THE -2518 A/G MCP-1 POLYMORPHISM AS A RISK FACTOR OF INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel diseases (IBD) are disorders originated from immune disturbances. The aim of the study was to evaluate the association between the -2518 A/G MCP-1 polymorphism and the risk of IBD development.

Material and methods. Genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Study group consisted of 197 subjects with IBD (120 with ulcerative colitis and 77 with Crohn’s disease) as well as 210 healthy controls.

Results. The presence of the -2518 G/G MCP-1 genotype in the investigated groups seems to be connected with higher risk of inflammatory bowel disease as well as Crohn’s disease only (OR 2.26; 95% CI 1.44-3.54 and OR 2.08; 95% CI 1.21-3.46, respectively).

Conclusions. Our data showed that the -2518 A/G MCP-1 polymorphism might be associated with the IBD occurrence and might be used as predictive factor of these diseases in a Polish population.

Key words: single nucleotide polymorphism, MCP-1 gene, inflammatory bowel disease

Colorectal cancer is one of the diseases with documented inflammatory background. One of the major causes of this entity is expression disturbances of cytokines related to induction of inflammation process. One of them is monocyte chemoattractant protein 1 (MCP-1/CCL-2). It is produced by a variety of cell types, either constitutively or after induction by oxidative stress, cytokines, endotoxin or other proinflammatory mediators (1, 2). Under inflammatory conditions MCP-1 plays a crucial role in recrutation of monocytes from bone marrow to the colon mucosa tissues (3). In situ hybridization and immunohistochemical assays showed an elevated level of MCP-1 mRNA and protein production by macrophages, smooth muscle, and endothelial cells in comparison to histologically normal mucosa (4).

Long lasting inflammation and permanent higher expression of MCP-1 may cause severe colitis and inflammatory bowel disease, probably as a result of prominent infiltrate of inflammatory cells including lymphocytes, macrophages, and neutrophils and alterations in enterochromaffin (EC) cells that produce 5-hydroxytryptamine (5-HT) (5). It is unclear whether the pro-inflammatory response associated with CCL-2 may cause the exacerbation of the disease or act protective. It is supposed that at the molecular background of these disturbances may lay the polymorphism or mutations in genes that encode cytokines and other
proinflammatory mediators. Cytokines are pleiotropic and their multiple overlapping functions often depend on their local concentration. The changes of cytokine expression level are mainly caused by polymorphisms in the promoter region of the gene. One way to explore the role of this chemokine is to determine whether the -2518 A/G MCP-1 polymorphism that affects gene expression is associated with the disease outcome.

The aim of our research study was to evaluate the role of the -2518 A/G MCP-1 polymorphism as the risk factor for inflammatory bowel disease in a case-control setting.

MATERIAL AND METHODS

Patients

Peripheral blood samples were obtained from 197 IBD patients (102 men and 95 women; median age 44, quartiles: 29, 56 years). Among them 120 subjects have ulcerative colitis (67 men and 53 women; median age 46, quartiles: 32, 57 years), 77 subjects have been diagnosed with Crohn’s disease (33 men and 44 women; median age 40, quartiles: 27, 51 years). Control group consisted of age (± 5 years), sex and ethnicity-matched 210 blood donors, with no cancer cases and no inflammatory diseases. Patients with inflammatory bowel diseases were selected during direct colonoscopy, histological examination and CRP level assay. Prior to examination, patients and control subjects did not receive any drugs like antibiotics or steroids. All patients and control subjects were recruited from Department of General and Colorectal Surgery, Medical University in Łódź, Poland and Department of Gastroenterology and Internal Diseases, Medical University in Łódź, Poland. All subjects involved in the study were unrelated Caucasians and resided in the Łódź district, Poland. The study was approved by the Local Ethic Committee and written consent from each patient or healthy blood donor was obtained before participating in the study.

Genotyping

Genomic DNA was prepared using the QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA (Qiagen, Chatsworth, CA, USA). PCR – Restriction fragments length polymorphism (RFLP) was employed to determine the genotypes of the -2518 A/G MCP-1 polymorphism. Each 20 µl of the PCR reaction contained 10 ng genomic DNA, 1.25 U Taq polymerase in 1×PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 11 mM MgCl₂, 0.1% gelatine), 1.5 mM MgCl₂, 50 mM dNTPs, and 250 nM of each primer (Qiagen, Chatsworth, CA, USA). Thermal cycling conditions for the fragment containing the MCP-1 gene were as follows: an initial denaturation step at 94°C for 1 min, followed by 35 cycles of 94°C for 30 sec and 30 sec at an annealing temperature of 55°C, and 72°C for 30 sec. The final extension was performed at 72°C for 7 min. The PCR was carried out in a MJ Research, INC thermal cycler; model PTC-100 (Waltham, MA, USA). The -2518 A/G polymorphism of the MCP-1 gene was determined using the following primers (Sigma-Aldrich, St. Louis, MO, USA): sense, 5’-CCG AGA TGT TCC CAG CAC AG-3’ and antisense 5’-CTG CTT TGC TTG TGC TTC TT-3’. The 930 bp product was digested for 6 hours at 37ºC with 5 U of the PvuII restriction enzyme. Restriction fragments were separated on a 4% agarose gel in a TAE buffer. The gel was stained with ethidium bromide and visualized under UV light. More than 10% of the samples were repeated, and the results were 100% concordant.

Statistical analysis

To compare the distributions of demographic variables and selected risk factors between patients and controls, chi-square tests were used. The Hardy–Weinberg equilibrium (HWE; \( p^2 + 2pq + q^2 = 1 \), where p is the frequency of the variant allele and \( q = 1 - p \)) was tested by a goodness-of-fit chi-square test to compare the observed genotype frequencies with expected genotype frequencies in cancer-free controls. Potential linkage between genotype and cancer was assessed by the logistic regression. Analyses were performed using STATISTICA 6.0 package (Statsoft, Tulsa, OK, USA).

RESULTS

The observed genotype frequencies of the -2518 A/G MCP-1 polymorphism in the control
subjects were not in agreement with HWE ($P = 0.002$; $\chi^2 = 9.467$). It is caused by the low abundance of the *MCP-1* -2518 G/G genotype in the examined Polish population. According to NCBI database (access on 20th February 2012), there is a very low frequency of -2518 G/G genotype in European population (less than 5%) and this is the reason why obtained results are not consistent with Hardy-Weinberg equilibrium (6).

All results are presented in tab. 1. Research revealed that the presence of -2518 G/G *MCP-1* genotype in the investigated groups seems to be connected with higher risk of IBD (OR 3.563; 95% CI 1.138-11.159; tab. 1). When this group was subdivided into two groups: Crohn’s disease and ulcerative colitis subjects we found an association between the -2518 G/G polymorphic variant and CD occurrence (OR 4.207; 95% CI 1.176-15.053, tab. 1). No such correlation was observed in UC patients (tab. 1).

### DISCUSSION

Our outcomes show that polymorphic variant of the the -2518 A/G *MCP-1* polymorphism is correlated with higher risk of inflammatory bowel disease. The -2518 G allele was found to increase *MCP-1* expression (7). This confirms results of Reinecker et al. (1995) as well as Grimm et al. (1996) that show elevated levels of both MCP-1 mRNA and protein in inflammed colon (4, 8). Herfarth et al. (2003), opposite to our research, did not find any association between the -2518 A/G *MCP-1* polymorphic variants and Crohn’s Disease occurrence in German population (9). In Italian population Palmieri et al. (2010) also did not find any statistical differences between genotype frequencies in inflammatory bowel disease and ulcerative colitis subjects compared to controls (10). Moreover, presence of the -2518 G/G genotype in Crohn’s Disease patients was even lower than in control group. Other trials also showed lack of correlation between inflammatory bowel diseases (ulcerative colitis and Crohn’s Disease) and the -2518 A/G *MCP-1* polymorphism in Tunisian population (11).

All menioned trials were conducted on different populations and that may be the key reason of results disagreement between compared groups.

Our work revealed that the -2518 A/G polymorphism of the *MCP-1* gene influences the susceptibility of inflammatory bowel disease. Our results compared to those obtained from other nations suggest that -2518 G/G *MCP-1* genotype may be connected with IBD and CD only in Poles, what makes it a specific marker of those diseases in Polish population. It also suggests that -2518 G/G *MCP-1* genotype may have an impact on the development of IBD and may affect the turn of epithelial as well as subepithelial cells of inflamed colon into cancer cells, but this point should be confirmed by correlation of our outcomes with histopathological trials.
REFERENCES


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