CONTRIBUTION OF THE -173 G/C POLYMORPHISM OF MACROPHAGE MIGRATION INHIBITORY FACTOR GENE TO THE RISK OF INFLAMMATORY BOWEL DISEASES*

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Inflammatory bowel disease (IBD) represents a heterogeneous group of chronic disorders characterized by inflammation of gastrointestinal tract, typically with a relapsing and remitting clinical course of unknown etiology. Presumably, IBD develops with response exogenous environmental factors only in persons with genetic predisposition. This predisposition was suggested to be associated with polymorphism and mutations in genes encoding proinflammatory immune system proteins. Enhanced production of macrophage migration inhibitory factor (MIF) was found in patients with inflammatory bowel disease (IBD) and mice with experimental colitis. These results suggest that MIF plays a critical role in etiology of the colitis.

The aim of the study was to determine whether the MIF -173 G/C gene polymorphism is associated with the susceptibility to inflammatory bowel disease (IBD).

Material and methods. A total of 99 IBD patients, including 58 patients with ulcerative colitis (UC) and 41 with Crohn’s disease (CD) and 436 healthy controls recruited from the Polish population, were genotyped for MIF polymorphisms. Genotyping of MIF gene polymorphism was performed by a RFLP-PCR.

Results. We found an increased risk of UC for the C allele of the MIF-173 G/C polymorphism. The distribution of the genotypes was not significantly different in the CD group compared with the controls.

Conclusions. We demonstrated that the C allele is associated with an increased risk for development of UC. This suggests that the G/C polymorphism in the MIF gene promoter may be a potential risk factor for UC in Polish population.

Key words: inflammatory bowel disease, MIF -173 G/C gene polymorphism, Crohn’s disease

Inflammatory bowel disease (IBD) represents a heterogeneous group of chronic disorders that lead to cachexia and are very bothersome and completely incurable, typically with a relapsing and remitting clinical course of unknown etiology. Its two major forms include ulcerative colitis (UC) and Crohn’s disease (CD). Etiology of inflammatory bowel disease is related to immune factors in the intestinal mucosa, intestinal microflora and genetic predisposition (1). Chronic bowel inflammation is presumed to result from imbalance between pro- and anti-inflammatory agents in the large intestine mucosa (UC) or in the whole wall of the gastrointestinal system (CD). Currently these disorders are believed to be related to polymorphisms and mutations of genes encoding protein responsible for the development of

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inflammatory processes that change expression or activity of these proteins, which in turn contributes to chronic inflammation in the intestinal mucosa (2). Genetic etiology of inflammatory bowel disease is supported by racial and ethnical difference in their incidence and marked increase of their risk in relatives of the patients suffering from these diseases (3). Therefore, there is a need for genetic risk factors of IBD that could identify subjects at risk and subject them to prophylactic management involving avoidance of known environmental risk factors or early therapy reducing complications of these disorders.

The recent studies indicate significant role of macrophage migration inhibitory factor (MIF) in the development of IBD: high level of this protein was found in the serum of IBD patients and in the mucosa of the large intestine of mice with experimentally induced IBD (4, 5).

In view of recently observed increased incidence of IBD in Poland, the aim of our study was to assess functional significance of G/C polymorphism at position 173 of the promoter region in the gene encoding macrophage migration inhibitory factor (MIF) for the development of IBD in the Polish population. G/C substitution at position 173 of the MIF gene results in appearance of AP-4 binding sequence that markedly increases MIF expression (6). Increased serum level was found in C allele carriers with various diseases (7, 8, 9). Since MIF plays a significant role in inflammatory disorders, including IBD, the studies polymorphism may become a genetic marker of these disorders.

MATERIAL AND METHODS

*MIF* polymorphism was analyzed in 99 patients with IBD, including 58 patients with ulcerative colitis and 41 patients with Crohn’s disease. The control group included 123 apparently healthy subjects with no signs of intestinal mucosa inflammation. Subjects enrolled to the study were from 18 to 60 years old, were hospitalized in Department of Gastroenterology and Internal Medicine, Medical University in Łódź and in Department of General and Colorectal Surgery, Medical University in Łódź and in Department of General and Colorectal Surgery, Medical University in Łódź. UC 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.

Bioethics Committee of Medical University in Łódź approved the study on 15.12.2009 (approval number RNN/835/09/KB).

Five ml 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 Gdanskit, according to manufacturer’s instructions. Analysis of polymorphic variants for *MIF* was performed using RFLP-PCR method (10). PCR reaction used oligonucleotide pairs restricting the polymorphic site of the studied gene to the following sequence: MIF173:F; 5’ACTAAGAAAGACCCGAGGC-3’, MIF173:R; 5’GGGGCACGTTGGTGTATA-3’.

The PCR reaction was performed in a MultiGene thermocycler (Labnet International Inc) in a total volume of 20 µl, containing 100 ng of genomic DNA, 1.45 nmol of any oligonucleotide (Eurogentec, Seraing, Belgium), 200 mM of any deoxynucleotide dATP, dCTP, dGTP and dTTP (Qiagen, Germany), 20 mM Tris-HCl (pH 8.4) 50 mM KCl, 1.5 µl MgCl$_2$ and 1 U Taq polymerase (Qiagen, Germany). The reactions were conducted under the following temperature conditions: predenaturation step at 94°C for 5 min, and then in 35 cycles: denaturation at 94°C for 30 s, oligonucleotide annealing: 51°C for 30 s, amplification of PCR products at 72°C for 30 s, and final incubation at 72°C for 5 minutes. Products of PCR reaction were subjected to digestion by a restrictive enzyme AluI (1.5 U) at 37°C. After 16 hours of digestion by a restrictive enzyme, the samples were separated by electrophoresis on a 8% polyacrylamide gel and obtained restrictive fragments were visualize by staining with ethidium bromide. In G/G homozygotes, electrophoresis resulted in 2 bands at the level of 268, 98 base pairs; G/C heterozygotes had 4 bands at the level of 268, 206, 98, 62 base pairs. C/C homozygotes had 3 bands at the level of 268, 206, 98, 62 base pairs. Figure 1 presents a representative electrophoregram obtained after digestion of PCR products.

Chi$^2$ test was used to test significance of differences in the allele frequency and genotype distribution between the two study groups. Hardy-Weinberg equilibrium test was performed for both study groups. Odds ratio (OR) and corresponding 95% confidence inter-
vals (CI) were used to assess correlations between genotypes and alleles and IBD.

RESULTS

Table 1 presents distribution of genotypes and allele in the IBD group and control group. Distribution of genotypes in the IBD group and control group was compatible with Hardy-Weinberg equilibrium (p=0.57071 and 0.42721, respectively). Statistically significant differences in the distribution and frequency of alleles between the study groups were shown. Risk of IBD was approximately two-fold higher for the G/C genotype and C allele (OR=2.02 (1.08;1.93); OR=1.89 (1.09;3.29), respectively). IBD patients were subdivided into UC and CD subgroups. Statistically significant differences in the distribution of genotypes between UC subgroup and the control group were found (tab. 2). Our results indicate that high C allele rate is correlated with UC (genotype G/C: OR=2.15 (1.05;4.39); allele C: OR=1.95 (1.04, 3.66) p=0.03). No statistically significant differences were found between CD patients and the control group with regard to genotype distribution and frequency of alleles of G/C polymorphism of MIF gene (tab. 3).

DISCUSSION

Macrophage migration inhibitory factor (MIF) is a key proinflammatory mediator, an

Table 1. The genotype and allele frequency and odds ratios (OR) of the MIF G/C polymorphism in inflammatory bowel disease (IBD) patients and controls

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>IBD patients (n=99)*</th>
<th>Controls (n=123)*</th>
<th>OR (PU 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>66 (0.67)</td>
<td>99 (0.80)</td>
<td>Ref.</td>
</tr>
<tr>
<td>G/C</td>
<td>31 (0.31)</td>
<td>23 (0.19)</td>
<td>2.02 (1.08; 1.93) p=0.03</td>
</tr>
<tr>
<td>C/C</td>
<td>2 (0.02)</td>
<td>1 (0.01)</td>
<td>3.00 (0.27; 33.75) p=0.36</td>
</tr>
<tr>
<td>G</td>
<td>163 (0.82)</td>
<td>221 (0.90)</td>
<td>Ref.</td>
</tr>
<tr>
<td>C</td>
<td>35 (0.18)</td>
<td>25 (0.10)</td>
<td>1.89 (1.09; 3.29) p=0.02</td>
</tr>
</tbody>
</table>

a (p>0.05) and b (p>0.05) consistent with Hardy-Weinberg equilibrium

Table 2. The genotype and allele frequency and odds ratios (OR) of the MIF G/C polymorphism in ulcerative colitis (UC) patients and controls

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>UC patients (n=58)</th>
<th>Controls (n=123)</th>
<th>OR (PU 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>38 (0.66)</td>
<td>99 (0.80)</td>
<td>Ref.</td>
</tr>
<tr>
<td>G/C</td>
<td>19 (0.33)</td>
<td>23 (0.19)</td>
<td>2.15 (1.05; 4.39) p=0.03</td>
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<tr>
<td>C/C</td>
<td>1 (0.02)</td>
<td>1 (0.01)</td>
<td>2.60 (0.16; 42.71) p=0.48</td>
</tr>
<tr>
<td>G</td>
<td>95 (0.82)</td>
<td>221 (0.90)</td>
<td>Ref.</td>
</tr>
<tr>
<td>C</td>
<td>21 (0.18)</td>
<td>25 (0.10)</td>
<td>1.95 (1.04; 3.66) p=0.03</td>
</tr>
</tbody>
</table>

Table 3. The genotype and allele frequency and odds ratios (OR) of the MIF G/C polymorphism in Crohn's disease (L-C) patients and controls

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>L-C patients (n=41)</th>
<th>Controls (n=123)</th>
<th>OR (PU 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>28 (0.68)</td>
<td>99 (0.80)</td>
<td>Ref.</td>
</tr>
<tr>
<td>G/C</td>
<td>12 (0.29)</td>
<td>23 (0.19)</td>
<td>1.84 (0.81; 4.16) p=0.21</td>
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<tr>
<td>C/C</td>
<td>1 (0.02)</td>
<td>1 (0.01)</td>
<td>3.53 (0.21; 58.34) p=0.40</td>
</tr>
<tr>
<td>G</td>
<td>68 (0.83)</td>
<td>221 (0.90)</td>
<td>Ref.</td>
</tr>
<tr>
<td>C</td>
<td>14 (0.17)</td>
<td>25 (0.10)</td>
<td>1.82 (0.88; 3.69) p=0.09</td>
</tr>
</tbody>
</table>
important cytokine involved in regulation of innate immunity. MIF is also known as a pituitary hormone released in response to endotoxin stimulation, responsible for controlling immune response and through inhibition of anti-inflammatory effects of glucocorticosteroids (11, 12). MIF may be synthesized by various cell types in response to their stimulation by proinflammatory cytokines and its release induces production of TNF-α by macrophages (13). Abnormal MIF expression plays an important role in the pathogenesis of chronic inflammatory disease such as rheumatoid arthritis (6), acute respiratory diseases (7) or glomerulonephritis (8). High serum MIF levels were also shown in IBD patients and in experimentally induced colitis (4, 5, 14). Marked increase of MIF expression was demonstrated in epithelial cells and immune cells infiltrating the large intestine, in the colitis model (4). Furthermore, blockade of biological activity of MIF through antibodies markedly limited production of TNF-α, interferon (IFN)-γ and matrix metalloproteinases in the large intestinal tissues in experimentally induced murine colitis model (4). Results of these studies indicate significance of MIF for the progression of inflammation in the large intestine.

Therefore it should be tested if C allele in the position 173, correlating with increased MIF gene expression, could result in excessive activation and proliferation of T cells and increased production of proinflammatory cytokines, eventually resulting in chronic inflammation of intestinal mucosa.

Here we tested if G/C polymorphism at position 173 of the promoter region in the MiF gene predisposes to IBD development. Our study demonstrated much higher C allele frequency in the IBD group where it correlated with UC. Available data suggest that this polymorphism is associated with increased incidence of ulcerative colitis in the Polish population. Contribution of any polymorphism to the risk of occurrence of a particular disorder is known to vary between populations. Results obtained in various populations are unclear. Literature data indicate that G/C polymorphism at position 173 of the MiF gene is associated with higher predisposition or intensity of inflammatory disorders, including UC (15). Correlation between C/C genotype at position 173 of the MiF gene and predisposition to UC was also identified in the Chinese population (16). Studies in Japanese population did not demonstrate any significant differences in the distribution of genotypes between UC patients and healthy subjects (17). Similarly Shiroeda H. et al. demonstrated that the discussed polymorphism had no effect on predisposition and phenotyping of UC in the Japanese population (18).

Studies in Polish population of IBD patients did not find any correlation between the analyzed polymorphism and Crohn’s disease. This indicates lack of C allele on incidence of this disease. Other researchers also did not conform a correlation between increased incidence of CD and G/C polymorphism at position 173 of the MiF gene (19).

In summary, demonstration of a correlation between C allele and increased risk of UC indicates potential significance of G/C polymorphism at position 173 of the promoter region in the MiF gene as a genetic risk factor of this disease in Polish population. However, lack of correlation between the studied polymorphism and CD may suggest markedly lower contribution of MIF to pathogenesis of this disorder, which is supported by previous publications.

REFERENCES

3. Pena AS et al.: Genetics and epidemiology may contribute to understanding the pathogenesis of IBD– a new approach is now indicated. Can J Gastroenterol 1993; 7: 71-75.


