Original Article
Identification of SNPs in displacement loop region of mitochondrial DNA as risk factors for rheumatoid arthritis

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Abstract: Rheumatoid arthritis (RA) is chronic inflammatory synovitis characterized by oxidative damage that associated with joint deformities and joint dysfunction. Single nucleotide polymorphisms (SNPs) in the displacement loop (D-loop) region of peripheral blood mitochondrial DNA (mtDNA) were sequenced in RA patients to evaluate the predictive value for RA in a case-control study. SNPs of nucleotides 146T/C, 150C/T, 195T/C, 16260C/T, and 16519T/C were found to be closely related to RA risk at statistical levels (p < 0.05). The major alleles of nucleotides 195T/C, 16260C/T and 16519C/T, the minor alleles of nucleotides 146T/C and 150C/T were associated with increased risk for RA. In conclusion, The SNPs of mtDNA D-loop may play roles in pathogenesis of RA.

Keywords: D-loop, RA, SNP, onset risk

Introduction

Rheumatoid arthritis (RA), one of the most common autoimmune diseases characterized by synovial hyperplasia and joint disability, is related to early death, systemic complications, and high socioeconomic costs [1]. The prevalence of RA is around 0.5-1% worldwide and the prevalence in females is four times that of males [2]. The incidence of RA in China has ranged from 3.9 to 6.3 million new cases per year with a higher prevalence found in city [1, 3, 4]. Many factors, including ROS overproduction, contribute to the pathogenesis of RA [5].

The human mitochondrial genome is a length of 16 kb closed loop molecule consists of two ribosomal RNAs and a complete 22 tRNAs [6]. Due to high ROS content, lack of histone protection, and limited repair ability, mtDNA is more susceptible to DNA damage and is more prone to mutation than nuclear DNA [7-9]. The noncoding region of mtDNA which is called D-Loop, contains the origin of the leading chain of replication and the main promoter of transcription, is important to regulate replication and expression of the mitochondrial genome [10]. Many diseases including Parkinson’s disease [11], coronary artery disease [12], chronic kidney disease [13] and renal cell carcinoma [14] have relationships with SNPs of mitochondrial D-loop. Several studies have focused on the predictive value of SNPs in the mtDNA D-loop region for some chronic disease [11-14], but the predictive value of SNPs in the mtDNA D-loop region for RA has not been evaluated. In this study, approximately 1 kb gene fragment of D-loop was sequenced to evaluate the role of D-loop SNPs in the pathogenesis of RA.

Materials and methods

Tissue specimens

Blood samples from 85 patients including 16 males and 69 females, confirmed to RA based on the 2010 revised criteria of the American College of Rheumatology [15], were obtained
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Table 1. Clinical data in RA patients and control

<table>
<thead>
<tr>
<th>Items</th>
<th>RA patients (n = 85)</th>
<th>Control (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.92 ± 11.47</td>
<td>54.86 ± 10.22</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>16/69</td>
<td>20/80</td>
</tr>
<tr>
<td>Other immunological disease (yes/no)</td>
<td>46/39</td>
<td>-</td>
</tr>
<tr>
<td>DAS28 ≤ 2.6</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>&gt; 2.6DAS28 ≤ 3.2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>&gt; 3.2DAS28 ≤ 5.1</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>&gt; 5.1</td>
<td>61</td>
<td>-</td>
</tr>
<tr>
<td>Anti-CCP (+/-)</td>
<td>41/44</td>
<td>-</td>
</tr>
<tr>
<td>CRP (+/-)</td>
<td>63/22</td>
<td>-</td>
</tr>
<tr>
<td>RF (+/-)</td>
<td>63/22</td>
<td>-</td>
</tr>
</tbody>
</table>

from the Department of Rheumatology in the Second Hospital of Hebei Medical University between 2017-5 (May 2017) and 2017-12 (Dec 2017). The clinical manifestations of RA patients including age, sex, disease activity score (DAS28), and the laboratory test results such as anti-CCP, CRP and ESR were collected. Disease activity was assessed by DAS28. DAS28 less than or equal to 2.6 was defined as clinical remission, 2.6-3.2 as low activity, 3.2-5.1 as moderate activity and higher than 5.1 as high activity [http://www.das28.nl/das28/DAScalculators/dascalculators.html]. The blood of age and sex matched healthy controls was also collected at the same time. The study was performed in accordance with the 1964 Declaration of Helsinki, and also was approved by the Institutional Research Ethics Committee and the Human Tissue Research Committee of the Second Hospital of Hebei Medical University. All patients provided written informed consent for the collection of samples and subsequent analysis. Wizard Genomic DNA extraction kit (Promega, Madison, WI, USA) was used for mtDNA extraction. mtDNA was stored in -20°C after extraction.

**Statistical analysis**

Student’s t-test and rank sum test were used to determine differences in SNP distribution within the D-Loop region and the number of SNPs per patient among groups. Fisher’s exact test and Chi-square was used to detect differences in individual SNPs for each patient group. Due to the non-normality of data distribution, the Rank sum test was used to determine the differences of SNP distribution within the D-Loop region and the number of SNPs per patient among groups. Chi-square test was used to identify the differences of age and sex distributions among groups and to determine the relationship between the SNPs of mtDNA D-loop and RA. A p value of less than 0.05 was considered statistically significant. All of the statistical analyses were done with the SPSS 21.0 software (SPSS Inc, Chicago, IL).

**Results**

The age and gender distributions between RA patients and controls were compared by the Chi-square test, and no statistical difference was found to exist between these two groups (Table 1).

The differences of SNPs distribution in the mitochondrial D-loop region was compared subsequently. SNPs were detected in 112 sites, the average SNPs frequency (the sum of SNPs per person) was not statistically different between RA patients and the controls (data not shown). The 25 SNPs with minor allele frequency higher than 5% were used to analyze their association with RA risk (Figure 1).

Five SNPs, were found in the D-loop that were significantly different by Chi-square test between RA and controls (p < 0.05). The major alleles of nucleotides 195T/C, 16260C/T and 16519C/T, the minor alleles of nucleotides...
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146T/C and 150C/T were associated with an increased risk for RA (Table 2).

Discussion

The current study shows SNPs of nucleotides 146T/C, 150C/T, 195T/C, 16260C/T and 16519C/T that were associated with RA. Four of five RA risk associated SNPs were located in the hypervariable (HV) segment region of the D-loop, the site of 16260 located in HV-I, while the sites of 146, 150, 195 located in HV-II [https://mitomap.org/MITOMAP].

The D-loop of mtDNA plays an important role in regulating mitochondrial genome duplication and expression, so the SNPs in D-loop might inhibit the function of the electron transport chain that results in the release of a large amount of ROS thereby to interfere the process of oxidative phosphorylation and initiate the vicious cycle between D-loop SNP and ROS to induce nuclear genome damage and illness progress [17-20]. The allele 146C, 150T were more likely to develop chronic diseases such as Leber hereditary optic neuropathy [21, 22] and Parkinson’s disease [23, 24], and the nucleotide 16260C/T was associated with schizophrenia [25]. In our previous study, the allele 16519C was identified as associated with the risk of gastric cancer due to its association with oxidative damage and ROS generation [26, 27]. A large amount of literature indicates that ROS can act as a secondary messenger in activating of nuclear factor kappa-B (NF-κB) to induce a variety of inflammatory mediators transcription so as to initiate the pathogenesis of RA [17, 28-32]. The mechanism of how normal alleles contributed to the pathogenesis of RA remains uncertain, and normal alleles in the mitochondrial D-loop associated with disease risk [26, 33] were not found. These alleles might link with candidate genes of diseases in mitochondrial coding region. Furthermore, complementary alleles might exhibit an inhibitory effect on the disease process. Subsequent functional studies are needed to validate the predictive values of these SNPs for RA.

Conclusion

Taken together, SNPs in D-loop play a role in the pathogenesis of rheumatic diseases.

Acknowledgements

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Disclosure of conflict of interest

None.

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Figure 1. Distribution of D-loop SNPs at 25 sites (X axis) and their relative frequencies in percentage within each group (Y axis).

Table 2. RA Onset risk associated SNPs in RA

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>RA (n = 85)</th>
<th>Control (n = 100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>16260</td>
<td>C/C</td>
<td>85 (100%)</td>
<td>93 (93%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>0 (0%)</td>
<td>7 (7%)</td>
<td>0.016</td>
</tr>
<tr>
<td>16519</td>
<td>T/T</td>
<td>38 (45%)</td>
<td>63 (63%)</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>47 (55%)</td>
<td>37 (37%)</td>
<td></td>
</tr>
<tr>
<td>146</td>
<td>T/T</td>
<td>67 (79%)</td>
<td>90 (90%)</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>18 (21%)</td>
<td>10 (10%)</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>C/C</td>
<td>59 (70%)</td>
<td>82 (82%)</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>26 (30%)</td>
<td>18 (18%)</td>
<td></td>
</tr>
<tr>
<td>195</td>
<td>T/T</td>
<td>84 (99%)</td>
<td>90 (90%)</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>1 (1%)</td>
<td>10 (10%)</td>
<td></td>
</tr>
</tbody>
</table>
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References


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