavljaju multifaktorski poremećaj klinička manifestacija kojega ovisi o interakciji imunog odgovora te genetskih i okolišnih čimbenika. Rezultati više novijih istraživanja upućuju na ulogu citokina i polimorfizama gena u patogenezi bolesti u različitim populacijama, iako molekularni mehanizmi njihova singaliziranja i interakcije još nisu dovoljno poznati. Cilj ovoga istraživanja bio je ispitati učinke interleukina-6, C-reaktivnog proteina i interleukin-6 rs1800795 na razvoj Crohnove bolesti, ulceroznoga kolitisa i upalnih bolesti crijeva općenito te utvrditi razlike između skupine ispitanika oboljelih od upalnih bolesti crijeva i kontrolne skupine ispitanika. U istraživanje je uključeno ukupno 132 oboljela od upalnih bolesti crijeva i 71 zdravi davatelj krvi. Serumska koncentracija interleukina-6 određena je kemiluminiscentnom sekvencijskom imunometričnom, a koncentracija C-reaktivnog proteina imunoturbidimetrijskom metodom. Analiza polimorfizma rs1800795 provodila se na uređaju LightCyclerTM PCR 1.2 u stvarnome vremenu temeljem prijenosa energije uslijed fluorescentne rezonancije. Serumske koncentracije interleukina-6 i C-reaktivnoga proteina bile su povišene i u oboljelih od Crohnove bolesti i oboljelih od ulceroznoga kolitisa. Utvrđene su pozitivne korelacije između serumskih koncentracija C-reaktivnoga proteina i interleukina-6 i indeksa aktivnosti ulceroznoga kolitisa mjerenoga prema ljestvici MTWSI. Serumska koncentracija C-reaktivnog proteina bila je viša u oboljelih od Crohnove bolesti bez prethodne resekcije crijeva u usporedbi s oboljelima od Crohnove bolesti s prethodnom resekcijom crijeva. U oboljelih od ulceroznoga kolitisa serumske koncentracije interleukina-6 i C-reaktivnog proteina bile su statistički značajno više kod CC genotipa interleukina-6 u usporedbi s genotipom GG+GC. Analizom polimorfizma promotorske regije IL-6 rs1800795 nisu uočene razlike u učestalosti alela između oboljelih od Crohnove bolelsti, oboljelih od ulceroznoga kolitisa i kontrolne skupine ispitanika, ni razlike između kontrolne skupine ispitanika i oboljelih od upalnih bolesti crijeva općenito. Rezultati ove studije pružaju potencijalno važan uvid u ulogu signaliziranja interleukina-6 i C-reaktivnoga proteina te polimorfizma interleukina-6 u patogenezi Crohnove bolesti i ulceroznoga kolitisa.

Ključne riječi: *Crohnova bolest; Kolitis, ulcerozni; Interleukin-6; C-reaktivni protein; Polimorfizam jednog nukleotida; Upalne bolesti crijeva*