Correlation of NLRP3 polymorphism with inflammasome activity and endothelial damage in patients with acute coronary syndrome

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ARTICLE INFO

Article history:
Received 13 Apr 2017
Received in revised form 17 Apr 2017
Accepted 19 Apr 2017
Available online 24 May 2017

Keywords:
Acute coronary syndrome
Nod-like receptor protein 3
Inflammasome
Gene polymorphism

ABSTRACT

Objective: To study the correlation of Nod-like receptor protein 3 (NLRP3) polymorphism with inflammasome activity and endothelial damage in patients with acute coronary syndrome.

Methods: Patients diagnosed with acute coronary syndrome and stable angina pectoris in Mianyang Central Hospital between May 2013 and August 2016 were selected and included in ACS group and SAP group respectively, and healthy volunteers who received physical examination during the same period were selected as control group. Peripheral blood was collected to detect NLRP3 gene rs10754558 loci polymorphism, and serum was separated to determine inflammasome activity indexes and endothelial injury indexes.

Results: NLRP3 gene GG genotype and GC genotype constituent ratio of ACS group were significantly higher than those of SAP group and control group while CC genotype constituent ratio was significantly lower than that of SAP group and control group, and serum IL-1\(\beta\), IL-18, E-selectin, vWF and ET-1 levels were significantly higher than those of SAP group and control group while serum NO level was significantly lower than that of SAP group and control group; serum IL-1\(\beta\), IL-18, E-selectin, vWF and ET-1 levels in ACS patients with GG genotype and GC genotype were significantly higher than those in patients with CC genotype while NO levels were significantly lower than those in patients with CC genotype, and serum IL-1\(\beta\), IL-18, E-selectin, vWF and ET-1 levels in ACS patients with GG genotype were significantly higher than those in patients with GC genotype while NO level was significantly lower than that in patients with GC genotype.

Conclusions: The increased NLRP3 gene rs10754558 loci alleles G in patients with ACS will increase inflammasome activity and endothelial injury.

1. Introduction

Acute coronary syndrome (ACS) is a group of clinical syndromes developed on the basis of coronary atherosclerosis, including unstable angina, non-ST-elevation myocardial infarction and ST-elevation myocardial infarction. The inflammation activation and endothelial injury are closely related to the lower coronary atheromatous plaque stability in the development and change of ACS, and inflammation can also increase endothelial damage\cite{1,2}.

Therefore, the inflammatory response is thought to be the key link in ACS development and changes. But at present, the regulatory mechanism of inflammation in patients with ACS is not yet clear. Nod-like receptor protein 3 (NLRP3) inflammasome is the important mechanism regulating the inflammatory response in the body, and it activates caspase-1 to mediate IL-1\(\beta\) and IL-18 generation\cite{3,4}. NLRP3 gene Rs10754558 loci polymorphism is considered as an important factor causing the increased formation of IL-1\(\beta\) and IL-18 as well as the abnormal activation of inflammatory reaction in ACS\cite{5,6}. In the following study, the correlation of NLRP3 gene Rs10754558 loci polymorphism with inflammasome activity and endothelial damage in patients with ACS was analyzed.

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Fund Project: Scientific Research Fund Support Project of Mianyang Health Bureau No: 13M011.}
2. Information and methods

2.1 Clinical case information

Patients diagnosed with acute coronary syndrome and stable angina pectoris in Mianyang Central Hospital between May 2013 and August 2016 were selected and included in ACS group and SAP group respectively. ACS group (n=79) included 49 male cases and 30 female cases that were 49-67 years old; SAP group (n=93) included 58 male cases and 35 female cases that were 45-65 years old; 100 healthy volunteers who received physical examination in our hospital during the same period were selected as control group, including 62 male cases and 38 female cases that were 45-68 years old. ACS group, SAP group and control group were not significantly different in general information (P>0.05).

2.2 NLRP3 polymorphism detection methods

Peripheral blood was collected from ACS group and SAP group on admission, peripheral blood was collected from control group during physical examination, a total of 5 mL was collected, whole blood genomic extraction kits were used to separate genomic DNA, primers for rs10754558 loci were designed for PCR reaction, the obtained PCR products were used for gene sequencing to determine rs10754558 loci polymorphism.

2.3 Serum index detection methods

5 mL of peripheral blood was collected in the same method as that of NLRP3 polymorphism detection and centrifuged to separate serum, and then ELISA kits were used to determine IL-1β, IL-18, E-selectin, vWF, ET-1 and NO levels.

2.4 Statistical methods

SPSS 20.0 software was used for variance analysis of measurement data and chi-square test of count data among three groups and P<0.05 indicated statistical significance in differences.

3. Results

3.1 NLRP3 polymorphism of three groups of subjects

NLRP3 gene rs10754558 loci CC genotype, GG genotype and GC genotype constituent ratio of ACS group were (17/79), (28/79) and (34/79) respectively; NLRP3 gene rs10754558 loci CC genotype, GG genotype and GC genotype constituent ratio of control group were (79/100), (9/100) and (12/100) respectively. After chi-square test, NLRP3 gene GG genotype and GC genotype constituent ratio of ACS group were significantly higher than those of SAP group and control group while CC genotype constituent ratio was significantly lower than that of SAP group and control group (P<0.05).

3.2 Serum inflammasome activity indexes of three groups of subjects

Analysis of serum inflammasome activity indexes IL-1β (ng/mL) and IL-18 (pg/mL) levels among three groups of subjects was as follows: serum IL-1β and IL-18 levels of ACS group and SAP group were significantly higher than those of control group and serum IL-1β and IL-18 levels of ACS group were significantly higher than those of SAP group. Differences in pair-wise comparison of serum IL-1β and IL-18 levels were statistically significant among three groups of subjects (P<0.05), shown in Table 1.

Analysis of serum inflammasome activity indexes IL-1β and IL-18 levels among ACS groups of patients with different NLRP3 genotypes was as follows: serum IL-1β and IL-18 levels in ACS patients with GG genotype and GC genotype were significantly higher than those in patients with CC genotype, and serum IL-1β and IL-18 levels in ACS patients with GG genotype were significantly higher than those in patients with GC genotype. Differences in pair-wise comparison of serum IL-1β and IL-18 levels were statistically significant among ACS groups of patients with different NLRP3 genotypes (P<0.05), shown in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IL-1β (ng/mL)</th>
<th>IL-18 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS group</td>
<td>79</td>
<td>2.68±0.42</td>
<td>236.59±41.26</td>
</tr>
<tr>
<td>SAP group</td>
<td>93</td>
<td>1.45±0.22</td>
<td>124.52±16.68</td>
</tr>
<tr>
<td>Control group</td>
<td>100</td>
<td>1.02±0.17</td>
<td>89.35±11.25</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; &: compared with SAP group, P<0.05.

<table>
<thead>
<tr>
<th>NLRP3 genotypes</th>
<th>n</th>
<th>IL-1β (ng/mL)</th>
<th>IL-18 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG genotype</td>
<td>28</td>
<td>3.68±0.52</td>
<td>352.12±46.97</td>
</tr>
<tr>
<td>GC genotype</td>
<td>34</td>
<td>2.75±0.36</td>
<td>246.69±34.58</td>
</tr>
<tr>
<td>CC genotypes</td>
<td>17</td>
<td>1.89±0.25</td>
<td>169.64±22.12</td>
</tr>
</tbody>
</table>

*: compared with CC genotype, P<0.05; &: compared with GC genotypes, P<0.05.
3.3 Serum endothelial injury indexes of three groups of subjects

Analysis of serum endothelial injury indexes E-selectin (ng/mL), vWF (mU/mL), ET-1 (ng/mL) and NO (pg/mL) levels among three groups of subjects was as follows: serum E-selectin, vWF and ET-1 levels of ACS group and SAP group were significantly higher than those of control group while NO levels were significantly lower than that of control group; serum E-selectin, vWF and ET-1 levels of ACS group were significantly higher than those of SAP group while NO level was significantly lower than that of SAP group. Differences in pair-wise comparison of serum E-selectin, vWF, ET-1 and NO levels were statistically significant among three groups of subjects (P<0.05), shown in Table 3.

Analysis of serum endothelial injury indexes E-selectin, vWF, ET-1 and NO levels among ACS groups of patients with different NLRP3 genotypes was as follows: serum E-selectin, vWF and ET-1 levels in ACS patients with GG genotype and GC genotype were significantly higher than those in patients with CC genotype while NO levels were significantly lower than that in patients with CC genotype; serum E-selectin, vWF and ET-1 levels in ACS patients with GC genotype were significantly higher than those in patients with CC genotype while NO level was significantly lower than that in patients with CC genotype. Differences in pair-wise comparison of serum E-selectin, vWF, ET-1 and NO levels were statistically significant among ACS groups of patients with different NLRP3 genotypes (P<0.05), shown in Table 4.

4. Discussion

NLRP3 inflammasome is an important mechanism regulating the inflammatory response in the body, it is the complex made up of NLRP3, CARD, Caspase-1 and ASC, and the activated NLRP3 can interact with CARD8 and then cause Caspase-1 activation, and thus result in the increased generation of downstream inflammatory mediators IL-1\(\beta\) and IL-18[7,8]. The IL-1\(\beta\) and IL-18 generation mediated by NLRP3 is closely related to the genetic polymorphism, and NLRP3 gene rs17054558 loci polymorphism can affect the function of NLRP3 to cause the change in IL-1\(\beta\) and IL-18 generation and the inflammatory response. In order to define the correlation between NLRP3 gene rs10754558 loci polymorphism and ACS condition, the genotypes and alleles of the polymorphic loci were analyzed, and the results showed that NLRP3 gene GG genotype and GC genotype constituent ratio of ACS group were significantly higher than those of SAP group and control group while CC genotype constituent ratio was significantly lower than that of SAP group and control group. It means that NLRP3 gene rs10754558 loci GG genotype and GC genotype increase and allele G increase are closely associated with the occurrence of ACS.

NLRP3 gene polymorphism can directly cause the change of NLRP3 function, and then affect the generation and secretion of downstream IL-1\(\beta\) and IL-18[9,10]. IL-1\(\beta\) and IL-18 are the important inflammatory mediators in the body that can activate the cascade amplification of the inflammatory reaction, induce inflammatory cell infiltration in the lesions and increase the secretion of a variety of adhesion molecules and chemokines. In the development and change of cardiovascular and cerebrovascular diseases, IL-1\(\beta\) and IL-18 can not only mediate inflammatory reaction activation and promote foam cell formation and atheromatous plaque deposition in the process of atherosclerosis plaque formation, but can also affect the plaque stability and increase the risk of plaque rupture and local thrombosis[11-13]. In order to define whether the IL-1\(\beta\) and IL-18 generation changed in ACS patients, serum IL-1\(\beta\) and IL-18 levels were analyzed, and the result showed that serum IL-1\(\beta\) and IL-18 levels of ACS group were significantly higher than those of SAP group and control group. This means that the excessive generation of IL-1\(\beta\) and IL-18 is closely related to the occurrence of ACS. Further analysis of the relationship of NLRP3 gene rs10754558 loci polymorphism with IL-1\(\beta\) and IL-18 generation showed that serum IL-1\(\beta\) and IL-18 levels in

**Table 3.**

Comparison of serum endothelial injury indexes among three groups of subjects.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>E-selectin</th>
<th>vWF</th>
<th>ET-1</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS group</td>
<td>79</td>
<td>18.49±2.35*</td>
<td>39.41±6.52*</td>
<td>125.63±17.85*</td>
<td>44.58±7.59*</td>
</tr>
<tr>
<td>SAP group</td>
<td>93</td>
<td>11.24±1.64*</td>
<td>26.53±6.67*</td>
<td>79.58±9.21*</td>
<td>72.41±9.87*</td>
</tr>
<tr>
<td>Control group</td>
<td>100</td>
<td>5.65±0.77</td>
<td>15.65±2.21</td>
<td>45.68±6.72</td>
<td>90.37±10.27</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; *: compared with SAP group, P<0.05.

**Table 4.**

Comparison of serum endothelial injury indexes among ACS groups of patients with different NLRP3 genotypes.

<table>
<thead>
<tr>
<th>NLRP3 genotypes</th>
<th>n</th>
<th>E-selectin</th>
<th>vWF</th>
<th>ET-1</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG genotype</td>
<td>28</td>
<td>25.73±4.15*</td>
<td>47.85±7.81*</td>
<td>158.65±21.45*</td>
<td>24.59±5.58*</td>
</tr>
<tr>
<td>GC genotype</td>
<td>34</td>
<td>17.98±2.52*</td>
<td>39.11±6.24*</td>
<td>128.59±16.72*</td>
<td>46.12±6.21*</td>
</tr>
<tr>
<td>CC genotypes</td>
<td>17</td>
<td>13.58±1.89</td>
<td>30.28±4.73</td>
<td>95.51±10.25</td>
<td>65.73±8.96</td>
</tr>
</tbody>
</table>

*: compared with CC genotype, P<0.05; *: compared with GC genotypes, P<0.05.
ACS patients with homozygous GG genotype and heterozygous GC genotype were significantly higher than those in patients with CC genotype, and serum IL-1 β and IL-18 levels in ACS patients with homozygous GG genotype were significantly higher than those in patients with heterozygous GC genotype. It means that the increased NLRP3 gene rs10754558 loci alleles G will enhance the activity of NLRP3 inflammasome, and promote the IL-1 β and IL-18 generation and release into the blood circulation.

One of the important ways for inflammation to cause coronary atherosclerosis is to cause endothelial damage. In the process of endothelial cell damage, the destruction of the endothelial structure integrity will increase the chance of foam cell infiltration and platelet adhesion, which is conducive to the formation of atheromatous plaque. E-selectin is a cytokine specifically expressed in the activated endothelial cells, and it is regarded as the marker of endothelial injury[14]; vWF is the importance molecule that mediates platelet and vascular endothelial cell adhesion, and its generation significantly increases in the process of endothelial injury[15]; ET-1 and NO is a pair of molecules that regulate the endothelial diastolic and systolic state, the former has strong vasoconstrictive effect and can cause endothelial damage, and the latter is an important vasodilator molecule and can protect vascular endothelium[16,17].

In the study, the analysis of serum endothelial damage indexes such as E-selectin, vWF, ET-1 and NO showed that serum E-selectin, vWF and ET-1 levels of ACS group were significantly higher than those of SAP group and control group while serum NO level was significantly lower than that of SAP group and control group. This means that endothelial injury is closely related to the occurrence of ACS. Further analysis the correlation between NLRP3 gene rs10754558 loci polymorphism and endothelial injury showed that serum E-selectin, vWF, ET-1 and NO showed that serum E-selectin, vWF and ET-1 levels of ACS patients with GG genotype and GC genotype were significantly higher than those in patients with CC genotype while NO levels were significantly lower than that in patients with CC genotype; serum E-selectin, vWF and ET-1 levels in ACS patients with homozygous GG genotype were significantly higher than those in patients with heterozygous GC genotype while NO level was significantly lower than that in patients with heterozygous GC genotype. It means that the increased NLRP3 gene rs10754558 loci alleles G will not only enhance the activity of NLRP3 inflammasome and increase the generation of IL-1 β and IL-18, but can also activate the inflammatory response to cause endothelial damage in patients with ACS.

In conclusion, it is believed that NLRP3 gene rs10754558 loci polymorphism is closely related to the occurrence and development of ACS; rs10754558 loci alleles G increase as well as GC genotype and GG genotype increase will increase the inflammasome activity as well as IL-1 β and IL-18 generation, and increase endothelial damage.

References