Polymorphisms of the TAP1 and TAP2 transporter genes in Japanese SLE

F Takeuchi, K Nakano, H Nabeta, G H Hong, S Kuwata, K Ito

Abstract

Objective—To determine how polymorphisms of transporter associated with antigen processing 1 and 2 (TAP1 and TAP2) alleles contributed to the pathogenesis of systemic lupus erythematosus (SLE) in Japanese patients.

Methods—TAP1 and TAP2 typing was carried out in 52 Japanese patients with SLE and 95 normal subjects by the PCR-RFLP (restriction fragment length polymorphism) method. HLA-DR typing and HLA-DRB1*15 genotyping were carried out by the PCR method and PCR-SSCP (single stranded DNA conformation polymorphism) method, respectively.

Results—No particular TAP 1 allele was associated with Japanese SLE or with an immunological subgroup of SLE. TAP2H showed a tendency towards increased frequency in SLE (5.8% vs 0% in control), but the corrected P value was not significant. No other particular association of TAP2 allele was observed. Furthermore, there was no evidence for linkage disequilibrium between any TAP1/TAP2 alleles and HLA-DRB1*1501—which is reported to be weakly but significantly associated with Japanese SLE—in either the normal control or the SLE patient group.

Conclusions—Neither the TAP1 nor the TAP2 gene appears to determine disease susceptibility to SLE in Japanese, and these results are in keeping with those reported in Caucasian SLE patients.

Methods

Patients

Genomic DNA was obtained from the peripheral white blood cells of 52 randomly selected unrelated Japanese SLE patients (aged 41.5 (SD 12.0) years; 49 women and three men), diagnosed according to the criteria of the American Rheumatism Association. The control population comprised 95 unrelated healthy volunteers.

Genotyping of TAP1 and TAP2 Genes

TAP1 and TAP2 genotyping was performed by the PCR-RFLP method using the specific primers described previously. In brief, two dimorphic sites of TAP1—codons 333 and 637—were digested with Sau3AI and AccI, respectively, and four dimorphic sites of TAP2—codons 379, 565, 665, and 687—were digested with NcoI, Rsal, MspI, and BfaI, respectively, after specific amplification. Four possible TAP1 alleles (TAP1A-D) and eight possible TAP2 alleles (TAP2A-H) were then determined and assigned according to the nomenclature proposed by Powis et al. as described previously.
Table 1 Phenotype frequencies (%) of TAP1 alleles in SLE and control populations

<table>
<thead>
<tr>
<th>Allele</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE (n=52)</td>
<td>100</td>
<td>26.9</td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dsDNA (n=25)</td>
<td>100</td>
<td>28.0</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>SS-A (n=18)</td>
<td>100</td>
<td>16.7</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>SS-B (n=2)</td>
<td>100</td>
<td>50.0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RNP (n=17)</td>
<td>100</td>
<td>23.5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sm (n=5)</td>
<td>100</td>
<td>40.0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control (n=95)</td>
<td></td>
<td></td>
<td></td>
<td>96.8</td>
<td>26.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

dsDNA, circulating antibodies to dsDNA; SS-A, circulating antibodies to SS-A; SS-B, circulating antibodies to SS-B; RNP, circulating antibodies to U1-RNP; Sm, circulating antibodies to Sm.

HLA-DR typing

HLA-DR typing was performed on the second exon of HLA-DRB1 by the PCR method using the specific primers described previously.14

Genotyping of DR15

HLA-DR15 genes were genotyped by the PCR-SSCP (single stranded DNA conformation polymorphism) method using the specific primers and electrophoresis condition described previously.14

Detection of autoantibodies

Anti SS-A, SS-B, U1-RNP, and Sm antibodies (a-SS-A, a-SS-B, a-RNP, a-Sm, respectively) were detected by the double immunodiffusion method using ENA-1 and ENA-2 test kit (MBL Co, Nagoya, Japan). Anti double stranded DNA antibody (a-dsDNA) was detected and quantified using an enzyme linked immunosorbent assay (ELISA) anti dsDNA kit (Japan DPC Co, Tokyo, Japan). These methods are standard in the Central Laboratory Service of Tokyo University Hospital.14

Statistics

The $\chi^2$ test (with Yates correction) was used for comparison. When a value below 5 was contained in the 2 × 2 table, Fisher's exact test was used. Relative risk (RR) was calculated by standard Woolf's formula or, when "0" was included by Haldane's modified formula. Probability value (P) was corrected occasionally for the number of comparisons made [corrected probability (PC)]. In this report, the P value was used primarily for comparison and discussion in order to avoid missing any clinically important associations, although PC was used occasionally for statistical decisions.

Results

The phenotype frequency of TAP1 in patients with SLE and controls are shown in Table 1. No TAP1 allele was found to be significantly associated with SLE. TAP1 phenotype frequencies were also determined in various immunological subgroups of SLE. TAP1B frequency was slightly decreased in patients with circulating anti SS-A antibody (P = NS). No significant associations of TAP1 alleles were observed in any immunological subgroup with autoantibody circulating to each autoantigen.

Table 2 shows TAP2 phenotype frequencies in SLE and controls. In SLE patients, the phenotype frequency of TAP2H was increased in comparison with control (5.8% vs 0%, P < 0.05), but the increase was not statistically significant when the P value was corrected (Pc = NS). Phenotype frequency of TAP2E was slightly decreased in SLE in comparison with control, but again the difference was not significant. No associations were observed between TAP2 alleles and overall SLE either. TAP2 phenotype frequencies were also determined in various immunological subgroups of SLE. Patients with a-RNP showed slight but not significant increase in the phenotype frequency of TAP2H (11.8%, P = NS). SLE patients with a-SS-A showed a slightly higher prevalence of TAP2A and low prevalence of TAP2E, but these differences were not significant. The frequency of TAP2E was not significantly increased in patients with circulating a-Sm. No TAP2 alleles were significantly associated in any immunological subgroup with autoantibody circulating to each autoantigen studied.

In control, associations of TAP1 and TAP2 alleles with DRB1*1501, which is reported to be associated with Japanese and Korean SLE,12 were estimated. No significant association was observed between any TAP1 or TAP2 allele and DRB1*1501 in either control or SLE.

Discussion

Because of their roles in the processing of antigen, TAP1 and TAP2 genes are potentially important in immune responses.44 Associations of TAP1 and TAP2 alleles have been studied in many diseases, including various autoimmune diseases, based on the hypothesis that TAP genes may represent candidate susceptibility genes or serve as useful markers for MHC linked diseases.9

In this experiment, a slightly increased frequency of TAP2H was observed in SLE, but the increase was not statistically significant (P < 0.05, Pc = NS). Additionally, the prevalence of TAP2H in SLE was too small (5.8%) to consider it a disease susceptible factor. Our study ultimately indicates that no TAP1 and TAP2 alleles are associated with Japanese SLE. Neither was there any significant association of any TAP1 or TAP2 allele in any of the immunological subgroups of Japanese SLE studied. These results strongly suggest that TAP 1 and TAP2 are not genetically involved in the pathogenesis of Japanese SLE.

Davis et al indicated that TAP2 is not involved in determining genetic predisposition to SLE in Caucasians.9 Our results further supported their findings, adding to the report.
of Chinese SLE, though the genetic backgrounds of autoimmune diseases often differ between Japanese and Caucasians. In their report, no significant increases of TAP2C or TAP2D were observed in patients with a-SS-A or a-RNP. TAP2C was not statistically frequent in the Japanese a-SS-A subgroup we observed. TAP2D is a rare allele in Japanese and was not increased in Japanese patients with a-RNP. Several weak associations (not significant) observed in this study were not observed in their report. TAP2A, which was somewhat increased in Japanese SLE patients with a-SS-A, has been reported to be decreased in Caucasian SLE patients with a-SS-A.

These findings in Japanese and Caucasian SLE patients' strongly suggested that TAP2 (and TAP1) genes are not involved in the autoantibodies in the SLE studied.

A general association of TAP1 and TAP2 genes with HLA-DR in Japanese was reported previously. In this paper, the association of TAP alleles with HLA DRB1*1501 was studied because a weak but significant association of HLADRB1*1501 had been observed in Japanese and Korean SLE patients. Although Singal et al. additionally implicated TAP genes together with HLA-DR in rheumatoid arthritis, TAP genes did not contribute to the pathogenesis of Japanese SLE along with HLA DRB1*1501. These findings essentially supported the previous observations by Davis et al. Davis reported a tendency towards increased frequency of TAP2D in SLE with a-RNP and the linkage disequilibrium between TAP2D and DR4 in control. Although the main genotypes of HLADRB1*04 differ between Japanese and Caucasians, TAP2D is a rare allele and is not associated with DR4 in Japanese controls. In the Chinese TAP2D is also a rare allele and is not associated with DR4 either.

It had been reported that no particular TAP2 allele is associated with vasculitis and renal disease in SLE. In our study, associations between TAP alleles and vasculitis or renal disease are not shown since biopsies were not carried out in all SLE patients and accurate discrimination of vasculitis or renal disease was difficult clinically in several patients.

Our study strongly suggests that TAP1 and TAP2 genes do not determine disease susceptibility to Japanese SLE. As indicated in the Discussion of the previous report by Davis et al., these results will prove useful in improving our understanding of the participation of TAP genes in the pathogenesis of SLE in a diverse range of ethnic groups.

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