Relative serum amyloid A (SAA) values: the influence of SAA1 genotypes and corticosteroid treatment in Japanese patients with rheumatoid arthritis

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Abstract

Objectives—(1) To determine whether serum concentration of serum amyloid A (SAA) protein is influenced by the SAA1 allele in Japanese patients with rheumatoid arthritis (RA) as previously shown in a healthy control group; and (2) to analyse what factors, based on such an allelic bias, influence the relative SAA values of those patients.

Methods—SAA and C reactive protein (CRP) concentrations together with SAA1 genotypes were determined in 316 Japanese patients with RA. The relative SAA values were evaluated as an SAA/CRP ratio.

Results—Comparison of the three SAA1 homozygote groups showed that the SAA/CRP ratio was highest in the 1.5/1.5 group (mean 9.0, p<0.01 v the other two homozygote groups) followed by the 1.3/1.3 group (mean 7.2, NS v the 1.1/1.1 group) and the 1.1/1.1 group (mean 4.0). The SAA/CRP ratio was significantly higher in patients receiving corticosteroids regardless of the presence of allele 1.5. No clear differences in the ratio between patients with or without amyloidosis were found.

Conclusion—The SAA1.5 allele and corticosteroid treatment had a positive influence on SAA concentrations in serum. These findings are important when evaluating SAA concentration in inflammatory diseases and when considering the cause or treatment of amyloidosis.

Relative serum amyloid A values

Table 1 Clinical profiles of Japanese patients classified by SAA1 genotype

<table>
<thead>
<tr>
<th>SAA1 genotype</th>
<th>No (male)</th>
<th>Class*</th>
<th>Duration† (years)</th>
<th>Amyloidosis‡ (No (%))</th>
<th>Treatment (No (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
</tr>
<tr>
<td>1.1/1.1</td>
<td>27 (3)</td>
<td>63.4</td>
<td>12</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>1.1/1.2</td>
<td>3 (1)</td>
<td>53.0</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1.1/1.3</td>
<td>73 (11)</td>
<td>65.5</td>
<td>4</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>1.1/1.5</td>
<td>55 (5)</td>
<td>63.2</td>
<td>6</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>1.2/1.3</td>
<td>3 (0)</td>
<td>57.3</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1.1/1.5</td>
<td>5 (3)</td>
<td>66.3</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1.3/1.3</td>
<td>44 (4)</td>
<td>64.6</td>
<td>0</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>1.3/1.5</td>
<td>78 (11)</td>
<td>67.6</td>
<td>5</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>1.5/1.5</td>
<td>29 (3)</td>
<td>66.7</td>
<td>2</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>316 (38)</td>
<td>65.2</td>
<td>17.7</td>
<td>66</td>
<td>21</td>
</tr>
</tbody>
</table>

*Class = Steinbrocker functional class.
†Age and duration are mean values.
‡CS = corticosteroid; DM = DMARDs; IS = immunosuppressive agents.

Methods

PATIENTS
Three hundred and sixteen Japanese subjects (38 male, 278 female, aged 16–89), both inpatients and outpatients at the Centre for Rheumatism, Dohgo Spa Hospital, Matsuyama, Japan during the months of June and July 1999, took part in this study. Informed consent was obtained from all subjects. All patients met the 1987 revised RA criteria of the American Rheumatism Association. All patients had undergone gastroduodenal endoscopic biopsy within the last two years; amyloidosis was diagnosed histologically from the biopsy specimens. Therapeutic drugs were grouped into three categories: corticosteroids, disease modifying antirheumatic drugs (DMARDs), and immunosuppressive agents. Corticosteroid was given as oral prednisolone. DMARDs included intramuscular gold, d-penicillamine, auranofin, bucillamine, salazosulfapyridine, and actarit (4-acetylamino phenylacetic acid). Immunosuppressive agents included methotrexate, azathioprine, cyclophosphamide, and tacrolimus (FK-506). DMARDs or immunosuppressive agents were given according to the commonly recommended regimen, and doses of corticosteroids varied among subjects. Table 1 shows the frequency of use.

ANALYSIS
Blood was drawn from inpatients at the monthly examination and from outpatients at the regular visit. SAA1 genotypes were determined using the genomic DNA of the patients by a polymerase chain reaction–restriction fragment length polymorphism analysis as described elsewhere. The SAA and CRP concentrations in serum were determined by a latex agglutination turbidimetric immunoassay, which is currently available from Eiken Chemicals Co Ltd, Tokyo, Japan. A statistical comparison of data between the groups was carried out with a χ² test or Mann-Whitney U test.

Table 2 SAA1 allele frequency and amyloid involvement

<table>
<thead>
<tr>
<th>SAA1 allele</th>
<th>Amyloidosis (No (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
</tr>
<tr>
<td>1.1</td>
<td>162 (0.331)</td>
</tr>
<tr>
<td>1.3</td>
<td>184 (0.376)</td>
</tr>
<tr>
<td>1.5</td>
<td>144 (0.293)</td>
</tr>
</tbody>
</table>

Results

CLINICAL FEATURES OF SUBJECTS BY SAA1 GENOTYPES
Data were assessed according to SAA1 genotype (table 1). There were no apparent differences in age, Steinbrocker functional class, duration of disease, use of corticosteroid (%), DMARDs (%), or immunosuppressive agents (%). The SAA1 allele distributions in patients with amyloidosis were compared with those in subjects without (table 2). The allele 1.1 was negatively associated with amyloidosis (p<0.01) while 1.5 was positively associated (p<0.01). There was no statistical difference in the 1.3 frequency between the groups.

INFLUENCE OF SAA1 GENOTYPES ON SAA AND CRP VALUES AND THE SAA/CRP RATIO
To assess the influence of the SAA1 allele on SAA and CRP values and on the SAA/CRP ratio, data from three homozygote groups were compared (table 3, fig 1). CRP concentrations did not differ between the three groups, whereas SAA concentrations and SAA/CRP ratios were significantly higher in the 1.5/1.5 than in the other groups.

FACTORS AFFECTING THE SAA/CRP RATIO
Because the allelic bias on the SAA/CRP ratio was proved, subjects were divided into two groups according to the presence or absence of the SAA1.5 allele and their data were analysed. Under these conditions the allele 1.2 was regarded as equivalent to the 1.5 as this allele is rare and has exon 3 structures, which distinguish the three common alleles, identical to

Table 3 Mean serum amyloid A (SAA) and C reactive protein (CRP) concentrations classified by SAA1 genotype

<table>
<thead>
<tr>
<th>SAA1 genotype</th>
<th>No</th>
<th>SAA (mg/l)</th>
<th>CRP (mg/l)</th>
<th>SAA/CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1/1.1</td>
<td>27</td>
<td>96</td>
<td>23</td>
<td>4.0</td>
</tr>
<tr>
<td>1.1/1.2</td>
<td>3</td>
<td>185</td>
<td>35</td>
<td>8.7</td>
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<tr>
<td>1.1/1.3</td>
<td>73</td>
<td>104</td>
<td>30</td>
<td>4.1</td>
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<td>1.1/1.5</td>
<td>55</td>
<td>193</td>
<td>29</td>
<td>6.8</td>
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<td>1.2/1.5</td>
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<td>195</td>
<td>11</td>
<td>14.8</td>
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<tr>
<td>1.3/1.3</td>
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<td>71</td>
<td>19</td>
<td>7.2</td>
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<td>1.3/1.5</td>
<td>78</td>
<td>105</td>
<td>16</td>
<td>11.0</td>
</tr>
<tr>
<td>1.5/1.5</td>
<td>29</td>
<td>232*</td>
<td>25</td>
<td>9.0†</td>
</tr>
</tbody>
</table>

Allele
1.5 (+) 172 157‡ 16 9.1‡
1.5 (−) 144 93 26 5.0

*p=0.022 v group 1.1/1.1, p=0.009 v group 1.3/1.3.
†p=0.001 v group 1.1/1.1, p=0.007 v group 1.3/1.3.
‡p=0.005, ‡p=0.0001 v group 1.5 (−).
the plot because of isolated data. (n=111), but not in those without it (n=138). Data from five subjects were not included in those groups divided by SAA1 allele when all the subjects or the groups divided by SAA1 allele were analysed (data not shown). The ratios were significantly higher (p<0.05) in patients with amyloidosis than in those without it when all the subjects were analysed (table 4). However, such differences did not reach significance in any of the divided groups. The use of DMARDs or immunosuppressive agents did not affect the SAA/CRP ratio, though the use of corticosteroids had a positive influence. This was seen both in all the subjects and in each of the divided groups (table 4). The SAA/CRP ratio correlated weakly with the dose of corticosteroid in the SAA1.5 group, but not in the allele negative group (fig 2).

Discussion
Two previous reports, including one by us,20 21 suggest that the SAA1.3 and SAA1.1 alleles are positive and negative risk factors for amyloidosis in Japanese patients with RA. One study21 showed an increased frequency, though not significant, of the SAA1.5 allele in subjects with amyloidosis. In the present study, similarly, a negative association of the allele 1.1 with amyloidosis was found. The increased frequency of 1.3, however, was not statistically significant. Instead, a significant association of 1.5 was shown. These controversial findings may be due to the subjects selected. Because this study did not aim at assessing susceptibility to amyloidosis, there may be some imbalance in the clinical profiles of the patients with or without amyloidosis. Nevertheless, the data show again the low frequency of 1.1. Perhaps, this may be a stronger genetic indication for amyloidogenesis in Japanese patients with RA than the high frequency of the allele 1.3.

In a previous report we described an allelic bias in plasma SAA concentrations of a healthy Japanese population sample.23 Our preliminary experiments with recombinant SAA1 isotypes suggested that the difference in plasma clearance among the allele-corresponding SAA phenotypes was responsible for this (unpublished findings). Whatever the mechanism, the possibility that such allelic bias is shown in subjects with abnormally raised SAA concentrations should be assessed. Because these concentrations change according to the inflammatory activity present when the blood sample is drawn, it was difficult to evaluate the relation between SAA values and SAA1 genotypes. Therefore, we introduced the SAA/CRP ratio. CRP correlated markedly with SAA in most of the inflammatory disorders. To date, no study has reported the participation of a genetic factor in producing a variation in serum CRP concentrations. By using this ratio, it was shown that SAA responds more sensitively than CRP to a kidney allograft rejection.5

The clinical profiles of the patients with RA in this study showed no apparent differences among the six major genotype groups. In addition, no statistical difference in CRP values was noted among the groups, suggesting that disease activity was also equally distributed. To
examine the allelic influence on relative SAA values, the SAA/CRP ratios were compared in three homozygote groups: SAA1.1/1.1, SAA1.3/1.3, and SAA1.5/1.5. The results showed that SAA1.5 homozygotes had higher SAA/CRP ratios than the others. Consequently, the positive influence by allele 1.5 was confirmed not only in healthy (or minimally pathological) states but also in inflammatory conditions.

The above results indicate the need to evaluate SAA concentrations in the light of genetic information about subjects. A recent finding suggests that SAA is a better prognostic marker than CRP.26 Combining SAA1 genotypes and SAA values would be interesting. In this study we assessed which factors influenced the relative SAA values in subjects divided by SAA1 genotype. Because the number of each genotype, including three homozygote groups, was not enough for further analyses, we divided the subjects into two groups based on the presence of the allele 1.5.

As a result, the use of corticosteroid had an enhancing effect on the SAA/CRP ratio. The mechanism by which the SAA/CRP ratio is raised by corticosteroids is unknown. The agent may affect the synthesis of these proteins transcriptionally or post-transcriptionally, or it may have an effect by altering production of several inflammatory cytokines. Nevertheless, our findings support the concept that SAA responds more than CRP during the administration of corticosteroids. Dramatically high responses of SAA have been described for renal allograft rejection.27,28 Although this investigation did not assess the influence of cyclosporin, the agent most often used in renal transplantation, the high dose of corticosteroids, together with the effect of the SAA1 allele, may be responsible for this response. If the alteration of SAA levels is implicated in the development of amyloidosis, the propriety of giving corticosteroid treatment to patients at risk should concern investigators.

The subjects with amyloidosis in this study had higher SAA concentrations than those without it, but significance was borderline (table 4). The comparatively high prevalence of the SAA1.5 allele in this group may, in part, account for such an increase. The SAA/CRP ratios of subjects with amyloidosis did not differ from the ratio in those subjects without amyloidosis either in the groups divided by the presence of the SAA1.5 allele (table 4) or in the two homozygote groups, SAA1.3/1.3 and SAA1.5/1.5 (data not shown). Thus a strong linkage between the relative SAA values and presence of amyloidosis seems unlikely. However, as seen in Japanese subjects with the SAA1.1 allele, one cannot rule out the possibility that allele regulated SAA levels represent the allelic bias affecting their susceptibility to amyloidosis. To assess this further, other populations, such as white subjects, in whom SAA1 allele distribution and genetic background for amyloidogenesis differ from those of Japanese subjects, need to be analysed.

In conclusion, our study confirms a positive influence of the SAA1.5 allele and corticosteroid treatment on relative SAA concentrations. These findings are important when evaluating SAA concentrations in diseases and when considering treatment in amyloidosis. Further elucidation of the allelic bias in amyloidogenicity is due to the allele regulated SAA levels or to other factors should be further investigated.

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