Correlation of interleukin-6 572C/G gene polymorphism with airway inflammation and remodeling in patients with COPD

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ABSTRACT

Objective: To study the correlation of interleukin-6 (IL-6) 572C/G gene polymorphism with airway inflammation and remodeling in patients with COPD. Methods: Patients with stable COPD who were treated in Hanzhong Central Hospital between March 2015 and December 2017 were selected and enrolled in the COPD group of the study, and healthy volunteers who received physical examination in Hanzhong Central Hospital during the same period and had general information matched with that of patients with COPD were selected as the control group. The peripheral blood was collected to detect the IL-6 gene 572 C/G locus polymorphism, and the serum was collected to detect the levels of inflammatory response mediators and airway remodeling indexes. Results: The proportion of GG genotype in COPD group was higher than that in control group, and the proportion of GC+CC genotype was lower than that in control group; serum IL-6, IL-21, IFN-γ, CXCL13, CTRP4, CTRP5, TGF-β1, VEGF, MMP2 and NE levels of COPD group were significantly higher than those of control group whereas 1-AT and TIMP1 levels were significantly lower than those of control group, and serum IL-6, IL-21, IFN-γ, CXCL13, CTRP4, CTRP5, TGF-β1, VEGF, MMP2 and NE levels of COPD patients with GG genotype were higher than those of COPD patients with GC+CC genotype whereas 1-AT and TIMP1 levels were lower than those of COPD patients with GC+CC genotype. Conclusion: The mutation from IL-6 gene 572C/G locus allele C to G can aggravate the inflammatory response and airway remodeling in the course of COPD.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a common respiratory chronic disease among the elderly, and it is mainly characterized by incompletely reversible airway limitation and airway remodeling, the inflammatory cell infiltration as well as inflammatory response and immune response disorder in airway is directly related to the changes of the airway function, and the secretion of various cytokines increases significantly in the course of COPD[1,2]. Interleukin-6 (IL-6) is a cytokine with multiple biological activities and plays an important regulatory role in both inflammatory response and immune response[3]. The secretion of IL-6 significantly increases in the process of COPD and it is closely related to the degree of airway inflammation and remodeling, but the mechanism of IL-6 changes in the pathogenesis of COPD remains unclear. The study on the change of IL-6 secretion in inflammatory diseases and autoimmune diseases in recent years has shown that gene polymorphism directly influences the expression and secretion of IL-6, and the promoter region -572 C/G mutations can affect the start of gene transcription and change the expression and secretion of IL-6[4]. In the following studies, we specifically analyzed the correlation of interleukin-6 572C/G gene polymorphism with airway inflammation and remodeling in patients with COPD.

2. Research subjects and methods

2.1 General information of research subjects

Patients with stable COPD who were treated in Hanzhong Central Hospital between March 2015 and December 2017 were chosen and enrolled in the COPD group of the research, all the patients were in accordance with the diagnosis of COPD and the patients...
combined with the diseases such as bronchial asthma, pulmonary tuberculosis or malignant tumor were eliminated. Healthy volunteers who received physical examination in Hanzhong Central Hospital during the same period and had general information matched with that of patients with COPD were chosen as the control group. There were 64 cases in COPD group, including 36 males and 28 females who were 53-68 years old; there were 76 cases in the control group, including 46 males and 30 females who were 51-66 years old. There was no significant difference in general data between the two groups ($P>0.05$).

2.2 Gene polymorphism detection

1mL of cubital venous blood was collected from the two groups respectively, genomic DNA kit was used to extract genomic DNA from the peripheral blood, then the primers of IL-6 gene 572C/G locus were designed for PCR amplification, the PCR products were incubated with restriction enzyme BsmAI and BsrBI for 3 to 6 h at 55 °C, finally the enzyme-digested products underwent electrophoresis within agarose gel, and the gene polymorphism was judged according to the electrophoresis images.

2.3 Serum index detection

5-6 mL of cubital venous blood was collected from the two groups respectively and centrifuged to separate serum, and Elisa kit instructions were referred to detect IL-6, IL-21, IFN-γ, CXCL13, CTRP4, CTRP5, TGF-β1, VEGF, MMP2, NE, 1-AT and TIMP1 levels.

2.4 Statistical methods

Software SPSS 21.0 was used to input data, the measurement data were analyzed by $t$ test, the count data were analyzed by chi-square test and $P<0.05$ meant statistical significance in the differences.

3. Results

3.1 IL-6 gene 572 C/G locus polymorphism

19 cases in COPD group were with IL-6 gene 572 C/G locus GG genotype and the proportion was 29.69%, 22 cases were with GC genotype and the proportion was 34.38%, 23 cases were with CC genotype and the proportion was 35.94%. 2 cases in control group were with IL-6 gene 572 C/G locus GG genotype and the proportion was 2.63%, 33 cases were with GC genotype and the proportion was 43.42%, 41 cases were with CC genotype and the proportion was 53.95%. After chi-square test, the proportion of GG genotype in COPD group was higher than that in control group, the proportion of GC+CC genotype was lower than that in control group and the differences were statistically significant ($P<0.05$).

3.2 Serum inflammatory response mediator levels

Analysis of serum inflammatory response mediators IL-6 (ng/L), IL-21 (μg/L), IFN-γ (μg/L), CXCL13 (ng/L), CTRP4 (μg/L) and CTRP5 (μg/L) between the two groups of subjects was as follows: serum IL-6, IL-21, IFN-γ, CXCL13, CTRP4 and CTRP5 levels of COPD group were significantly higher than those of control group. Analysis of serum inflammatory response mediators IL-6, IL-21, IFN-γ, CXCL13, CTRP4 and CTRP5 between COPD group of patients with different IL-6 gene polymorphisms was as follows: serum IL-6, IL-21, IFN-γ, CXCL13, CTRP4 and CTRP5 levels of COPD patients with GG genotype were higher than those of COPD patients with GC+CC genotype.

3.3 Serum airway remodeling index levels

Analysis of serum airway remodeling indexes TGF-β1 (μg/L), VEGF (ng/L), MMP2 (ng/L), NE (ng/L), 1-AT (ng/L) and TIMP1 (ng/L) between the two groups of subjects was as follows: serum TGF-β1, VEGF, MMP2 and NE levels of COPD group were significantly higher than those of control group whereas 1-AT and TIMP1 levels were significantly lower than those of control group.

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IL-6</th>
<th>IL-21</th>
<th>IFN-γ</th>
<th>CXCL13</th>
<th>CTRP4</th>
<th>CTRP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD group</td>
<td>64</td>
<td>227.4±31.6</td>
<td>3.71±0.52</td>
<td>0.88±0.11</td>
<td>37.12±5.52</td>
<td>12.71±1.52</td>
<td>0.64±0.08</td>
</tr>
<tr>
<td>Control</td>
<td>76</td>
<td>109.3±14.7</td>
<td>1.67±0.22</td>
<td>0.37±0.05</td>
<td>20.35±3.27</td>
<td>4.77±0.52</td>
<td>0.30±0.05</td>
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<td>$&lt;0.05$</td>
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<td>$P$</td>
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<td>$&lt;0.05$</td>
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</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>n</th>
<th>IL-6</th>
<th>IL-21</th>
<th>IFN-γ</th>
<th>CXCL13</th>
<th>CTRP4</th>
<th>CTRP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>19</td>
<td>299.3±42.7</td>
<td>5.27±0.68</td>
<td>1.13±0.15</td>
<td>46.98±6.42</td>
<td>18.12±2.03</td>
<td>0.82±0.11</td>
</tr>
<tr>
<td>GG+GC</td>
<td>45</td>
<td>157.2±18.9</td>
<td>2.33±0.37</td>
<td>0.66±0.08</td>
<td>28.33±3.94</td>
<td>7.05±0.89</td>
<td>0.48±0.06</td>
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<tr>
<td>$t$</td>
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</table>
Table 3.
Comparison of serum airway remodeling indexes between the two groups of Subjects.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TGF-β 1</th>
<th>VEGF</th>
<th>MMP2</th>
<th>NE</th>
<th>1-AT</th>
<th>TIMP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD group</td>
<td>64</td>
<td>64.13±8.92</td>
<td>267.3±31.5</td>
<td>0.42±0.07</td>
<td>32.62±4.62</td>
<td>9.93±1.03</td>
<td>13.52±1.77</td>
</tr>
<tr>
<td>Control group</td>
<td>76</td>
<td>30.43±4.26</td>
<td>135.2±15.2</td>
<td>0.21±0.03</td>
<td>17.51±2.52</td>
<td>20.12±2.62</td>
<td>28.93±4.12</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.
Comparison of serum airway remodeling indexes between COPD group of Patients with different IL-6 gene polymorphisms.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>TGF-β 1</th>
<th>VEGF</th>
<th>MMP2</th>
<th>NE</th>
<th>1-AT</th>
<th>TIMP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>19</td>
<td>81.42±10.25</td>
<td>331.2±40.8</td>
<td>0.51±0.09</td>
<td>40.82±6.12</td>
<td>5.23±0.77</td>
<td>8.69±1.03</td>
</tr>
<tr>
<td>GG+GC</td>
<td>45</td>
<td>47.51±6.21</td>
<td>203.5±25.6</td>
<td>0.34±0.05</td>
<td>24.12±3.42</td>
<td>14.21±1.96</td>
<td>19.31±2.36</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of serum airway remodeling indexes TGF-β 1, VEGF, MMP2, NE, 1-AT and TIMP1 between COPD group of patients with different IL-6 gene polymorphisms as was as follows: serum TGF-β 1, VEGF, MMP2 and NE levels of COPD patients with GG genotype were higher than those of COPD patients with GC+CC genotype whereas 1-AT and TIMP1 levels were lower than those of COPD patients with GC+CC genotype.

4. Discussion

COPD is the chronic respiratory system disease with the basic pathological feature of chronic airway inflammation, and the mass infiltration of inflammatory cells and the mass secretion of inflammatory cytokines in airway can further cause airway remodeling and lead to irreversible airflow limitation[5]. IL-6 is a cytokine that plays an important role in the COPD process, and it has multiple biological activities and can mediate the cascade amplification of inflammatory response and the disorder of immune response. It has been reported that the levels of IL-6 abnormally increase in the serum of COPD patients[6,7], but the specific mechanism regulating the secretion of IL-6 is still not completely clear. Gene polymorphism is the newly discovered way to regulate IL-6 secretion in recent years, and the polymorphism of 572C/G locus is confirmed to be related to the occurrence of various chronic inflammatory diseases and autoimmune diseases. IL-6 gene 572C/G locus is located in gene promoter regions, and after the allele of the mutation from IL-6 gene 572 C/G locus allele C to G and the emergence of GG mutant can enhance the binding activity of the corresponding promoter regions to promote the transcription of IL-6 gene, increase the secretion of IL-6 and promote the disorder of inflammatory response and immune response in the course of COPD.

The sustained activation of chronic airway inflammation is the basic pathophysiological change in the course of COPD, the cytokine IL-6 encoded by IL-6 gene can participate in the regulation of inflammation cascade reaction after it is released into the blood circulation, and it has a promoting effect on the activation of a variety of inflammatory cells and the release of inflammatory factors. IL-21 is cytokine secreted by lymphocytes and NK-like T cells, which has powerful pro-inflammatory activity and can induce the mass infiltration of neutrophils in the airway and enhance local airway inflammation; another type of pro-inflammatory cytokines IFN-γ is massively secreted in the early stage of inflammatory response, and can participate in the cascade activation and amplification process of inflammatory response in local airway[10]; CXCL13 is an inflammatory mediator with chemotactic activity, and its combination with inflammatory cell surface receptor CXCR5 can enhance the activity of the cellular chemotactic movement, facilitate the inflammatory cell infiltration in the airway and participate in the abnormal activation of inflammatory response[11]; CTRP4 and CTRP5 are the CTRP family members involved in the regulation of inflammation, and they can amplify the inflammatory response through downstream NF-κB and STAT3 signaling molecules[12].

Analysis of the changes of above serum inflammatory response mediators in patients with COPD showed that serum IL-6, IL-21, IFN-γ, CXCL13, CTRP4 and CTRP5 levels of COPD group were significantly higher than those of control group. This indicates that the mass secretion of various inflammatory response mediators is related to the occurrence of COPD. Further analysis of the correlation between IL-6 gene 572 C/G locus polymorphism and inflammatory response mediator secretion indicated that serum IL-6, IL-21, IFN-γ, CXCL13, CTRP4 and CTRP5 levels of COPD patients with GG genotype increased significantly. This means that the mutation from IL-6 gene 572 C/G locus allele C to G and the emergence of GG mutant can promote the secretion of a variety of inflammatory response mediators in the course of COPD, and help to enhance the chronic airway inflammation in COPD patients.

The continuous activation of inflammatory response in the course
of COPD can further cause the airway remodeling and affect the airway function. The excessive proliferation of fibroblasts and the abnormal synthesis and degradation of collagens in pulmonary interstitium are the important pathological links in the process of airway remodeling, and the abnormal secretion of a variety of cytokines and proteases is closely related to it. TGF-β1 and VEGF are the cytokines mediating fibroblast proliferation, the former can promote fibroblast proliferation through the downstream Smad2/3, and can also increase the secretion of collagen and polysaccharide in pulmonary interstitium, and play the role of promoting airway remodeling in the course of COPD[15,16]. α1-AT and TIMP1 are protease inhibitors that can reduce the hydrolytic activity of proteases and hinder airway remodeling[17]. Analysis of the change trends of above serum airway remodeling indexes in COPD patients showed that serum TGF-β1, VEGF, MMP2 and NE levels of COPD group were significantly higher than those of control group whereas α1-AT and TIMP1 levels were significantly lower than those of control group. This indicates that the abnormal secretion of various cytokines and proteases is related to the occurrence of airway remodeling in the course of COPD. Further analysis of the correlation between IL-6 gene 572 C/G locus polymorphism and airway remodeling showed that serum TGF-β1, VEGF, MMP2 and NE levels of COPD patients with GG genotype increased significantly whereas 1-A and TIMP1 levels decreased significantly. This means that the mutation from IL-6 gene 572C/G locus allele C to G and the emergence of GG mutant can cause the abnormal secretion of airway remodeling-associated cytokines and proteases in the course of COPD to aggravate the airway remodeling in COPD patients.

The results of above gene polymorphism and serum indexes can be concluded as follows: there is IL-6 gene 572C/G locus polymorphism in patients with COPD, and the mutation from allele C to G increases; the existence of above polymorphism can aggravate the inflammatory response and airway remodeling in the course of COPD.

References