A large-scale association study identified multiple HLA-DRB1 alleles associated with ACPA-negative rheumatoid arthritis in Japanese subjects.

Author(s)
Terao, Chikashi; Ohmura, Koichiro; Kochi, Yuta; Ikari, Katsunori; Maruya, Etsuko; Katayama, Masaki; Shimada, Kota; Murasawa, Akira; Honjo, Shigeru; Takasugi, Kiyoshi; Matsuo, Keitaro; Tajima, Kazuo; Suzuki, Akari; Yamamoto, Kazuhiko; Momohara, Shigeki; Yamanaka, Hisashi; Yamada, Ryo; Saji, Hiroo; Matsuda, Fumihiko; Mimori, Tsuneyo

Citation
Annals of the rheumatic diseases (2011), 70(12): 2134-2139

Issue Date
2011-12

URL
http://hdl.handle.net/2433/152548

Right
© 2011 BMJ Publishing Group Ltd & European League Against Rheumatism.

Type
Journal Article

Textversion
publisher
A large-scale association study identified multiple HLA-DRB1 alleles associated with ACPA-negative rheumatoid arthritis in Japanese subjects

Chikashi Terao,1,2 Koichiro Ohamura,1 Yuta Kochi,3 Katsunori Ikari,4 Etsuko Maruya,5 Masaki Katayama,1 Kota Shimada,6 Akira Murasawa,7 Shigeru Honjo,8 Kiyoshi Takasugi,9 Keitaro Matsuo,10 Kazuo Tajima,10 Akari Suzuki,3 Kazuhiko Yamamoto,11 Shigeki Momohara,4 Hisashi Yamanaka,4 Ryo Yamada,2 Hiroo Saji,5 Fumihiko Matsuda,2,12,13 Tsuneyo Mimori1

ABSTRACT

Background HLA-DRB1 is associated with rheumatoid arthritis (RA). However, it has recently been suggested that HLA-DRB1 is only associated with patients with RA who have anticitrullinated peptide/protein antibodies (ACPA), which are specific to RA.

Objective To elucidate whether specific HLA-DR alleles are associated with ACPA-negative RA development.

Methods HLA-DRB1 typing was carried out in 368 Japanese ACPA-negative patients with RA and 1508 healthy volunteers as the first set, followed by HLA-DRB1 typing of 501 cases and 500 controls as the second set. The HLA-DRB1 allele frequency and diplotype frequency were compared in each group, and the results of the two studies were combined to detect HLA-DRB1 alleles or diplotypes associated with ACPA-negative RA.

Results HLA-DRB1*12:01 was identified as a novel susceptibility allele for ACPA-negative RA (p = 0.000088, OR = 1.72, 95% CI 1.31 to 2.26). HLA-DRB1*04:05 and *14:03 showed moderate associations with ACPA-negative RA (p = 0.0063, OR = 1.26, 95% CI 1.07 to 1.49 and p = 0.0043, OR = 1.81, 95% CI 1.20 to 2.73, respectively). The shared epitope was weakly associated with ACPA-negative RA, but no dosage effect was detected (p = 0.016, OR = 1.17, 95% CI 1.03 to 1.34).

A combination of HLA-DRB1*12:01 and DRB1*09:01 showed a strong association with susceptibility to ACPA-negative RA (p = 0.00013, OR = 3.62, 95% CI 1.79 to 7.30). Homozygosity for HLA-DR8 was significantly associated with ACPA-negative RA (p = 0.0070, OR = 2.16, 95% CI 1.22 to 3.82). It was also found that HLA-DRB1*15:02 and *13:02 were protective against ACPA-negative RA (p = 0.00010, OR = 0.68, 95% CI 0.56 to 0.83 and p = 0.00059, OR = 0.66, 95% CI 0.52 to 0.84, respectively).

Conclusions In this large-scale association study multiple alleles and diplotypes were found to be associated with susceptibility to, or protection against, ACPA-negative RA.

INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common causes of chronic arthritis and results in severe joint damage and a shorter life span.1 Genetic factors have been shown to contribute to the onset of RA.2 Among the genetic susceptibility loci detected to date, HLA-DRB1 has a strong impact on the predisposition to RA and has been repeatedly shown to be associated with RA in an ethnicity-independent manner.2 It is widely accepted that the shared epitope (SE), a common amino acid sequence located from the 70th to the 74th amino acids of the HLA-DR β chain, explains the associations of specific HLA-DRB1 alleles with RA.4 Anticitrullinated protein antibodies (ACPA) are a highly specific marker of RA.5 6 Recent data have shown that the SE is associated with ACPA-positive RA but not associated or only weakly associated with ACPA-negative RA.7 8 Many of the non-HLA susceptibility genes for RA detected to date, such as PTPN22 and CTLA4, have been shown to be associated with ACPA-positive RA alone, and no association between these genes and ACPA-negative RA has been detected. These findings suggest that ACPA-negative RA is genetically distinct from ACPA-positive RA.

Among HLA-DRB1 molecules, HLA-DR3 and HLA-DR15 were reported to be associated with ACPA-negative RA in populations of European descent, but the same results were not obtained in a meta-analysis of a large Caucasian cohort.14 In Asian populations, there has only been a small study which showed that HLA-DRB1*09:01 might be associated with ACPA-negative RA.15 While SEs, especially DRB1*04:05, *04:01 and *01:01, were associated with RA and ACPA-positive RA,15 16 Thus, no specific alleles that convey susceptibility to, or are protective against, ACPA-negative RA have been identified in populations of European or Asian descent. In this large-scale Japanese case–control association study, we show that HLA-DRB1*12:01, *14:03 and *04:05 are susceptibility alleles for ACPA-negative RA and that HLA-DRB1*13:02 and *15:02 are protective against ACPA-negative RA. We also identified multiple diplotypes that convey susceptibility to, or are protective against, ACPA-negative RA.

MATERIALS AND METHODS

Study subjects

DNA samples were collected at Kyoto University Hospital from 184 patients with RA who were negative for ACPA, as reported previously,7 and another 184 patients with RA without ACPA were recruited at Tokyo Women’s Medical University. These two sample groups were used as the first
set. Independent DNA samples were collected from 501 ACPA-negative patients with RA at RIKEN under the support of BioBank Japan and were used as the second set. The 501 cases in the second set are a fraction of 2410 RA cases included in another manuscript (K Shimane et al, unpublished data). All patients were Japanese and diagnosed by rheumatologists to fulfill the 1987 American College of Rheumatology revised criteria for RA.17 A first set of control DNA samples were collected from 1508 healthy control subjects at Aichi Cancer Center Hospital and from the DNA banks of the Pharma SNP Consortium, which contains DNA samples from healthy Japanese volunteers.18 The second set of control DNA samples were collected from 500 healthy volunteers at the HLA laboratory. This study was approved by the local ethical committees at each institution, and written informed consent was obtained from all patients. Basic information about cases and controls is shown in table 1.

ACPA detection
ACPA were detected with the MESACUP CCP ELISA kit (Medical and Biological Laboratories Co, Ltd, Nagoya, Japan) according to the manufacturer’s instructions at each institution. A cut-off value of 4.5 U/ml was used to assess ACPA positivity.

HLA-DRB1 genotyping
HLA-DRB1 typing was carried out with the WAKFlow system and described in detail elsewhere.7 In the 184 cases collected at Tokyo University and all the controls in the two sets, genotyping was performed at the HLA laboratory (Kyoto, Japan), whereas it was carried out at RIKEN for all 501 cases in the second set. HLA-DRB1 genotyping in the 184 cases collected at Tokyo Women’s Medical University was performed by a sequencing-based typing method using the AlleleSEQR HLA-DRB1 typing kit (Abbott, Tokyo, Japan), and allele assignment was performed using the Assign software.


Statistical analysis
The frequency of each genotype or diplotype among the ACPA-negative patients with RA was compared with that in the controls using a χ² test or Fisher’s exact test. Ninety-five percent CIs, p values and ORs were also calculated. The relative risk (RR) of ACPA-negative susceptibility induced by homozygosity for each allele was calculated to estimate the dosage effect. We performed 1000 permutation tests to confirm the associations of these five alleles (figure 1). Among the protective alleles detected in the first set, the protective effect of DRB1*04:03 was confirmed in the second set and combined study, respectively (table 2). Although the protective effect of DRB1*13:02 was not replicated in the second set, the combined analysis showed a significant protective effect (p=0.0059). The protective effect of DRB1*04:05 was confirmed in the second set, and the combined study demonstrated a weak protective association (p=0.038). To exclude the possibility that the associations of the susceptibility alleles were induced by the absence of protective alleles or vice versa, we applied logistic regression analysis. The logistic regression analysis suggested that none of the allelic associations—namely, those of HLA-DRB1*12:01, *14:03, *04:05, *13:02, and *15:02, depended on the effects of other alleles (online supplementary table 3). In addition, the permutation tests confirmed the associations of these five alleles (permutation p<0.0070, data not shown).

Next, we analysed the dosage effects of each protective or susceptibility allele. DRB1*12:01 showed a potential dosage effect, but only two patients were homozygous for DRB1*12:01 (figure 1). We could not detect any dosage effects of HLA-DRB1*04:05 or the SE. No patients were homozygous for *14:03

Replication in the second set and combined analysis
We performed HLA-DRB1 genotyping of samples in the second set to replicate the results found in the first set, using the DNA samples from 501 ACPA-negative patients with RA and 500 sex-matched healthy controls and combined the results of the two association studies. Among the susceptibility alleles found in the first set, HLA-DRB1*12:01 was confirmed to display a susceptible association (p=0.010 and 0.00088 for the second set and combined study, respectively; table 2). The susceptibility tendencies of *04:05 and *14:03 were replicated in the second set, and these alleles showed moderate associations with susceptibility to ACPA-negative RA in the combined analysis (p=0.0063 and 0.0043, respectively). DRB1*09:01 and *14:05 showed potential susceptibility to ACPA-negative RA in the pooled study (p=0.062 and 0.080, respectively). The SE showed a weak association with susceptibility to ACPA-negative RA in the combined study (p=0.016), but we could not detect any dosage effect (table 3 and figure 1). Among the protective alleles detected in the first set, the protective effect of DRB1*15:02 was successfully replicated (p=0.002 and 0.00010 in the second set and combined study, respectively; table 2). Although the protective effect of DRB1*13:02 was not replicated in the second set, the combined analysis showed a significant protective effect (p=0.0059). The protective effect of DRB1*04:05 was confirmed in the second set, and the combined study demonstrated a weak protective association (p=0.038). To exclude the possibility that the associations of the susceptibility alleles were induced by the absence of protective alleles or vice versa, we applied logistic regression analysis. The logistic regression analysis suggested that none of the allelic associations—namely, those of HLA-DRB1*12:01, *14:03, *04:05, *13:02, and *15:02, depended on the effects of other alleles (online supplementary table 3). In addition, the permutation tests confirmed the associations of these five alleles (permutation p<0.0070, data not shown).

Next, we analysed the dosage effects of each protective or susceptibility allele. DRB1*12:01 showed a potential dosage effect, but only two patients were homozygous for DRB1*12:01 (figure 1). We could not detect any dosage effects of HLA-DRB1*04:05 or the SE. No patients were homozygous for *14:03

<table>
<thead>
<tr>
<th>Classification</th>
<th>ACPA-negative RA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>368</td>
<td>1508</td>
</tr>
<tr>
<td>Female (%)</td>
<td>79.7</td>
<td>52.9</td>
</tr>
<tr>
<td>Age (mean±SD)</td>
<td>54.7±16.1</td>
<td>46.5±15.3</td>
</tr>
<tr>
<td>Set 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>501</td>
<td>500</td>
</tr>
<tr>
<td>Female (%)</td>
<td>80.8</td>
<td>80.0</td>
</tr>
<tr>
<td>Age (mean±SD)</td>
<td>62.4±12.2</td>
<td>NA</td>
</tr>
</tbody>
</table>

ACPA, anticitrullinated peptide/protein antibody; NA, not available; RA, rheumatoid arthritis.
in the cases or controls. Both DRB1*13:02 and *15:02 showed potential dosage effects.

### Diplootype analysis

When we analysed the effects of HLA-DRB1 allele diplotypes on the predisposition to ACPA-negative RA, we found that a combination of DRB1*09:01 and *12:01 demonstrated susceptible effects in both sets (p=0.025, 0.020 and 0.00013 in the first, second and combined study, respectively; table 3). DRB1*08:03 homozygosity showed a weak susceptible association without any dosage effects (table 3, supplementary table 1). Although we found no susceptibility effect of DRB1*08:02 homozygosity, the combination of DRB1*08:02 and *08:03 also resulted in weak susceptibility (supplementary table 2). When we analysed DR8 allele homozygosity, we found that it displayed a moderate susceptibility association in the combined analysis (p=0.0070, table 3). Any combination of two of the three susceptibility alleles—namely, HLA-DRB1*12:01, *14:03, and *04:05, showed a potentially susceptible effect (supplementary table 2).

The HLA-DRB1*08:03 and *15:02 diplootype showed the strongest protective effect (p=0.00011, table 3). We found that the diplotypes with protective effects (*08:03/*15:02, *15:02/*15:02 and *15:02/*15:02) all included HLA-DRB1*15:02 (table 3).

### DISCUSSION

Recent studies have suggested that ACPA-negative RA is a genetically different subset of RA.7 8 While SE is very strongly associated with ACPA-positive RA, it is reported as not associated or only weakly associated with ACPA-negative RA. In populations of European descent, HLA-DR3 and DR13 were reported to be susceptibility alleles,12 13 but a recent meta-analysis of a large Caucasian cohort did not find any such association.14 In Japanese subjects, only DRB1*09:01 was reported to be associated with ACPA-negative RA, using small numbers of patients and controls (28 and 265, respectively).15 16 HLA-DR3 is rare in the Japanese population, and we found only one HLA-DR3 allele in our cohorts.

Although genetic factors contribute to the development of ACPA-negative RA as much as ACPA-positive RA,19 little is known about the ACPA-negative RA susceptibility alleles of HLA and non-HLA genes.

Here, we performed a case–control association study using a large number of ACPA-negative patients with RA and controls and showed that multiple alleles and diplotypes are associated
Extended report


with ACPA-negative RA in Japanese people. Although the
controls in the first set had different age and sex ratios from
those of the patients and we could not obtain age data for the
500 controls in the second set, the effects of the above-men-
tioned difference and lack of data on our results were considered
to be limited. The HLA locus is located on chromosome 6 and
is not affected by sex or age. Indeed, regression analysis did not
significantly alter our association results (data not shown).

Our study showed that HLA-DRB1*12:01 is strongly associ-
ated with ACPA-negative RA and that HLA-DRB1*14:03 and
HLA-DRB1*04:05 in SE are moderately associated with ACPA-
negative RA in Japanese people. All three susceptibility alleles
showed susceptibility associations with ACPA-negative RA
when found in combination with one of the other two alleles.

Our data also suggested a dosage effect of HLA-DRB1*12:01,
while no dosage effect of HLA-DRB1*04:05 was detected, with
decreased OR of DRB1*04:05 in homozygotes compared with
heterozygous patients. In addition, we showed that the HLA-
DRB1*09:01 and HLA-DRB1*12:01 diplotype and HLA-DR8
homozygosity are strong susceptibility combinations for ACPA-
negative RA. We also determined HLA-DRB1*13:02 and *15:02
as protective alleles against ACPA-negative RA with a potential
dosage effect. The combination of DRB1*08:03 and *15:02 had
a strong protective effect in our study. Using logistic regres-
sion analysis, we confirmed that the effects of these suscepti-

Table 3  Associations between HLA-DRB1 allele diplotypes and ACPA-negative RA

<table>
<thead>
<tr>
<th>Effect</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Set 1 N (%), OR (95% CI)</th>
<th>p Value</th>
<th>Set 2 N (%), OR (95% CI)</th>
<th>p Value</th>
<th>Pooled study N (%), OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-SE</td>
<td>*09:01</td>
<td>*12:01</td>
<td>7 (1.9), 0.025, 2.90 (1.1 to 7.68)</td>
<td>0.025</td>
<td>13 (2.6), 0.020, 4.41 (1.25 to 15.58)</td>
<td>0.0013</td>
<td>20 (2.3), 0.00013, 3.62 (1.79 to 7.30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*08:03</td>
<td>*12:01</td>
<td>5 (1.4), 0.054, 2.95 (0.93 to 9.36)</td>
<td>0.36</td>
<td>7 (1.4), 0.026, 2.51 (0.48 to 13.00)</td>
<td>0.033</td>
<td>12 (1.4), 0.021, 2.54 (1.12 to 5.78)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*04:05</td>
<td>*14:05</td>
<td>5 (1.4), 0.054, 2.95 (0.93 to 9.36)</td>
<td>0.026</td>
<td>5 (1.0), 0.026, 2.51 (0.48 to 13.00)</td>
<td>0.033</td>
<td>12 (1.4), 0.021, 2.54 (1.12 to 5.78)</td>
<td></td>
</tr>
<tr>
<td>Protective</td>
<td>*08:03</td>
<td>*15:02</td>
<td>3 (0.8), 0.0095, 0.95 (0.00 to 1.13)</td>
<td>0.00047</td>
<td>10 (2.0), 0.00048, 0.95 (0.00 to 1.13)</td>
<td>0.00047</td>
<td>20 (4.0), 0.00048, 0.95 (0.00 to 1.13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*15:02</td>
<td>*12:01</td>
<td>2 (0.5), 0.36, 0.51 (0.12 to 2.23)</td>
<td>0.011</td>
<td>10 (2.0), 0.011, 0.51 (0.12 to 2.23)</td>
<td>0.011</td>
<td>20 (4.0), 0.011, 0.51 (0.12 to 2.23)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*13:02</td>
<td>*12:01</td>
<td>3 (0.8), 0.16, 0.43 (0.13 to 1.44)</td>
<td>0.036</td>
<td>3 (0.6), 0.16, 0.43 (0.13 to 1.44)</td>
<td>0.036</td>
<td>6 (1.2), 0.036, 0.43 (0.13 to 1.44)</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>SE</td>
<td>SE</td>
<td>26 (7.1), 0.35, 1.24 (0.79 to 1.95)</td>
<td>0.68</td>
<td>27 (5.4), 0.35, 1.24 (0.79 to 1.95)</td>
<td>0.68</td>
<td>53 (11.6), 0.35, 1.24 (0.79 to 1.95)</td>
<td></td>
</tr>
<tr>
<td>Serotype</td>
<td>DR8</td>
<td>DR15</td>
<td>8 (2.2), 0.027, 0.44 (0.21 to 0.95)</td>
<td>0.021</td>
<td>10 (2.0), 0.021, 0.44 (0.21 to 0.95)</td>
<td>0.021</td>
<td>18 (2.1), 0.021, 0.44 (0.21 to 0.95)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DR13</td>
<td>DR15</td>
<td>6 (1.6), 0.051, 0.44 (0.19 to 1.02)</td>
<td>0.22</td>
<td>6 (1.2), 0.051, 0.44 (0.19 to 1.02)</td>
<td>0.22</td>
<td>12 (1.4), 0.051, 0.44 (0.19 to 1.02)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DR8</td>
<td>DR8</td>
<td>13 (3.5), 0.16, 0.43 (0.13 to 1.44)</td>
<td>0.036</td>
<td>10 (2.0), 0.16, 0.43 (0.13 to 1.44)</td>
<td>0.036</td>
<td>23 (2.7), 0.16, 0.43 (0.13 to 1.44)</td>
<td></td>
</tr>
</tbody>
</table>

*Number of alleles (allele frequency).

ACPA, anticitrullinated peptide/protein antibody; SE, shared epitope; RA, rheumatoid arthritis.

Figure 1  Suggestive dosage effect of associated alleles on anticitrullinated peptide/protein antibody (ACPA)-negative rheumatoid arthritis susceptibility. The OR for each diplotype is shown. Different colours indicate the number of copies of each allele. The numbers of homozygotes of *12:01, *14:05, *15:02 and *13:02 in cases are limited (3, 2, 2 and 3, respectively). Since no patients in this study were homozygous for DRB1*14:05, only the result for *15:05 is shown in this figure. SE in the figure includes DRB1*04:05, which is shown separately.
is 1–4%, and so far there are no reports showing an association with ACPA-positive RA. Although HLA-DRB1*12:01 showed a potential dosage effect, further confirmation is necessary as only two homozygous patients were among the cases. The allele frequency of HLA-DRB1*12:01 in a European population is 1–4%, and so far there are no reports showing an association with ACPA-negative RA. As a potential common risk allele for both T1D and ACPA-negative RA, HLA-DRB1*12:01 showed no association with ACPA-negative RA. HLA-DRB1*14:03 was reported to be associated with ACPA-negative RA, while *14:01 and *14:07 did not. In total, DR14 alleles, including *14:06 in SE, showed moderate susceptibility effects on ACPA-negative RA (supplementary table 1).

Although one European study suggested that HLA-DR13 alleles, including DRB1*13:02, were reported to be protective against ACPA-negative RA, its effect on ACPA-negative RA has not been fully examined. We showed that HLA-DR15 has strong protective effect against ACPA-negative RA and a possible dosage effect. HLA-DRB1*15:02 is reported to be associated with Japanese T1D in a protective manner.

Among HLA-DR13 alleles, HLA-DRB1*13:02 was reported to be protective against ACPA-positive RA. Its protective effect was also reported in Japanese patients with RA. Its effect on ACPA-negative RA has not been established. Our study suggested that HLA-DRB1*13:02 has a protective effect against ACPA-negative RA. As the second set in our study did not show any differences in allele frequency between the patients and controls, further validation of our findings is necessary. HLA-DRB1*13:01, a major component of DR13 in populations of European descent, had no effect in our study, where we included DRB1*13:01 in eight alleles in cases and 23 alleles in controls (p=0.59).

HLA-DRB1 has also been reported to be associated with some arthropathic autoimmune diseases, such as juvenile idiopathic arthritis and psoriatic arthritis in European subjects. The associations indicate that these arthropathies share common pathological mechanisms. Interestingly, the combination of DR8 and DR15 had a strong protective effect against ACPA-negative RA. Considering that DR8 did not show susceptibility association as a single allele, it seems to induce ACPA-negative RA susceptibility in a recessive manner. Among the DR8 alleles, DRB1*08:03 appeared to have a strong effect on ACPA-negative RA susceptibility.

Although we did not detect a dosage effect of HLA-DRB1*08:03, it showed a potentially protective effect against ACPA-negative RA in the combined study. Further studies are necessary to confirm the association.
Competing interests  None.

Patient consent  Obtained.

Ethics approval  This study was approved by the local ethical committees at each institution.

Provenance and peer review  Not commissioned; externally peer reviewed.

REFERENCES


A large-scale association study identified multiple HLA-DRB1 alleles associated with ACPA-negative rheumatoid arthritis in Japanese subjects

Chikashi Terao, Koichiro Ohmura, Yuta Kochi, et al.

Ann Rheum Dis 2011 70: 2134-2139 originally published online August 27, 2011
doi: 10.1136/annrheumdis-2011-200353