A novel polymorphism of the SSA1 gene is associated with anti-SS-A/Ro52 autoantibody in Japanese patients with primary Sjögren’s syndrome

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This work was partly supported by a Grant-in Aid for Scientific Research (11670446) from the Japan Society for the Promotion of Science.

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Key words: Sjögren’s syndrome, SSA1 gene, SNP, anti-SS-A/Ro52 antibody.

ABSTRACT

Objective. To investigate the association of polymorphisms of the SSA1 gene (OMIM 109092) with primary Sjögren’s syndrome (SS) and anti-SS-A/Ro52 antibody production.

Methods. Polymorphisms of SSA1 gene in 111 Japanese SS patients and in 97 healthy controls were analyzed with polymerase chain reaction and automated DNA sequencing.

Results. A new single-nucleotide polymorphism (SNP) was identified in intron 1 at position 7216. The allele frequency and genotype of 7216A/G were not significantly different between SS patients and control subjects. However, the allele frequency and genotype of 7216A/G were associated with the presence of anti-SS-A/Ro52 antibody among primary SS patients. The association was not found in patients with SLE, suggesting the limited role for the SNP in anti-SS-A/Ro52 antibody production. The 9571C/T polymorphism, which has been shown to associate with anti-SS-A/Ro52 antibody in Caucasian patients, was not associated with the presence of anti-SS-A/Ro52 antibody in Japanese patients.

Conclusions. 7216A/G polymorphism of SSA1 gene may be one of the genetic factors that determine the presence of anti-SS-A/Ro52 antibody in patients with primary SS.

Introduction

Sjögren’s syndrome (SS) is an autoimmune exocrinopathy characterized by lymphocyte infiltration into salivary glands and polyclonal B cell activation with autoantibody production including anti-SS-A and SS-B autoantibodies (1). Anti-SS-A antibody is involved in sicca symptoms, subacute cutaneous lupus, and neonatal lupus erythematosus, indicating the roles in the related conditions (2,3). Anti-SS-A antibody has been shown to react Ro52 and/or Ro60 proteins (4,5).

Genetic factors contribute to the autoantibody production as well as disease susceptibility in SS. HLA has been shown to be associated with the presence of anti-SS-A and SS-B antibodies (6). Recently cytokine gene polymorphisms have been shown to be associated with anti-SSB antibody (7). We have previously shown the association of Glutathione S-transferase gene polymorphism with anti-SSA antibody production (8).

Polymorphisms of SSA1 gene, which encodes Ro52 protein, seem to be one of the candidate genes to determine the SS-A antibody production. Polymorphism at 7219 of SSA1 gene is strongly associated with the susceptibility of systemic lupus erythematosus (SLE) in African Americans (9). The 9571C/T polymorphism has been reported to be strongly associated with the presence of anti SS-A/Ro52 autoantibodies in Australian patients (11). The discrepancy may be due to the different ethnic backgrounds and indicate that further studies in different ethnic groups are important to confirm the genetic contribution of SSA1 gene to autoantibody profile.

Thus we set out to determine whether the polymorphisms of SSA1 gene contribute to disease susceptibility and anti-SS-A/Ro52 antibody production in Japanese SS patients. Here we report a new polymorphism in intron 1 at position 7216 in Japanese population and association between 7216A/G polymorphism and anti-SS-A/Ro52 antibody production in SS patients.

Materials and methods

Patients and controls

111 Japanese patients with primary SS and 97 healthy Japanese control subjects were enrolled in the study. The patients were followed up at Kobe University Hospital, Kanazawa Medical School Hospital, and Kyoto University Hospital. All patients had symptomatic dry eyes and/or dry mouth, and were diagnosed as having SS by the preliminary classification criteria proposed by Vitali et al. (12). Cases of Secondary SS were excluded from study. Seventy-three patients with SLE without SS were also enrolled for the study. Peripheral blood mononuclear cells (PBMC) were isolated from heparin-
ized blood by standard Ficoll-Hypaque gradient centrifugation. Sera of the SS patients were obtained and stored at −20°C until assayed.

Autoantibody profile
Anti-SS-A/Ro52 and anti-SS-A/Ro60 antibodies were measured for 62 patients whose sera were available, using commercially available EIA (Medical & Biological Laboratories, Nagoya, Japan) (13).

Polymerase chain reaction (PCR)
DNA extraction was performed using the QIAamp Blood Midi kit (Qiagen, Hilden, Germany). SSA1 gene fragments were amplified by polymerase chain reaction (PCR). PCR was performed in a 50 µl reaction volume consisting of 5 units Ex Taq polymerase (Takara Shuzo, Otsu, Japan), 0.25mM dNTP, 100 pmoles of each primer, and 1 µl sample DNA. Samples were subjected to 30 cycles of denaturing (94°C for 1 minute), annealing (60°C for 1 minute), and extension (72°C for 2 minutes), with an initial denaturation at 94°C for 4 minutes and final elongation at 72°C for 10 minutes, using a thermal cycler (Perkin Elmer GeneAmp System 9600, Emeryville, CA).

To amplify the SSA1 gene, four pairs of PCR primers were used. The sequences of the primer pairs were: 1, CCAACAGCATATGAGTGATACCCTTT and CACTCTTATGTCCTCAGCTATACAA; 2, CGTCAGATGATGTGATTTCCAG and AACGAAGCCTCACCAGGTGTC; 3, ACCCTCTGGATTGGAGGGGTTAG and AGCTGTTTCTGAGGACTAGACG; 4, TCACGTCCCCAGAAACTCTAACC and AAATGGCACTTCCAGATAGCTGT. The amplified genomic regions were: 1, nucleotides 6789-7321; 2, nucleotides 7590-8250; 3, nucleotides 8319-8699; 4, nucleotides 9522-9991. Nucleotides are numbered according to the sequence contained in Accession No. U01882.

Restriction enzyme fragment length polymorphism (RFLP) technique
To determine the 7219 polymorphism, amplified products were digested with Bgl II, and their RFLP genotype was determined by 2% agarose gel electrophoresis.

DNA sequencing
Amplified PCR products were subjected to DNA sequencing using ABI prism Big Dye Terminator Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA). Cycle Sequencing was performed in 20 µl reaction volume consisting of 8 µl BigDye Terminator, 3.2 picomoles of primers, and 10 µl PCR products. Samples were subjected to 25 cycles of denaturing (96°C for 10 seconds), annealing (50°C for 5 seconds), and extension (60°C for 4 minutes), with an initial denaturation at 96°C for 5 minutes.

Statistical analysis
Chi-square test or Fisher’s exact test was used for statistical analyses. The significance level was set at p-values < 0.05. The odds ratio (OR) was calculated with respect to the minor allele compared with the major allele. Estimation of haplotype frequency was performed by the maximum-likelihood method using SNPAlyze (DYNACOM Co., Ltd. Yokohama, Japan).

Results
Identification of a new polymorphism at position 7216 in Japanese individuals
We carried out RFLP and DNA sequencing analysis of exons including exon-intron boundaries on 111 Japanese patients with primary SS and 97 healthy Japanese control subjects. We investigated 4 SNPs (single nucleotide polymorphism) at positions 7219, 7649, 8463, and 9571 of SSA1 gene (Fig.1a). The 7219A/G polymorphism has been reported to be associated with disease susceptibility to SLE in African Americans (9). However, RFLP analysis using BglII restriction enzyme revealed that there were no polymorphisms at position 7219 in Japanese healthy individuals, SS patients, or SLE patients. Then we carried out direct DNA sequencing and confirmed that all the samples showed C allele at position 7219. Interestingly, direct DNA sequencing revealed a new polymorphism at position 7216 in Japanese population. Representative sequencing results of A/A, A/G, and G/G genotypes were shown (Fig. 1b). In Caucasian SS patients, 7649 A/G,
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Table I. Genotype frequencies between SS patients and control subjects.

<table>
<thead>
<tr>
<th>Location*</th>
<th>Nucleotide change</th>
<th>Genotype frequency, n (%)</th>
<th>χ² test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SS (n = 111)</td>
<td>Control (n = 97)</td>
</tr>
<tr>
<td>Intron 1 (7216)</td>
<td>A/A</td>
<td>18 (16.2)</td>
<td>16 (16.5)</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>59 (53.2)</td>
<td>44 (45.4)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>34 (30.6)</td>
<td>37 (38.1)</td>
</tr>
<tr>
<td>Intron 1 (7649)</td>
<td>A/A</td>
<td>63 (56.8)</td>
<td>49 (50.5)</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>38 (34.2)</td>
<td>39 (40.2)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>10 (9.0)</td>
<td>9 (9.3)</td>
</tr>
</tbody>
</table>

* Numbers indicate the position in the gene according to GeneBank accession no. U01882.

Table II. Genotype frequencies between the anti-SSA/Ro 52kD-positive and -negative patients.

<table>
<thead>
<tr>
<th>Location* no</th>
<th>Nucleotide change</th>
<th>Allele frequency, n (%)</th>
<th>χ² test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron 1 (7216)</td>
<td>A/A</td>
<td>10 (25.6)</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>7 (17.9)</td>
<td>10 (43.5)</td>
</tr>
<tr>
<td>Intron 1 (7649)</td>
<td>A/A</td>
<td>16 (41.0)</td>
<td>15 (65.2)</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>17 (43.6)</td>
<td>7 (30.4)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>6 (15.4)</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Intron 3 (9571)</td>
<td>C/C</td>
<td>13 (56.5)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>9 (39.1)</td>
<td>5 (31.3)</td>
</tr>
</tbody>
</table>

* Numbers indicate the position in the gene according to GeneBank accession no. U01882.

Table III. Genotype frequencies between the anti-SSA/Ro 52kD-positive and -negative SLE patients.

<table>
<thead>
<tr>
<th>Location* no</th>
<th>Nucleotide change</th>
<th>Allele frequency, n (%)</th>
<th>χ² test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron 1 (7216)</td>
<td>A/A</td>
<td>4 (19.1)</td>
<td>7 (13.5)</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>10 (47.6)</td>
<td>27 (51.9)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>7 (33.3)</td>
<td>18 (34.6)</td>
</tr>
</tbody>
</table>

* Numbers indicate the position in the gene according to GeneBank accession no. U01882.

8463 C/G, and 9571 C/T polymorphisms have been reported (10). We found that Japanese have 7649 A/G and 9571 C/T, but not 8463 polymorphisms by PCR-direct sequencing, showing that not all the SNPs are conserved among different ethnic groups.

Association of polymorphisms in SSA1 gene with disease susceptibility to SS

We next analyzed whether the new SNP, as well as 7649 SNP, is associated with primary SS. The genotype frequencies of 7216 and 7649 polymorphisms were not different between SS patients and healthy controls (Table I). Also the allelic frequencies did not differ significantly (p = 0.454 and 0.459 for 7216 and 7649, respectively). Thus the 7216 and 7649 SNPs were not associated with disease susceptibility to SS in the group of Japanese patients studied.

Association of autoantibody production with new polymorphism of the SSA1 gene

We next analyzed the association of 7216A/G polymorphism with anti-SS-A antibody, anti-SS-A/Ro52 antibody, and anti-SS-A/Ro60 antibody production. The polymorphism was not associated with anti-SS-A/Ro autoantibody production in the patients (allele frequencies of 7216A were 44.0% and 37.8% in anti-SS-A/Ro-positive and -negative patients, respectively, p = 0.414; OR = 1.30; 95% CI 0.70 – 2.41). The genotype and allele frequencies of 7216 SNP were not different between the patients with anti-SS-A/Ro60-positive and -negative patients (allele frequencies of 7216A were 48.6% versus 40.7%, p = 0.385; OR = 1.37; 95% CI 0.67 – 2.81). However, genotype gave significant difference between anti-SS-A/Ro52 antibody-positive and -negative patients (Table II). The allele frequency of 7216A was significantly increased in the patients with anti-SS-A/Ro52 antibodies compared to the antibodies-negative patients (53.8% versus 30.4%, p = 0.011; OR = 2.67; 95% CI 1.25 – 5.70). Thus 7216 SNP is associated with anti-SS-A/Ro52, but not with anti-SS-A/Ro60 or anti-SS-A-antibody production.

Association of anti-SSA/Ro52 autoantibody production with 7649 and 9571 polymorphisms

The genotype frequency of 7649A/G polymorphism did not differ between the patients and controls (AA/AG/GG: 56.8%/34.2%/9.0% versus 50.5%/40.2%/9.3%, p = 0.645). The genotype frequencies of 9571 C/T polymorphism also did not differ (CC/CT/TT: 59.0%/35.9%/5.1% versus 68.3%/28.0%/3.7%, p = 0.600). Thus 7649 and 9571 polymorphisms were not associated with disease susceptibility to SS.

Importantly, the genotype frequencies of 7649 A/G and 9571 C/T polymorphisms did not differ between the patients with or without anti-SS-A/Ro52 antibody (Table II). The 7649A allele frequency was significantly lower in anti-SS-A/Ro52 antibody-positive patients compared to -negative patients (62.8% vs 80.4%, p = 0.040), indicat-
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Discussion

In this study, we have performed direct sequence in 3 exons with exon-intron boundaries of SSA1 gene and found a new SNP at position 7216 in Japanese population. We concluded that the new 7216 polymorphism of SSA1 gene is one of genetic factors involved in anti-SS-A/ Ro52 autoantibody production in Japanese SS but not in SLE patients.

There have been several studies investigating possible associations between SSA1 gene polymorphisms and disease susceptibility and/or autoantibody production. The 7219 SNP has been shown to be strongly associated with SLE in African Americans (9). However, Japanese and Caucasians do not have the 7219 SNP, it is limited to African Americans. Seven polymorphisms in the SSA1 gene have been identified in Norwegian population (10). Four of them, 4595, 7649, 9571, and 12986 have been shown to be associated with anti-SS-A/Ro52 antibody-positive SS patients. Among them, the 9571 polymorphism showed the strongest association with anti-SS-A/Ro52 autoantibody. However, we and others did not find any association of the polymorphism with anti-SS-A/Ro52 autoantibody production, suggesting that the role of this SNP is limited to Norwegian population (11). On the other hand, we have found significant association of 7649 SNP with anti-SS-A/52kD antibody production in Japanese patients confirming the contribution of 7649 SNP to SS-A/Ro52 antibody production.

We have previously shown that GSTM1 and TAP2 genes polymorphisms are associated with disease susceptibility to SS and the presence of anti-SSA- Antibody (8, 14). We did not find any association between 7216 SSA polymorphism and GSTM1 or TAP2 polymorphisms. We speculate that disease susceptibility to SS and anti-SS-A/ Ro52 antibody production are influenced by the polymorphisms of multiple genes. In conclusion, we found a new polymorphism at position 7216 of SSA1 gene and its association with anti-SS-A/ Ro52 antibody production in Japanese patients with SS. The relevance of this polymorphism should be examined in different ethnic groups because genetic predisposition might be different among different ethnic groups.

Further studies are needed to elucidate how polymorphisms of SSA1 gene influence anti-SS-A/Ro52 antibody production in patients with primary SS.

Acknowledgements

The authors thank Dr. S. Sugai at Kanazawa Medical University for patient samples.

References