Original Article
Association between CXCL12 and CXCR4 polymorphisms and inflammatory bowel disease risk in a Guangxi Zhuang population

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Abstract: Objective: To explore the association of the CXCL12 gene polymorphism rs1801157 and the CXCR4 gene polymorphism rs2228014 and susceptibility to inflammatory bowel disease (IBD) in a Zhuang population from Guangxi, China. Methods: A case control study was performed, which included intestinal tissue samples of 245 Zhuang patients with IBD in the experimental group and 202 healthy Zhuang subjects in the control group from January 2013 to February 2016. All patients and healthy subjects were from the Guangxi Zhuang Autonomous Region of China. Genomic DNA was extracted from intestinal tissue by the centrifugal column method. CXCL12 rs1801157 and CXCR4 rs2228014 polymorphisms were amplified by polymerase chain reaction (PCR), and then genotyped by restriction fragment length polymorphism (RFLP) analysis and sequencing. Results: The allele and genotype distributions of CXCL12 rs1801157 and CXCR4 rs2228014 were all in Hardy-Weinberg equilibrium for the IBD group and the control group. The genotypes of CXCL12 rs1801157 and CXCR4 rs2228014 polymorphisms were found in both IBD patients and healthy controls. No significant differences were noted in genotype and allele frequencies of CXCL12 rs1801157 among UC/CD and control groups (P > 0.05). Differences in allele frequency between UC/CD patients and control group for CXCR4 rs2228014 were statistically significant (χ² = 11.589, P = 0.001; χ² = 14.365, P < 0.001). Conclusion: The CXCL12 gene rs1801157 polymorphism may not be associated with IBD in the Guangxi Zhuang population, but CXCR4 rs2228014 may be associated with IBD in the Guangxi Zhuang population.

Keywords: Chemokine ligand 12, chemokine receptor 4, inflammatory bowel disease, single nucleotide polymorphisms

Introduction

Inflammatory bowel disease (IBD), the cause of which is not clear, is a chronic non-specific inflammatory bowel disease, including ulcerative colitis (UC) and Crohn’s disease (CD). Recently, the incidence of IBD has increased gradually, and its aetiology and pathogenesis is still unclear. A large number of studies suggest that the interaction of genetic, immunological and environmental factors is involved in the pathogenesis of IBD, which may determine the clinical phenotype [1-3]. Among them, gene mutation is the most direct cause of IBD at the molecular level. So far, genome-wide association studies (GWAS) have identified more than 160 susceptibility loci for IBD. Association of the CXC Chemokine Ligand 12 (CXCL12) gene polymorphism rs1801157 and the CXC chemokine receptor 4 (CXCR4) gene polymorphism rs2228014 and diseases such as autoimmune rheumatic disease, HIV infection, transplant rejection and cancer has been reported [4-5]; however, the relationship between these two genes and the incidence of IBD has rarely been reported, especially in the Guangxi Zhuang population. Among different ethnicities and regions, gene polymorphism frequencies vary. The Guangxi Zhuang Autonomous Region is one of the largest ghettos of Zhuang nationality in China, so the genetic diseases and genetic polymorphism of the Guangxi Zhuang population are unique [6]. Therefore, we investigated the association be-
Gene polymorphisms in inflammatory bowel disease

Table 1. Specific polymerase chain reaction primers and restriction enzymes for each single nucleotide polymorphism

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Sequence of primers (5’→3’)</th>
<th>Fragments (bp)</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL12</td>
<td>F: 5’GGGCAAAGCCTAGTGAAG3’</td>
<td>209</td>
<td>MspI</td>
</tr>
<tr>
<td></td>
<td>R: 5’GCAGAAGCTGGAGATGT3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCR4</td>
<td>F: 5’TGACGGCAAGTACAGGC3’</td>
<td>309</td>
<td>BccI</td>
</tr>
<tr>
<td></td>
<td>R: 5’CAAAGATGAAGTCGGGAAT3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The primers used to amplify the CXCL12 gene (rs1801157) and the CXCR4 gene (rs22280-14) were designed according to the National Center for Biotechnology Information gene database, and shown in Table 1 (primers were designed and synthesized in TAKARA Biotechnology co., Ltd., Dalian, China). Analysis of polymorphic variants for CXCL12 and CXCR4 was performed using the RFLP method.

Materials and methods

Patients and controls

The study group consisted of 245 IBD Zhuang patients without genetic kinship enrolled in the Gastroenterology Department, the First Affiliated Hospital of Guangxi Medical University, from January 2013 to February 2016. Among them, there were 120 males and 125 females, aged 38.9 ± 16.54 years, including 144 patients with UC and 101 patients with CD. All patients had a well-established diagnosis of UC or CD, according to standard clinical criteria drawn up by the Chinese Society of Gastroenterology in 2012 (Guangzhou consensus) [7], with other infectious and non-infectious colitis patients excluded [8, 9]. The control group included 202 healthy Zhuang subjects without genetic kinship who were healthy individuals or patients with functional dyspepsia. Among them, there were 99 males and 103 males, aged 39.2 ± 16.23 years. There were no significant differences in age and sex between the study group and the control group. All patients and healthy controls gave informed consent and the study was approved by the ethics committee of the hospital.

DNA extraction

Thirty to fifty milligrams of fresh intestinal mucosa was collected, and then stored in a liquid nitrogen tank. The specimens were washed with saline, cut into pieces and carefully ground into a powder using a mortar. DNA was extracted using Genomic DNA Extraction Kit (Takara, TAKARA Biotechnology co., Ltd., Dalian, China) according to the manufacturer’s instructions. DNA was added into 50-100 μL TE to dissolve, and the concentration and purity of DNA was measured, before being stored at -20°C.

Polymerase chain reaction

PCR reactions for all tested gene polymorphisms were performed in a thermo cycler (Veriti, Applied Biosystems, Inc.). The total reaction system was 50 μL, including 100 ng genomic DNA, 25 μL Premix Taq (TaKaRa Taq™ Version 2.0 plus dye, Takara), Forward primer 1 μL, Reverse primer 1 μL, and ddH₂O made up to 50 μL. CXCL12 and CXCR4 gene PCRs were performed under the follow temperature conditions: denaturation at 94°C for 5 min, followed by amplification for 35 cycles at 94°C for 30 s, at 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 7 min.

RFLP analysis and sequencing

PCR products of the CXCL12 gene were digested using the restriction enzyme MspI (5 U) at 37°C, and the PCR products of the CXCR4 gene were digested using the restriction enzyme BccI (5 U) at 37°C. After 5 hours of digestion, the samples were separated and visualised by 2% gel electrophoresis (Biowest Agarose), and the resulting fragments were stained with ethidium bromide. RFLP results were confirmed by direct sequencing of 100 randomly chosen samples for CXCL12 and CXCR4. PCR products of CXCL12 and CXCR4 with sequencing forward primer were then sent to GenScript Biotechnology Co., Ltd. (Nanjing, China) for sequencing.

Statistical analysis

The genetic equilibrium was tested using Hardy-Weinberg. Allele and genotype frequencies in
Gene polymorphisms in inflammatory bowel disease

Chicago, United States), and $P$ values were considered statistically significant at a level of less than 0.05.

The statistical methods of this study were reviewed by a biomedical statistician from Public Health of Guangxi Medical University, China.

Results

PCR products of all subjects were consistent with the fragment size. After amplification, the fragment lengths of CXCL12 and CXCR4 were 209 bp and 309 bp, respectively, which showed the success of amplification (Figures 1 and 2).

CXCL12 rs1801157 PCR–RFLP and sequencing

The MspI restriction enzyme was used to identify the sequence CCGG. Homozygous G/G produces two bands at 117 bp and 92 bp, the heterozygous state G/A yields three bands of 209 bp, 117 bp and 92 bp, while the homozygous genotype A/A only produces the 209 bp band, as this allele is not digested (Figures 3 and 4).

CXCR4 rs2228014 PCR–RFLP and sequencing

The BclI restriction enzyme was used to detect the sequence CCATCN. Homozygous C/C gives two bands at 105 and 204 bp, the heterozygous state, C/T, produces three bands of 309 bp, 105 bp and 204 bp, while the homozygous genotype T/T shows only band at 309 bp, as this allele is not digested (Figures 5 and 6).

Distribution of genotype and allele frequency

Genotype and allele frequency distribution of CXCL12 gene rs1801157 and CXCR4 gene rs2228014 were tested using Hardy-Weinberg; the differences between the two groups were not statistically significant (all $P > 0.05$), indicating that the case and control groups were genetic population that reach equilibrium and are representative.

Differences in allele frequencies of CXCL12 rs1801157 between patients with UC/CD and normal controls were not statistically significant ($\chi^2 = 2.327$, $\chi^2 = 0.459$, both $P > 0.05$), which suggested that the CXCL12 rs1801157 polymorphism is not associated with IBD in the Guangxi Zhuang population (Table 2). Differ-
Gene polymorphisms in inflammatory bowel disease

In the past, the incidence of IBD has been noted to be markedly high in European and North American populations, while the incidence in Asian populations is lower; however, in the last 20 years, the incidence of IBD in Asian populations has shown a significant increase. Currently, the prevalence of UC and CD in European and North American countries is 505/100,000 and 322/100,000 respectively [10], while the prevalence of UC and CD in the Asian population is 63.6/100,000 and 21.2/100,000, respectively [11]. The aetiology and pathogenesis of IBD is not entirely clear yet, and it is thought that this may be caused by a variety of environmental or other factors acting on those who are genetically susceptible. With the participation of the intestinal flora or as yet unidentified microorganisms, the intestinal immune and non-immune system is activated, eventually leading to immune response and inflammatory reaction. Monozygotic twins show a high frequency, and the incidence of IBD shows familial aggregation and ethnic differences, which indicates that the occurrence and genetic factors are closely related. In 2001, Hugot et al. [12] reported the first CD susceptibility gene, NOD2/CARD15; since then, other groups have confirmed more than 160 susceptibility loci for IBD by genome-wide association studies (GWAS). Among them, a number of chemokines were confirmed to be involved in the pathogenesis of IBD [13, 14], and blocking the interaction between these chemokines can be used as a means of treatment for IBD.

Chemokines are a class of cytokines that predominantly attracts and activates neutrophils, which is a class of small molecule polypeptides among members of the cytokine superfamily. These are secreted by a variety of tissue cells, such as lymphocytes, macrophages, stromal cells and so on, and play a role via seven transmembrane receptors [4]. Chemokines induce the directional migration of various types of immune cells, which play a wide range of physiological and pathological roles in the development of immune cells and organs, immune response, inflammation, pathogen infection, angiogenesis, wound healing, tumour formation and metastasis and so on [5]; they can also participate in regulation of T and B cell function [15]. CXCL12 was originally cloned from mouse bone marrow stromal cell lines, so it is also known as stromal cell–derived factor 1 (SDF-1) [16]. The gene encoding CXCL12 is located at 10q11.1, spanning 1776 bp, with the wild type G allele at position 801 of the 3' UTR

**Discussion**

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Gene polymorphisms in inflammatory bowel disease

The untranslated region being mutated to an A in rs1801157. The A allele may be the target by which cis-acting factors increase expression of the CXCL12 chemokine [17], so it produces more CXCL12 mRNA than the wild-type G allele, therefore expressing more CXCL12. Individuals who are homozygous for mutant AA and heterozygous for GA produce more CXCL12 product compared with the wild-type GG homozygotes [17-19].

Chemokines can recruit the cells, which have specific receptors at the cell surface, to participate in a variety of physiological and pathological processes. This specific receptor at the cell surface is called the chemokine receptor (CKR). CXCR4 is the specific receptor of CXCL12, while CXCL12 is the only ligand of CXCR4. CXCR4 is a seven-transmembrane G-protein-coupled receptor that was purified and extracted from the human blood mononuclear cell gene pool with the IL-8 receptor gene probe [20], which is known as Leukocyte expressed seven-transmembrane domain receptor (LESTR). They are expressed in bone marrow, umbilical cord blood, all cells mobilised into the peripheral blood stream (CD34+ cells, B cells, naive T cells and monocytes) and a variety of stromal cells and organs. CXCL12 shows high affinity for CXCR4. Signal transduction pathways will form after CXCL12 and CXCR4 binding, which can play an important role in biological function of immune and inflammatory response. CXCR4 is a cause for concern initially, because it participates in HIV infection and plays an important role in the process responsible for HIV invading T cells and diffusion throughout the body [21].

Impaired regulation of gene expression or structural mutations in the coding sequence can cause chronic inflammation-related diseases [22]. Cai et al. [23] found that CXCL12 rs1801157G/A and CXCR4 rs2228014C/T may be risk factors and deterioration indicators of renal cell carcinoma. De Oliveira et al. [21] found that the occurrence of breast cancer is associated with gene mutation and the low expression of CXCL12 rs1801157. Manetti et al. [25] found that the gene mutation is associated with systemic sclerosis. Xu et al. [26] induced lung injury with bleomycin in mice and then treated them with the CXCR4 antibody (TNF4003); after that, lung fibrosis in mice showed significant recovery, indicating that CXCL12 and CXCR4 play a key role in lung injury with fibrosis. Activation of the CXCL12/CXCR4 axis also play an important role in a number of inflammatory diseases, such as systemic lupus erythematosus (SLE), ankylosing spondylitis (AS), osteoarthritis, mycosis fungoides (MF) and IBD [26-28]. Cao et al. [30] detected the expression level of CXCL12/CXCR4 by immunohistochemistry and found that

![](image)

**Figure 6.** The sequencing results of CXCR4. A: The homozygous mutant; B: The wild-type homozygotes; C: The heterozygous mutant.

<p>| Table 2. The CXCL12 gene rs1801157 polymorphism in patients with IBD and the control group |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Genotype</th>
<th>UC patients (n = 144)</th>
<th>CD patients (n = 101)</th>
<th>Control (n = 202)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>49 (34.0)</td>
<td>46 (45.5)</td>
<td>88 (43.6)</td>
</tr>
<tr>
<td>G/A</td>
<td>79 (54.9)</td>
<td>49 (48.5)</td>
<td>95 (47.0)</td>
</tr>
<tr>
<td>A/A</td>
<td>16 (11.1)</td>
<td>6 (6.0)</td>
<td>19 (9.4)</td>
</tr>
<tr>
<td>G</td>
<td>177 (61.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141 (69.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>271 (67.1)</td>
</tr>
<tr>
<td>A</td>
<td>111 (38.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61 (30.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133 (32.9)</td>
</tr>
</tbody>
</table>

Note: <sup>a</sup>P > 0.05 vs control group.

<p>| Table 3. The CXCR4 gene rs2228014 polymorphism in patients with IBD and the control group |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Genotype</th>
<th>UC patients (n = 144)</th>
<th>CD patients (n = 101)</th>
<th>Control (n = 202)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>87 (60.4)</td>
<td>65 (64.4)</td>
<td>91 (45.0)</td>
</tr>
<tr>
<td>C/T</td>
<td>50 (34.7)</td>
<td>33 (32.6)</td>
<td>84 (41.6)</td>
</tr>
<tr>
<td>T/T</td>
<td>7 (4.9)</td>
<td>3 (3.0)</td>
<td>27 (13.4)</td>
</tr>
<tr>
<td>C</td>
<td>224 (77.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>163 (80.7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>266 (65.8)</td>
</tr>
<tr>
<td>T</td>
<td>64 (22.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39 (19.3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>138 (34.2)</td>
</tr>
</tbody>
</table>

Note: <sup>a</sup>P < 0.05 vs control group.
expression levels of CXCL12 and CXCR4 in the intestinal tissue of patients with active UC were significantly increased compared to the control group, but they did not analyse the gene mutation further. The study of Mrowicki et al. [31] used PCR-RFLP analysis method in the Polish population and showed that CXCR4/CXCL12 polymorphisms may be related to the occurrence and development of IBD and its activation may be the defence response of chronic persistent inflammation.

In this study, by testing correlation between two single nucleotide polymorphisms locus of CXCL12/CXCR4 and Guangxi Zhuang IBD patients in China using PCR-RFLP, we found that there was no significant association between mutation of the CXCL12 gene (rs1801157) and IBD in Guangxi Zhuang populations. However, mutation of the CXCR4 gene (rs2228014) showed an obvious correlation with the incidence of IBD in the Guangxi Zhuang population, which is consistent with the results of the study by Mrowicki et al. in the Polish population. In addition, the results of an association study of these two genes with IBD patients in the Guangxi Zhuang population were reported for the first time. Under the role of inflammatory mediators, the expression of these cytokines may be increased, leading to maintained chronic inflammation [32], which may be a defensive mechanism aimed at the reconstruction of damaged intestinal epithelium. Blockade of the CXCL12/CXCR4 axis by the administration of a CXCR4 antagonist ameliorated colonic inflammation in dextran sulphate sodium-induced colitis, which might have potential as a therapeutic target for the treatment of IBD [14]. The CXCL12/CXCR4 chemokine axis participates in angiogenic response, regeneration of the intestinal epithelium and regulation of basic functions of intestinal epithelial cells, which may play a significant role in intestinal homeostasis and inflammation in the restoration of the physiological human intestinal mucous membrane [33, 34].

In conclusion, the CXCR4 gene polymorphism rs2228014 may be a risk factor for IBD and related to the progression of IBD in the Guangxi Zhuang population. The results need to be confirmed in other populations or using larger scale studies in the future.

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Disclosure of conflict of interest
None.

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References
Gene polymorphisms in inflammatory bowel disease


