meta-analysis of a large Caucasian cohort [8]. In Asian populations, we recently reported that DRB1*12:01 is a HLA-DRB1 susceptibility allele for ACPA-negative RA in Japanese populations and that DRB1*04:05, the most common SE allele in Japanese, and *14:03 showed moderate associations with ACPA-negative RA susceptibility [14]. We also reported that DRB1*15:02 and *13:02 displayed protective associations with ACPA-negative RA and that being homozygous for HLA-DR8 was associated with ACPA-negative RA susceptibility. While a very small Japanese study suggested that HLA-DRB1*09:01 is associated with ACPA-negative RA [15], our study did not detect a significant association between them. These findings suggest that ACPA-negative RA is genetically different from ACPA-positive RA in terms of its associations with HLA-DRB1 alleles. While some specific alleles and diplotypes seem to be associated with ACPA-negative RA, the genetic characteristics of ACPA-negative RA have not been fully elucidated. Recently, UK group reported that SE is associated with ACPA-negative RF-positive RA in UK population [16]. However, whether this is true to other population is uncertain. Moreover, the associations of other alleles than SE with subgroups of ACPA-negative RA have never been reported. Here, we show that when we classified ACPA-negative RA into two subsets based on rheumatoid factor (RF) positivity, we were able to clearly distinguish them from each other according to their associations with HLA-DRB1 alleles, not only with SE, but with other alleles. We also compared ACPA-positive RA patients based on their RF positivity to examine whether we can apply this classification to ACPA-positive RA.

Results

HLA-DRB1 Alleles Associated with ACPA-negative RF-positive RA

We compared 179 ACPA-negative RF-positive RA with 1508 controls in collection 1 for their frequency of HLA-DRB1 alleles, followed by comparison of 267 ACPA-negative RF-positive RA with 500 controls in collection 2. Significant association was evaluated in the combined analysis. Regarding HLA-DRB1 alleles that were previously shown to be associated with ACPA-negative RA, we found that all of the alleles, namely, HLA-DRB1*12:01, *04:05, *13:02, *14:03, and *15:02 showed association tendency with ACPA-negative RF-positive RA in the combined study (Table 1). Interestingly, HLA-DRB1*04:05 ($p = 8.8 \times 10^{-6}$, odds ratio (OR): 1.57) showed the strongest association, while its association with entire ACPA-negative RA was moderate in the previous study. When we analyzed the associations of the SE, we found that it displayed a significant association ($p = 0.00013$, OR: 1.37). HLA-DRB1*04:05 was responsible for most of the association of SE because none of the other SE alleles showed significant associations with ACPA-negative RF-positive RA. We also found that HLA-DRB1*09:01, which was not associated with ACPA-negative RA as a single allele, was found to be significantly associated with ACPA-negative RF-positive RA ($p = 0.0011$, OR: 1.37). Importantly, these association tendencies written above were observed in both collections (Table 1). Logistic regression analysis was carried out to examine whether the susceptibility associations were dependent on a lack of protective alleles or vice versa. As a result, it was demonstrated that HLA-DRB1*04:05, *09:01, and *12:01 showed significant associations ($p<0.0005$), while the associations of HLA-DRB1*14:03, *13:02, and *15:02 were moderate to suggestive (Table S1). Next, we analyzed the dosage effects of the alleles and found that the association between HLA-DRB1*09:01 and ACPA-negative RF-positive RA showed a clear dosage effect (Figure S1). HLA-DRB1*12:01 also showed a dosage effect (data not shown due to small number). HLA-DRB1*04:05 did not show a dosage effect, suggesting that the effect of HLA-DRB1*04:05 on the predisposition to ACPA-negative RF-positive RA is a dominant effect.

HLA-DRB1 Alleles Associated with ACPA-negative RF-negative RA

Next we compared 274 ACPA-negative RF-negative RA with 1,508 controls, followed by comparison between 234 ACPA-negative RF-negative RA and 500 controls. Interestingly, we did not observe association of HLA-DRB1*04:05 and *09:01 with ACPA-negative RF-negative RA, while HLA-DRB1*12:01, *13:02, *14:03, and *15:02 were moderately associated with ACPA-negative RF-negative RA (Table 2). The SE was not associated with ACPA-negative RF-negative RA. DR14 was found to be significantly associated with ACPA-negative RF-negative RA and HLA-DRB1*14:03 and *14:06 comprised the association of HLA-DR14 (Table S2). These association tendencies in ACPA-negative RF-negative RA were observed in both sets (Table 2). Logistic regression analysis confirmed that none of the associations were mutually dependent and that the association of DR14 remained significant ($p = 0.00069$, Table S3). DR14 could not be evaluated the dosage effect because neither the cases nor controls included DRB1*14:03 or *14:06 homozygotes or the DRB1*14:03 and *14:06 diplotype.

HLA Diplotype Analysis: DR8 Homozygote and *12:01/*09:01 Diplotype

As we previously showed that the DR8 homozygote was significantly associated with susceptibility to ACPA-negative RA, we analyzed its associations with ACPA-negative RF-positive RA and RF-negative RA. As a result, we found that the HLA-DR8 homozygote is exclusively associated with ACPA-negative RF-negative RA in the combined study ($p = 0.00013$, OR: 3.08 for ACPA-negative RF-negative RA, Table 2; $p = 0.86$, OR: 1.08 for ACPA-negative RF-positive RA, Table 1). The effect of DR8 on the susceptibility to ACPA-negative RF-negative RA was not dose-dependent (OR: 1.04 for HLA-DR8 heterozygote).

We also found that the combination of HLA-DRB1*12:01 and *09:01, the diplotype that was most strongly associated with susceptibility to ACPA-negative RA in the previous study, was especially strongly associated with ACPA-negative RF-positive RA ($p = 5.0 \times 10^{-6}$, OR: 4.97 for ACPA-negative RF-positive RA; $p = 0.040$, OR: 2.46 for ACPA-negative RF-negative RA).

We found that the similar associations were seen between the alleles/diplotypes and ACPA-negative RF-positive erosive RA and ACPA-negative RF-negative erosive RA (except for that between HLA-DRB1*12:01 and the ACPA-negative RF-negative subset), even though the number of patients was limited (Table S4).

Comparison between ACPA-negative RF-positive RA and ACPA-negative RF-negative RA

To compare the usage of HLA-DRB1 allele between ACPA-negative RF-positive RA and ACPA-negative RF-negative RA, we directly compared the allele and diplotype frequencies between the two groups (Table 3). As expected, HLA-DRB1*09:01 and *04:05 showed significant differences in their frequencies between the two subsets ($p = 0.0018$ and 0.0034, respectively). The SE was more common in the ACPA-negative RF-positive RA patients ($p = 0.0047$), whereas DR14 was more prevalent in the ACPA-negative RF-negative RA patients ($p = 0.028$). The DR8 homozygote was more frequently seen in the ACPA-negative RF-negative RA patients than in the ACPA-negative RF-positive RA patients

Table 1. Association of HLA-DRB1 alleles with ACPA-negative RF-positive RA.

	1st set				2nd set				combined analysis			
HLA-DRB1 allele	$ACPA (-)RF(+)RA	$control	p	OR	$ACPA (-)RF(+)RA	$control	p	OR	$ACPA (-)RF(+)RA	$control	p	OR
*04:05	65 (18.2%)	340 (11.3%)	0.00015	1.75 (1.30–2.34)	88 (16.5%)	129 (12.9%)	0.055	1.33 (0.99–1.79)	153 (17.2%)	469 (11.7%)	8.8×10^{-6}	1.57 (1.28–1.91)
*09:01	70 (19.6%)	432 (14.3%)	0.0086	1.45 (1.10–1.92)	99 (18.5%)	154 (15.4%)	0.11	1.25 (0.95–1.65)	169 (18.9%)	586 (14.6%)	0.0011	1.37 (1.13–1.65)
*12:01	13 (3.6%)	91 (3%)	0.53	1.21 (0.67–2.19)	35 (6.6%)	37 (3.7%)	0.012	1.83 (1.14–2.93)	48 (5.4%)	128 (3.2%)	0.0014	1.73 (1.23–2.43)
*13:02	21 (5.9%)	273 (9.1%)	0.043	0.63 (0.40–0.99)	18 (3.4%)	52 (5.2%)	0.10	0.64 (0.37–1.1)	39 (4.4%)	325 (8.1%)	0.00013	0.52 (0.37–0.73)
*14:03	7 (2.0%)	39 (1.3%)	0.31	1.52 (0.68–3.43)	13 (2.4%)	14 (1.4%)	0.14	1.76 (0.82–3.77)	20 (2.2%)	53 (1.3%)	0.040	1.71 (1.02–2.88)
*15:02	43 (12.0%)	369 (12.2%)	0.90	0.98 (0.70–1.37)	37 (6.9%)	113 (11.3%)	0.0060	0.58 (0.4–0.86)	80 (9.0%)	482 (12.0%)	0.010	0.72 (0.56–0.93)
SE	106 (29.6%)	677 (22.4%)	0.0024	1.45 (1.14–1.85)	150 (28.1%)	233 (23.3%)	0.039	1.29 (1.01–1.63)	256 (28.7%)	910 (22.7%)	0.00013	1.37 (1.17–1.62)
DR14	29 (8.1%)	253 (8.4%)	0.85	0.96 (0.64–1.44)	48 (9.0%)	73 (7.3%)	0.24	1.25 (0.86–1.83)	78 (8.7%)	326 (8.1%)	0.55	1.08 (0.83–1.40)
Diplotype												
DR8/DR8	3 (1.7%)	17 (1.1%)	0.46	1.49 (0.28–5.24)	3 (1.1%)	8 (1.6%)	0.76	0.70 (0.12–2.94)	6 (1.3%)	25 (1.2%)	0.86	1.08 (0.44–2.65)
*12:01/*09:01	5 (2.8%)	10 (0.66%)	0.0041	4.30 (1.45–12.74)	9 (3.3%)	3 (0.60%)	0.0051	5.76 (1.42–33.42)	14 (3.1%)	13 (0.6%)	5.0×10^{-6}	4.97 (2.32–10.66)

OR: odds ratio.

SE: shared epitope: HLA-DRB1*01:01, *01:02, *04:01, *04:04, *04:05, *04:08, *04:10, *04:13, *04:16, *10:01, *14:02, and *14:06.

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(p = 0.021). When we applied logistic regression analysis to the HLA-DRB1*09:01, *04:05, and HLA-DR14, their associations were revealed to be significant and do not depend on each other (p = 0.00067 and 0.00072, respectively, Table S5), except for that of DR14 (p = 0.30).

Comparison between ACPA-positive RF-positive RA and ACPA-positive RF-negative RA

Next, we analyzed whether these allele usage differences are also seen in ACPA-positive RA. We collected data about the HLA-DRB1 genotypes of 154 ACPA-positive RF-negative RA patients and 531 ACPA-positive RF-positive RA patients. As the SE and HLA-DRB1*09:01 were found to be associated with ACPA-positive RA, we analyzed the differences in the frequencies of these alleles [17]. In comparison with the healthy controls, SE and HLA-DRB1*09:01 were associated with a predisposition to ACPA-positive RF-positive RA as well as ACPA-positive RF-negative RA and displayed comparable odds ratios in logistic regression analysis (Table 4). No HLA-DRB1 alleles showed a strong specific association with a particular subset. When we directly compared the two subsets of ACPA-positive RA, no alleles displayed significant associations (Figure 1, Table S6). However, whether the two subsets of ACPA-positive RA share most of HLA-DRB1 susceptibility associations is inconclusive due to the small number of RF-negative subset.

Discussion

In this study, we demonstrated that classifying Japanese ACPA-negative RA patients based on their RF positivity successfully divided them into two genetically different subsets, which displayed different associations with HLA-DRB1. We showed that HLA-DRB1*09:01 and *04:05, strong susceptibility alleles to ACPA-positive RA, were also associated with ACPA-negative RF-positive subset, and that DR14 and the DR8 homozygote were associated only with the ACPA-negative RF-negative subset (Figure 1). Since the titer of RF fluctuates along with disease activity much more than that of ACPA, we were very careful to take the maximum RF titer when multiple titers were available for a particular patient, in order to prevent the RF positive subset from being contaminated with RF negative RA patients. The Recent UK population study reported the association of SE with ACPA-negative RF-positive RA [16]. Our study not only confirmed this association in Japanese RA, but also showed that the association of SE with ACPA-negative RF-positive RA is mainly due to the effect of HLA-DRB1*04:05 and that HLA-DRB1*09:01, HLA-DR14, and homozygote of HLA-DR8 are specifically associated with subsets of ACPA-negative RA.

These above-mentioned association tendencies were observed in the first set and successfully replicated in the second set, indicating that we can avoid population stratification or sampling bias. The effect sizes (odds ratio) of the alleles were comparable in each cohort (Tables 1 and 2) and the associations in the combined analysis reached significant level, although the p-values in each set did not reach the significance level due to the limited number of samples they contained. These data indicate that our results are reliable, at least in Japanese populations, although further replication studies including other populations are favorable. In the current study, we used logistic regression analysis to confirm independency of associated alleles in each comparison. When we used relative predispositional effects (RPE) method [18] to stratify associated alleles, we obtained the similar results to those we obtained by logistic regression analysis (data not shown).

Table 2. Association of HLA-DRB1 alleles with ACPA-negative RF-negative RA.

	1st set				2nd set				combined analysis			
HLA-DRB1 allele	§ACPA(-)IRF (-)RA	§control	*p*	OR	§ACPA(-)IRF (-)RA	§control	*p*	OR	§ACPA(-)IRF (-)RA	§control	*P*	OR
*04:05	69 (12.6%)	340 (11.3%)	0.37	1.13 (0.86–1.49)	57 (12.2%)	129 (12.9%)	0.70	0.94 (0.67–1.31)	126 (12.4%)	469 (11.7%)	0.52	1.07 (0.87–1.32)
*09:01	74 (13.5%)	432 (14.3%)	0.61	0.93 (0.72–1.22)	65 (13.9%)	154 (15.4%)	0.45	0.89 (0.65–1.21)	139 (13.7%)	586 (14.6%)	0.46	0.93 (0.76–1.13)
*12:01	28 (5.1%)	91 (3.0%)	0.012	1.73 (1.12–2.67)	27 (5.8%)	37 (3.7%)	0.070	1.59 (0.96–2.65)	55 (5.4%)	128 (3.2%)	0.00071	1.74 (1.26–2.40)
*13:02	28 (5.1%)	273 (9.1%)	0.0023	0.54 (0.36–0.81)	34 (7.3%)	52 (5.2%)	0.070	1.59 (0.96–2.65)	62 (6.1%)	325 (8.1%)	0.033	0.74 (0.56–0.98)
*14:03	12 (2.2%)	39 (1.3%)	0.10	1.71 (0.89–3.29)	10 (2.1%)	14 (1.4%)	0.30	1.54 (0.68–3.49)	22 (2.2%)	53 (1.3%)	0.047	1.65 (1.00–2.73)
*15:02	51 (9.3%)	369 (12.2%)	0.051	0.74 (0.54–1.00)	36 (7.7%)	113 (11.3%)	0.033	0.65 (0.44–0.97)	87 (8.6%)	482 (12.0%)	0.0020	0.69 (0.54–0.87)
SE	131 (23.9%)	677 (22.4%)	0.45	1.09 (0.88–1.34)	103 (22%)	233 (23.3%)	0.58	0.93 (0.71–1.21)	234 (23.0%)	910 (22.7%)	0.80	1.02 (0.87–1.2)
DR14	69 (12.6%)	253 (8.4%)	0.0016	1.57 (1.19–2.09)	51 (10.9%)	73 (7.3%)	0.021	1.55 (1.07–2.26)	120 (11.8%)	326 (8.1%)	0.00022	1.52 (1.21–1.89)
Diplotype												
DR8/DR8	12 (4.4%)	17 (1.1%)	9.1×10^{-5}	4.02 (1.90–8.51)	7 (3.0%)	8 (1.6%)	0.21	1.90 (0.68–5.29)	19 (3.7%)	25 (1.2%)	0.00013	3.08 (1.68–5.64)
*12:01/*09:01	4 (1.5%)	10 (0.66%)	0.25	2.22 (0.50–7.76)	4 (1.7%)	3 (0.60%)	0.22	2.88 (0.48–19.80)	8 (1.6%)	13 (0.6%)	0.040	2.46 (1.01–5.96)

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In our previous study [14], HLA-DRB1*09:01 was not significantly associated with ACPA-negative RA, in spite of the association it displayed in combination with HLA-DRB1*12:01. In the current study, we showed that HLA-DRB1*09:01 displayed a strong dose-dependent association with ACPA-negative RF-positive RA, but not with ACPA-negative RF-negative RA. These findings were confirmed by a direct comparison between the two subsets. A small study in Japan suggested that HLA-DRB1*09:01 is associated with ACPA-negative RA [15]. Our results suggest that their study mainly included ACPA-negative RF-positive RA patients. HLA-DRB1*09:01 was shown to reduce the ACPA titer in Japanese ACPA-positive RA patients [19–20]. Therefore, HLA-DRB1*09:01 might increase the titer of RF and decrease that of ACPA, although our study also showed that HLA-DRB1*09:01 is associated with ACPA-positive RF-negative RA.

HLA-DRB1*04:05, which is a major component of the SE in Asians [17], was shown to be significantly associated with ACPA-negative RA in our previous study. The current study showed that it is only associated with ACPA-negative RF-positive RA. This predisposition was also confirmed by direct comparison of the two subsets. As we could not detect a dosage effect of HLA-DRB1*04:05, its susceptibility effect might occur in a dominant manner. It is interesting that of the many SE alleles only HLA-DRB1*04:05 is associated with ACPA-negative RF-positive RA. This does not seem to be due to the relatively low frequencies of the other SE alleles (Table 1). Therefore, the common amino acid sequence that extends from the 70th to the 74th amino acid of the HLA-DRβ chain might not be important for the development of ACPA-negative RF-positive RA. As immunization of citrullinated peptide induced arthritis in HLA-DR4 transgenic mice [21] and citrullinated peptides were shown to have higher affinity to HLA-DR4 [22], high affinity of SE to citrullinated antigen is hypothesized to be the link between SE and RA development. Our findings may raise possibility of another mechanism of SE in developing arthritis.

It is quite interesting that HLA-DRB1*04:05 and *09:01, strongly associated alleles with ACPA-positive RA, are associated with ACPA-negative RF-positive RA. Although there are genetic similarities between ACPA-negative RF-positive RA and ACPA-positive RA, they should be considered to be different subsets as SE alleles other than HLA-DRB1*04:05 are not associated with ACPA-negative RF-positive RA and the HLA-DRB1*09:01 and *12:01 diplotype is strongly associated with ACPA-negative RF-positive RA.

When we analyzed the HLA-DR14 serotype, it showed a strong association with ACPA-negative RF-negative RA, largely due to HLA-DRB1*14:03 and *14:06. When we compared the frequency of DR14 in each ACPA-negative subset after stratifying the data according to the presence of HLA-DRB1*09:01 and *04:05, DR14 did not display a significant effect. In this sense, the specific association of DR14 with ACPA-negative RF-negative RA needs to be confirmed.

The HLA-DR8 homozygote displayed an association with ACPA-negative RA in our previous study [14]. The current study demonstrated that its association is specific to ACPA-negative RF-negative RA. As the number of HLA-DR8 homozygote is limited, further replication is necessary for this association. No association between the HLA-DR8 and 14 diplotype and susceptibility to ACPA-negative RF-negative RA was found (data not shown).

It is interesting that HLA-DR14 and HLA-DR8, associated serotype with ACPA-negative RF-negative RA, were reported association with psoriatic arthritis [23]. HLA-DR14 is often linked with HLA-Cw*06, susceptibility serotype to psoriasis arthritis in European [24]. HLA-Cw*06 is rare in Japanese (<1%) and the

Table 3. Direct comparison of HLA-DRB1 allele frequency between ACPA-negative RF-positive RA and ACPA-negative RF-negative RA.

HLA-DRB1	ACPA(−)RF(+)RA Number of allele (%)	ACPA(−)RF(−)RA Number of allele (%)	*p*	OR (95%CI)
*09:01	169 (18.9%)	139 (13.7%)	0.0018	1.47 (1.15–1.88)
*04:05	153 (17.2%)	126 (12.4%)	0.0034	1.46 (1.13–1.89)
*08:02	24 (2.7%)	52 (5.1%)	0.0068	0.51 (0.31–0.84)
*14:06	8 (0.9%)	21 (2.1%)	0.037	0.43 (0.19–0.97)
SE	256 (28.7%)	234 (23.0%)	0.0047	1.35 (1.09–1.65)
DR14	78 (8.7%)	120 (11.8%)	0.028	0.72 (0.53–0.97)
DR8/DR8	6 (1.3%)	19 (3.7%)	0.021	0.35 (0.14–0.89)

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strong association between HLA-Cw*06 and HLA-DR14 is not observed in Japan (<10%). While psoriatic arthritis is not reported to be associated with these serotypes in Japan, association between these serotypes and arthritis is interesting.

It could be argued that ACPA-negative RA includes some non-RA arthritic diseases such as psoriasis, seronegative spondyloarthropathy and other collagen vascular diseases. Thus, we analyzed the associations between the above-mentioned alleles and diplotypes with ACPA-negative RA displaying bone erosion to examine whether the same association patterns were present in this strictly defined cohort. The typical bone erosions of RA are rarely seen in other arthritic disorders. As a result, we found the same associations. Therefore, we are convinced that our findings were not caused by the contamination of our study population by patients with other diseases. Since RF sometimes normalizes after treatment, the RF-negative RA patients whose RF titers were not measured at multiple points might not have been RF-negative. So, we re-analyzed our data by excluding the RA patients for whom consecutive RF titers were not available. As a result, we found the same tendency of associations for each allele and diplotype in each subset (data not shown), indicating that these subsets are stable.

Analysis using ACPA-positive RF-positive RA and ACPA-positive RF-negative RA patients compared with healthy controls did not result in distinct differences in HLA-DRB1 association. The SE is associated with both ACPA-positive RF-positive and RF-negative RA. HLA-DRB1*09:01 was found to be associated with both subsets after stratifying the patients according to their SE alleles. We also did not detect an association between HLA-DR14 or the HLA-DR8 homozygote and either subset. While 154 ACPA-positive RF-negative RA patients in our study are too small in number to detect the difference in HLA-DRB1 alleles with weak effect size between the two ACPA-positive subsets, these results suggest that there are no big differences in the HLA usage of the two subsets in ACPA-positive RA. To confirm our results and to detect possible different frequency of other HLA-DRB1 alleles in the two ACPA-positive subsets, replication study is necessary.

Table 4. Logistic regression analysis of HLA-DRB1 alleles with ACPA-positive RF-positive RA and ACPA-positive RF-negative RA.

	ACPA(+)RF(+)RA		ACPA(+)RF(−)RA	
HLA-DRB1	*p**	OR (95%CI)*	*p**	OR (95%CI)*
SE	$<2\times10^{-16}$	3.21 (2.72–3.78)	$<2\times10^{-16}$	3.03 (2.33–3.94)
*09:01	2.4×10^{-9}	1.83 (1.5–2.25)	0.0035	1.67 (1.17–2.37)

**p*-values and odds ratios in logistic regression analysis using SE and HLA-DRB1*09:01.

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In the current study, we performed multiple comparisons in each subset and between subsets. The associations should be evaluated in the combined analysis with significant level corrected by Bonferronii's method and independency of each association should be evaluated by logistic regression analysis or RPE method. In this sense, p-values around cut-off level in the combined analysis should be taken with caution and the associations should be confirmed by independent study.

We have shown that ACPA-negative RA includes two genetically distinct subsets in Japanese population: RF-positive and RF-negative RA. This is the first report in Asians to show that these subsets are genetically distinct. We have to clarify the clinical difference between these two subsets. We also have to clarify whether non-HLA genes display different associations with each subset. So far, many genome wide association studies (GWAS) of RA and ACPA-positive RA have been performed, and more than twenty genes or loci have been shown to be susceptibility loci [25–38]. However, no GWAS studies have detected susceptibility genes for ACPA-negative RA with genome-wide significance [39]. This is probably due to the relatively small number of patients studied, but it might be overcome by stratifying ACPA-negative RA patients into RF-positive and RF-negative subsets. Since RA susceptibility genes usually cross ethnic boundaries [40], global collaboration might result in a fruitful dissection of these minor subsets.

Materials and Methods

Ethics Statement

This study was approved by the local ethical committees at each institution, namely, Kyoto University Graduate School and Faculty of Medicine, Ethics Committee, Tokyo Women's Medical University Genome Ethics Committee, and the ethics committee of RIKEN, and written informed consent was obtained from all patients.

Study Subjects

DNA samples were collected from ACPA-negative RA patients at Kyoto University Hospital, Tokyo Women's Medical University [41], and RIKEN with the support of BioBank Japan. All patients were Japanese and had been diagnosed by rheumatologists

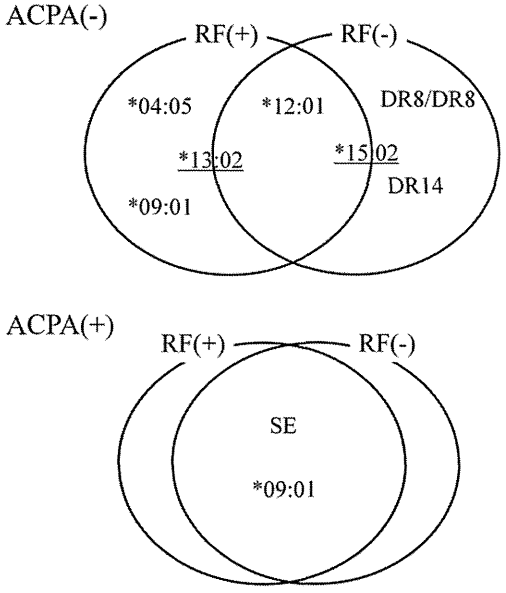

Figure 1. Summary of the HLA-DRB1 alleles associated with ACPA-negative RA and ACPA-positive RA. The relationships between the RF-positive and RF-negative subsets of ACPA-negative and ACPA-positive RA in terms of their associations with HLA-DRB1 alleles are illustrated. While the two subsets of ACPA-positive RA seem to share most associations with HLA-DRB1, the two ACPA-negative RA subsets possess specific alleles and HLA-DRB1 diplotypes. The underlined alleles are protective alleles.
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according to the 1987 American College of Rheumatology revised criteria for RA [42]. The control DNA samples were collected at Aichi Cancer Center Hospital, the DNA banks of the Pharma SNP Consortium [43], and HLA laboratory. A more detailed description of the collection procedure was given in a previous study [14]. We performed association studies using similar study design of the two collections to our previous study; namely, collection 1 for 456 ACPA-negative RA and 1508 healthy subjects, and collection 2 for 501 ACPA-negative RA and 500 healthy people. RF data were available for 453 out of 456 cases in collection 1 and all of 501 cases in collection 2. 179 patients were RF-positive in collection 1 and 267 patients were RF-positive in collection 2. We also collected DNA samples from 531 ACPA-positive RF-positive RA patients at Kyoto University Hospital and 154 ACPA-positive RF-negative RA patients at Kyoto University and Tokyo Women's Medical University.

ACPA Detection

The MESACUP CCP ELISA kit (Medical and Biological Laboratories Co., Ltd, Nagoya, Japan) was used to detect 2nd generation ACPA in each RA patient, according to the manufacturer's instructions. A cut-off value of 4.5 U/ml was used to define ACPA positivity.

RF Detection

The serum RF concentrations of samples in collection 1 were quantified using a latex agglutination turbidimetric immunoassay. An ELISA assay was used to determine the RF levels of samples in collection 2. When multiple values for RF had been obtained at different visits, we used the maximum RF value for each patient. The cut off values of each detection kit in each hospital were employed.

HLA-DRB1 Genotyping

The HLA-DRB1 typing methods were previously described [14]. Briefly, the WAKFlow system or the AlleleSEQR HLA-DRB1 typing kit (Abbott, Tokyo, Japan) was used for the HLA-DRB1 typing. The following HLA-DRB1 alleles were classified as belonging to the SE: DRB1*01:01, *01:02, *04:01, *04:04, *04:05, *04:08, *04:10, *04:13, *04:16, *10:01, *14:02, and *14:06.

Statistical Analysis

The frequency of each allele or diplotype was compared among the ACPA-negative RF-positive RA, ACPA-negative RF-negative RA patients, and the healthy controls in each set and combined set using the chi-square test or Fisher's exact test. The same analyses were performed in ACPA-positive RA patients classified according to their RF possession. Ninety-five percent confidence intervals (CI) for the OR were also calculated. Logistic regression analysis was used to evaluate the effects of each allele by adjusting for the influence of strongly-associated alleles. Single alleles were regarded as significant when they showed p-values of less than 0.0026 in a combined study, which is obtained by Bonferroni's correction. For diplotype analyses, we regarded 0.025 as the cut off level for significance because we performed just two tests. All statistical analyses were performed using the R statistic system (http://www.R-project.org) or SPSS (version 18).

Supporting Information

Figure S1 Dosage effects of HLA-DRB1*04:05 and *09:01 alleles on ACPA-negative RF-positive RA susceptibility. Each column represents the odds ratio for developing ACPA-negative RF-positive RA associated with possessing one (red column) or two (green column) alleles of HLA-DRB1*04:05 or *09:01.
(TIF)

Table S1 Logistic regression analysis of associated alleles with ACPA-negative RF-positive RA. *p-values and odds ratios in logistic regression analysis using the six alleles listed above.
(DOC)

Table S2 Association between HLA-DR14 and ACPA-negative RF-negative RA.
(DOC)

Table S3 Logistic regression analysis of associated alleles with ACPA-negative RF-negative RA. *p-values and odds ratios in logistic regression analysis using HLA-DR14 and three HLA-DRB1 alleles listed above.
(DOC)

Table S4 Association of HLA-DRB1 with ACPA-negative RA erosive subsets. [a)]Total allele number is 268. [b)]Total allele number is 212.
(DOC)

Table S5 Logistic regression analysis of assoicated alleles with ACPA-negative RF-positive RA, compared with ACPA-negative RF-negative RA. *p-values and odds ratios in logistic regression analysis using HLA-DRB1*09:01, *04:05, and HLA-DR14. [a)]HLA-DRB1 alleles which showed $p<0.05$ in Table 3 were used for analysis.
(DOC)

Table S6 Comparison between ACPA-positive RF-positive RA and ACPA-positive RF-negative RA. [a)] Alleles with frequency more than 1% in any groups are shown.
(DOC)

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Author Contributions

Conceived and designed the experiments: CT KO KI YK RY FM TM. Performed the experiments: CT KI YK EM K. Yurugi MK AS HS. Analyzed the data: CT. Contributed reagents/materials/analysis tools: KI EM KS AM SH K. Takasugi KM K. Tajima SM HY K. Yamamoto HS TM. Wrote the paper: CT KO.

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Quantitative effect of HLA-DRB1 alleles to ACPA levels in Japanese rheumatoid arthritis: no strong genetic impact of shared epitope to ACPA levels after stratification of HLA-DRB1*09:01

Anti-citrullinated peptide antibody (ACPA) is a highly specific serological marker for rheumatoid arthritis (RA).[1–3] Different HLA-DRB1 alleles have been shown to be associated with the susceptibility to ACPA-positive RA.[4 5] Former studies demonstrated that HLA-DRB alleles carrying a shared epitope (SE),[6] consisting of a conserved amino acid motif at positions 70–74 of the HLA-DRβ chain, were strongly associated with ACPA-positive RA and with higher ACPA levels in European and Japanese populations.[7–9] On the other hand, HLA-DRB1*09:01 was recently found to be negatively associated with ACPA levels in the Japanese.[9] These observations imply that combinations of HLA-DRB1 alleles differentially influence ACPA levels in ACPA-positive RA.

To address this question, we conducted a genetic association study employing 2457 ACPA-positive Japanese RA patients. ACPA was quantified by MESACUP CCP ELISA kit (MBL Co Ltd, Nagoya, Japan) with a cut-off level of 4.5 U/ml. The patients were then divided into three groups based on their ACPA titres: low (~4.5–13.5 U/ml), intermediate (~13.5–100 U/ml) and high (≥100 U/ml) groups. These groups were defined according to the 2010 ACR/EULAR classification criteria for RA and a measurement limit of the kit. HLA-DRB1 genotyping was carried out using either the Wakflow system (Wakunaga Pharmaceutical Co Ltd, Osaka, Japan) or the sequencing-based AlleleSEQR HLA-DRB1 typing kit (Abbott Japan, Nagoya, Japan). Frequencies of HLA-DRB1 alleles were compared among the three groups using the Cochran-Armitage Trend test. The relative predispositional effect (RPE) method was applied to identify the associations of more than one HLA-DBR1 allele sequentially according to their strength.[10] Briefly, associations of HLA-DRB1 alleles with ACPA categories were estimated for each allele using the Cochran-Armitage Trend test. When we detected the strongest association with a significant p value, the allele was excluded from the whole data and the same steps were repeated until no further significant alleles were found.

As expected from the previous studies,[9] HLA-DRB1*09:01 showed the strongest association with ACPA levels in a decreasing manner ($p=1.0\times10^{-21}$) and the SE alleles were significantly associated with an increasing effect ($p=3.2\times10^{-7}$) (table 1). In addition, HLA-DRB1*04:07 showed negative association with ACPA levels (p=0.0013), and HLA-DRB1*15:01 and HLA-DRB1*15:02 were positively associated with ACPA levels ($p=2.3\times10^{-5}$ and 0.0011, respectively) (table 1). Of note, the association between the SE and ACPA levels lost significance after stratification of HLA-DRB1*09:01 using RPE (p=0.16) whereas HLA-DRB1*04:07 and HLA-DRB1*15:01 remained significant after RPE (p=0.00034 and p=0.0011, respectively) (table 1). To confirm the dominant effect of HLA-DRB1*09:01

Table 1 Association of HLA-DRB1 alleles with ACPA levels

HLA-DRB1	Low n=594	Intermediate n=1510	High n=2810	p Value	RPE p Value	RPE (OR)	Effect on ACPA levels
SE							
SEall	216 (36.4%)	616 (40.8%)	1303 (46.4%)	3.2×10^{-7}	0.16†	1.08 (0.98–1.20)†	
DRB1*01:01	32 (5.4%)	96 (6.4%)	223 (7.9%)	0.0096			
DRB1*04:01	18 (3.0%)	47 (3.1%)	82 (2.9%)	0.78			
DRB1*04:04	2 (0.3%)	1 (0.1%)	14 (0.5%)	0.13			
DRB1*04:05	138 (23.2%)	409 (27.1%)	840 (29.9%)	0.00053			
DRB1*04:10	17 (2.9%)	33 (2.2%)	67 (2.4%)	0.71			
DRB1*10:01	6 (1.0%)	13 (0.9%)	28 (1.0%)	0.87			
DRB1*14:06	3 (0.5%)	14 (0.9%)	44 (1.6%)	0.013			
Non-SE							
DRB1*04:03	12 (2.0%)	30 (2.0%)	31 (1.1%)	0.019			
DRB1*04:06	17 (2.9%)	14 (0.9%)	57 (2.0%)	0.96			
DRB1*04:07	5 (0.8%)	11 (0.7%)	4 (0.1%)	0.0013	0.00034	0.30 (0.16–0.57)	(−)
DRB1*08:02	15 (2.5%)	30 (2.0%)	60 (2.1%)	0.74			
DRB1*08:03	36 (6.1%)	66 (4.4%)	119 (4.2%)	0.10			
DRB1*09:01	158 (26.6%)	334 (22.1%)	367 (13.1%)	1.0×10^{-21}	1.0×10^{-21}	0.56 (0.50–0.62)	(−)
DRB1*11:01	8 (1.3%)	27 (1.8%)	50 (1.8%)	0.57			
DRB1*12:01	14 (2.4%)	30 (2.0%)	68 (2.4%)	0.63			
DRB1*12:02	8 (1.3%)	26 (1.7%)	50 (1.8%)	0.52			
DRB1*13:02	22 (3.7%)	53 (3.5%)	102 (3.6%)	0.98			
DRB1*14:01	4 (0.7%)	32 (2.1%)	32 (1.1%)	0.64			
DRB1*14:03	6 (1.0%)	17 (1.1%)	37 (1.3%)	0.46			
DRB1*14:05	5 (0.8%)	19 (1.3%)	21 (0.7%)	0.36			
DRB1*15:01	20 (3.4%)	53 (3.5%)	180 (6.4%)	2.3×10^{-5}	0.0011	1.53 (1.21–1.92)	(+)
DRB1*15:02	36 (6.1%)	120 (7.9%)	276 (9.8%)	0.0011			
DRB1*16:02	4 (0.7%)	20 (1.3%)	29 (1.0%)	0.83			

HLA-DRB1 alleles with frequencies greater than 0.5% are shown. Significant levels were set as 0.0022 for HLA-DRB1 alleles after Bonferroni's correction for multiple testing.
†p Value and OR after removal of HLA-DRB1*09:01.
ACPA, anti-citrullinated peptide antibody; RPE, relative predispositional effect; SE, shared epitope.

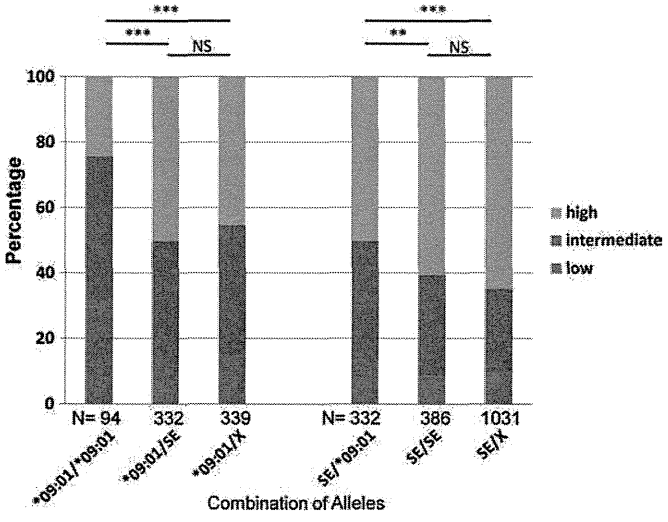

Figure 1 Comparisons of blood anti-citrullinated peptide antibody (ACPA) levels among HLA-DRB1*09:01, shared epitope (SE) and other alleles in combination. Frequencies of three rheumatoid arthritis subgroups based on ACPA levels were compared among different HLA-DRB1 combinations containing HLA-DRB1*09:01 and/or SE. X indicates HLA-DRB1 alleles other than HLA-DRB1*09:01 and SE. 'Low', 'intermediate' and 'high' categories correspond to patients with ACPA titres of ~4.5–13.5, ~13.5–100 and ≥100 U/ml, respectively. **p<0.005 and ***p<0.00005. NS, not significant.

on ACPA levels over SE, we compared ACPA levels in two sets: first between HLA-DRB1*09:01/*09:01 and HLA-DRB1*09:01/SE or HLA-DRB1*09:01/X, and second between SE/HLA-DRB1*09:01 and SE/SE or SE/X. We found that HLA-DRB1*09:01 showed a significant association with low ACPA category compared with the other two groups in both sets of analyses (p<0.005, figure 1). On the other hand, we could not observe any difference between SE and the other alleles.

In this study, we aimed to identify HLA-DRB1 alleles showing quantitative effects on ACPA levels using a large collection of Japanese ACPA-positive RA patients. RPE was applied to avoid misleading frequency deviation by the allele with the strongest association to other associated alleles. We demonstrated that HLA-DRB1*09:01 was the strongest genetic determinant for lower ACPA levels, and the quantitative effects of HLA-DRB1 alleles carrying the SE were not a primary effect but merely an expected consequence of the decreased frequency of HLA-DRB1*09:01. We also identified two novel HLA-DRB1 alleles, HLA-DRB1*04:07 and HLA-DRB1*15:01, being associated with ACPA levels. It is interesting and feasible to perform similar studies in other populations and investigate whether or not the same set of HLA-DRB1 alleles are related to the quantitative effects beyond ethnicities and to examine if such alleles share conserved amino acid motifs.

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

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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.[3] 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.[7–9] Many of the non-HLA susceptibility genes for RA detected to date, such as *PTPN22*[10] and *CTLA4*[11] 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[12] and HLA-DR13[13] 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 fulfil 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 Kyoto 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 of 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.

The following HLA-DRB1 alleles were classified as belonging to the SE: DRB1*01:01, *01:02, *04:01, *04:04, *04:05, *04:08, *04:10, *04:13, *04:16, *10:01, *13:03, *14:02 and *14:06.

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 χ^2 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 found for each allele. Logistic regression analysis was used to evaluate the effects of alleles by adjusting for the influence of other alleles. Statistical analysis was performed using the R statistic system (http://www.R-project.org) or SPSS (version 18). The power calculation was performed using an online power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/).

RESULTS

Genotyping of the first set

We performed HLA-DRB1 genotyping in the 368 ACPA-negative patients with RA and 1508 healthy controls in the first set to compare the allele frequency of each genotype between the cases and controls (table 1). Tables 2 and 3 show the main results of our association study for single alleles and diplotypes, respectively. More detailed results are given in the online supplementary tables 1 and 2.

The SE showed a weak association with moderate effect (p=0.039), mainly due to HLA-DRB1*04:05. Among the other HLA-DRB1 alleles, HLA-DRB1*14:03, *12:01, and *09:01 resulted in moderate to potential susceptibility to ACPA-negative RA (p=0.022, 0.10, and 0.10, respectively). DRB1*13:02, *04:03, and *15:02 showed moderate to potentially protective effects (p=0.0072, 0.059, and 0.12, respectively).

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 0.000088 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=0.00059). The protective effect of DRB1*04:03 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 1 Basic information for ACPA-negative patients with RA and controls

Classification	ACPA-negative RA	Control
Set 1		
Number	368	1508
Female (%)	79.7	52.9
Age (mean±SD)	54.7±16.1	46.5±15.3
Set 2		
Number	501	500
Female (%)	80.8	80.0
Age (mean±SD)	62.4±12.2	NA

ACPA, anticitrullinated peptide/protein antibody; NA, not available; RA, rheumatoid arthritis.

Table 2 Association of the HLA-DRB1 allele with ACPA-negative RA

	Set 1				Set 2				Pooled study			
	†ACPA-negative RA, N (%)	†Control, N (%)	p Value	OR (95% CI)	†ACPA-negative RA, N (%)	†Control, N (%)	p Value	OR (95% CI)	†ACPA-negative RA, N (%)	†Control, N (%)	p Value	OR (95% CI)
Non-SE Susceptible												
*12:01	31 (4.2)	91 (3.0)	0.10	1.41 (0.93 to 2.14)	62 (6.2)	37 (3.7)	0.010	1.72 (1.13 to 2.60)	93 (5.4)	128 (3.2)	0.000088	1.72 (1.31 to 2.26)
*14:03	18 (2.4)	39 (1.3)	0.022	1.91 (1.09 to 3.36)	23 (2.3)	14 (1.4)	0.14	1.65 (0.85 to 3.23)	41 (2.4)	53 (1.3)	0.0043	1.81 (1.20 to 2.73)
*09:01	123 (16.7)	432 (14.3)	0.10	1.20 (0.96 to 1.49)	164 (16.4)	154 (15.4)	0.55	1.08 (0.85 to 1.37)	287 (16.5)	586 (14.6)	0.062	1.16 (0.99 to 1.35)
*14:05	19 (2.6)	63 (2.1)	0.41	1.24 (0.74 to 2.09)	29 (2.9)	18 (1.8)	0.11	1.63 (0.9 to 2.95)	48 (2.8)	81 (2.0)	0.080	1.38 (0.96 to 1.98)
Protective												
*15:02	75 (10.2)	369 (12.2)	0.12	0.81 (0.63 to 1.06)	73 (7.3)	113 (11.3)	0.0020	0.62 (0.45 to 0.84)	148 (8.5)	482 (12.0)	0.00010	0.68 (0.56 to 0.83)
*13:02	44 (6.0)	273 (9.1)	0.0072	0.64 (0.46 to 0.89)	52 (5.2)	52 (5.2)	0.99	1.00 (0.67 to 1.48)	96 (5.5)	325 (8.1)	0.00059	0.66 (0.52 to 0.84)
*04:03	14 (1.9)	97 (3.2)	0.059	0.58 (0.33 to 1.03)	23 (2.3)	28 (2.8)	0.47	0.82 (0.47 to 1.43)	37 (2.1)	125 (3.1)	0.038	0.68 (0.47 to 0.98)
SE												
*04:05	103 (14.0)	340 (11.3)	0.040	1.28 (1.01 to 1.62)	145 (14.5)	129 (12.9)	0.31	1.14 (0.89 to 1.47)	248 (14.3)	469 (11.7)	0.0063	1.26 (1.07 to 1.49)
*14:06	16 (2.2)	37 (1.2)	0.051	1.79 (0.99 to 3.23)	14 (1.4)	9 (0.9)	0.30	1.56 (0.67 to 3.62)	30 (1.7)	46 (1.1)	0.076	1.52(0.95 to 2.41)
*10:01	8 (1.9)	13 (0.4)	0.032	2.54 (1.05 to 6.15)	6 (0.6)	5 (0.5)	0.76	1.20 (0.36 to 3.94)	14 (0.8)	18 (0.4)	0.094	1.80 (0.90 to 3.63)
*04:04	4 (0.5)	6 (0.2)	0.10	2.74 (0.77 to 9.74)	3 (0.3)	2 (0.2)	0.66	1.50 (0.25 to 8.99)	7 (0.4)	8 (0.2)	0.16	2.03 (0.73 to 5.60)
*01:01	43 (5.8)	183 (6.1)	0.82	0.96 (0.68 to 1.35)	50 (5.0)	64 (6.4)	0.17	0.77 (0.52 to 1.12)	93 (5.4)	247 (6.2)	0.24	0.86 (0.67 to 1.10)
*04:01	12 (1.6)	35 (1.2)	0.30	1.41 (0.73 to 2.73)	10 (1.0)	10 (1.0)	1.0	1.00 (0.41 to 2.41)	22 (1.3)	45 (1.1)	0.64	1.13 (0.68 to 1.89)
*04:10	6 (0.8)	63 (2.1)	0.021	0.39 (0.17 to 0.89)	25 (2.5)	14 (1.4)	0.076	1.80 (0.93 to 3.49)	31 (1.8)	77 (1.9)	0.73	0.93 (0.61 to 1.41)
All SE	192 (26.1)	677 (22.4)	0.036	1.22 (1.01 to 1.47)	253 (25.3)	233 (23.3)	0.31	1.11 (0.91 to 1.36)	445 (25.6)	910 (22.7)	0.016	1.17 (1.03 to 1.34)

Allele number and the frequency of each HLA-DRB1 allele in ACPA-negative patients with RA (n=368 and allele number=736 in the 1st set and n=501 and allele number=1002 in the 2nd set) and healthy controls (n=1508 and allele number=3016 in the 1st set and n=500 and allele number=1000 in the 2nd set) as well as the p value and OR of each allele for the development of ACPA-negative RA are shown. p Values were calculated using Fisher's exact test or the χ^2 test.
†Number of alleles (allele frequency).
ACPA, anticitrullinated peptide/protein antibody; SE, shared epitope; RA, rheumatoid arthritis.

in the cases or controls. Both DRB1*13:02 and *15:02 showed potential dosage effects.

Diplotype 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 diplotype 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 *13:02/*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

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

Effect	Allele 1	Allele 2	Set 1				Set 2				Pooled study			
			†ACPA-negative RA, N (%)	†Control, N (%)	p Value	OR (95% CI)	†ACPA-negative RA, N (%)	†Control, N (%)	p Value	OR (95% CI)	†ACPA-negative RA, N (%)	†Control, N (%)	p Value	OR (95% CI)
Non-SE														
Susceptible	*09:01	*12:01	7 (1.9)	10 (0.7)	0.025	2.90 (1.1 to 7.68)	13 (2.6)	3 (0.6)	0.020	4.41 (1.25 to 15.58)	20 (2.3)	13 (0.7)	0.00013	3.62 (1.79 to 7.30)
	*08:03	*08:03	5 (1.4)	7 (0.5)	0.054	2.95 (0.93 to 9.36)	7 (1.4)	4 (0.8)	0.36	1.76 (0.51 to 6.04)	12 (1.4)	11 (0.6)	0.021	2.54 (1.12 to 5.78)
	*04:05	*14:05	5 (1.4)	7 (0.5)	0.054	2.95 (0.93 to 9.36)	5 (1.0)	2 (0.4)	0.26	2.51 (0.48 to 13.00)	10 (1.2)	9 (0.5)	0.033	2.59 (1.05 to 6.39)
Protective	*08:03	*15:02	3 (0.8)	35 (2.3)	0.095	0.35 (0.11 to 1.13)	1 (0.2)	14 (2.8)	0.00047	0.070 (0.010 to 0.53)	4 (0.5)	49 (2.4)	0.00011	0.18 (0.07 to 0.51)
	*15:02	*15:02	2 (0.5)	16 (1.1)	0.36	0.51 (0.12 to 2.23)	1 (0.2)	9 (1.8)	0.011	0.11 (0.010 to 0.86)	3 (0.4)	25 (1.3)	0.024	0.27 (0.08 to 0.91)
	*13:02	*15:02	3 (0.8)	28 (1.9)	0.16	0.43 (0.13 to 1.44)	3 (0.6)	7 (1.4)	0.20	0.42 (0.11 to 1.65)	6 (0.7)	35 (1.7)	0.029	0.39 (0.16 to 0.94)
SE	SE	SE	26 (7.1)	87 (5.8)	0.35	1.24 (0.79 to 1.95)	27 (5.4)	30 (6.0)	0.68	0.89 (0.52 to 1.52)	53 (6.1)	117 (5.8)	0.78	1.05 (0.75 to 1.47)
Serotype	DR8	DR15	8 (2.2)	72 (4.8)	0.027	0.44 (0.21 to 0.93)	10 (2.0)	23 (4.6)	0.021	0.42 (0.20 to 0.90)	18 (2.1)	95 (4.7)	0.00075	0.43 (0.26 to 0.71)
	DR13	DR15	6 (1.6)	55 (3.7)	0.051	0.44 (0.19 to 1.02)	6 (1.2)	11 (2.2)	0.22	0.54 (0.20 to 1.47)	12 (1.4)	66 (3.3)	0.0039	0.41 (0.22 to 0.77)
	DR8	DR8	13 (3.5)	17 (1.1)	0.00097	3.21 (1.55 to 6.67)	10 (2.0)	8 (1.6)	0.64	1.25 (0.49 to 3.20)	23 (2.7)	25 (1.3)	0.0070	2.16 (1.22 to 3.82)

Diplotype number and the frequency of each HLA-DRB1 diplotype in ACPA-negative patients with RA (n=368 and 501 in the 1st and 2nd set, respectively) and healthy controls (n=1508 and 500 in the 1st set and 2nd set, respectively) as well as the p value and OR of each diplotype for the development of ACPA-negative RA are shown. p Values were calculated using Fisher's exact test or the χ^2 test.
†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 genotype 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 (2, 2, 3 and 3, respectively). Since no patients in this study were homozygous for DRB1*14:03, only the result for *14:05 is shown in this figure. SE in the figure includes DRB1*04:05, which is shown separately.

with ACPA-negative RA in Japanese people. Although the controls in the first set had different age and sex ratio values 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-mentioned 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 associated 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 HLADRB1*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 regression analysis, we confirmed that the effects of these susceptibility and protective alleles do not depend on each other (supplementary table 3). Although we searched for common amino acid sequences among the susceptibility alleles, we could not detect any meaningful sequences common to HLA-DRB1*12:01, *14:03, and/or *04:05. We also failed to detect a common amino acid sequence among the protective alleles HLA-DRB1*15:02 and *13:02.

Although the association of SE with ACPA-negative RA cannot be concluded, our large-scale study showed that it is weakly associated with ACPA-negative RA. As we observed a lower OR of the SE in homozygotes than in heterozygous patients, confirmation of this association in other studies are needed. We consider that the SE is associated with ACPA-negative RA but has a much weaker effect than in ACPA-positive RA. Both the

relatively small effect of SE on ACPA-negative RA and the small number of cases in previous reports might have resulted in non-significant p values for such tendencies.

HLA-DRB1*12:01, which was found to be associated with ACPA-negative RA susceptibility in our study, was reported to be associated with type 1 diabetes mellitus (T1D) in Latin America, but no similar association has been reported in Japan.[20 21] While a Japanese study showed RA with the anti-glucose-6-phosphate isomerase antibody is associated with HLA-DRB1*12:01,[22] no large-scale studies have reported an association between HLA-DRB1*12:01 and RA. As RA shares susceptibility genes with T1D such as *PTPN22*,[23] the determination of HLA-DRB1*12:01 as a potential common risk allele for both T1D and ACPA-negative RA is interesting. Although HLA-DRB1*12:01 showed a possible 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%,[24] and so far there are no reports showing an association with ACPA-negative RA.[14] HLA-DRB1*12:02, the other allele of HLA-DR12, showed no association with ACPA-negative RA.

HLA-DRB1*14:03 was reported to be associated with Grave's disease in Japanese patients,[25] but its role in RA is unknown. Although our samples did not contain any patients who were homozygous for the allele owing to its low allele frequency, it showed a moderate association with ACPA-negative RA susceptibility. Among the other non-SE DR14 alleles, DRB1*14:05 displayed a tendency towards ACPA-negative RA susceptibility, 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-DR15 has a protective effect against ACPA-negative RA, its effect on ACPA-negative RA has not been fully examined.[13] 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.[21]

Among HLA-DR13 alleles, HLADRB1*13:02 was reported to be protective against ACPA-positive RA.[26 27] Its protective effect was also reported in Japanese patients with RA.[16] Its effect on ACPA-negative RA has not been established.[13 14] 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-DR8 has also been reported to be associated with some arthropathic autoimmune diseases, such as juvenile idiopathic arthritis[28] and psoriatic arthritis[29] 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*04:03, it showed a potentially protective effect against ACPA-negative RA in the combined study. Further studies are necessary to confirm the association.

As DRB1*09:01 has been shown to be associated with a decreased ACPA titre in ACPA-positive RA,[30] it is likely to be associated with ACPA-negative RA. While DRB1*09:01 showed a potential susceptibility association (p=0.062), the combination of DRB1*09:01 and *12:01 showed strong susceptibility association (p=0.00013). DRB1*09:01 also showed a possible dosage effect. From this viewpoint, we consider that DRB1*09:01 has a potential susceptibility effect on ACPA-negative RA. Owing to the relatively high allele frequency of DRB1*09:01, another independent association study or appropriate classification of ACPA-negative RA could produce significant results.

In addition to the different associations of the SE with ACPA-negative RA and ACPA-positive RA, we found multiple alleles associated with ACPA-negative RA that are not shared by ACPA-positive RA. These showed that ACPA-negative RA is a distinct subset of RA. Moreover, when we focused on ACPA-negative erosive RA to exclude the possibility of our results being affected by non-RA arthritic diseases, the effects of all the following alleles were maintained: *12:01, *14:03, *04:05, *13:02 and *15:02 (data not shown).

This is the first large-scale association study involving Japanese ACPA-negative patients with RA and the detection of multiple alleles and diplotypes associated with susceptibility to, or protection against, ACPA-negative RA. To evaluate whether our cohort had sufficient power to detect HLA-DRB1 genotype associations, we applied a risk allele with 5% frequency in the general population (see 'Materials and methods'). Our power calculation showed that this study had power values of 81% for finding genotype associations with an OR of 1.4 at the 0.05 significance level. When we set the OR to 1.2, our study had power values of 31%. These results suggest that our study has sufficient power to detect associated alleles that are present in relatively high frequencies (such as 5%) and a moderate OR of 1.4. On the contrary, our study has insufficient power to detect associations involving a weak OR such as 1.2. There is a possibility that ACPA-negative RA is associated with more HLA-DRB1 alleles or diplotypes that display a low allele frequency and/or a low OR. Further studies using ACPA-negative RA samples in Japan are necessary to find such associations.

While association studies using ACPA-negative patients with RA of European descent only found a few weak associations and none of them were subsequently replicated, our study successfully determined multiple alleles with relatively strong effects on ACPA-negative RA. From this viewpoint, we suppose that Japanese ACPA-negative patients with RA have a relatively similar genetic background compared to European patients. Population stratification within European population may also be assumed. Nevertheless, the validation of our results in Asian countries is necessary, and large-scale genome-wide association studies of ACPA-negative RA are also required to elucidate the pathogenesis of ACPA-negative RA.

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LETTER

Non-synonymous variant (Gly307Ser) in *CD226* is associated with susceptibility in Japanese rheumatoid arthritis patients

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Autoimmune diseases (ADs) are characterized by an abnormal immune response to self-antigens, and are believed to share a common pathogenesis. For example, the *PTPN22* risk allele (620Trp) dramatically increases susceptibility to rheumatoid arthritis (RA), type 1 diabetes (T1D), systemic lupus erythematosus (SLE) and autoimmune thyroid disease [1], and *STAT4* is also associated with RA, SLE, and systemic sclerosis (SSc) [2, 3]. A genome-wide association study in a Caucasian population also associated susceptibility to T1D with an SNP (rs763361; Gly307Ser) in the *CD226* gene [4]. The *CD226* glycoprotein, a 67 kDa a member of the immunoglobulin superfamily, is involved in regulating T-cell adhesion and activation [5]. The *CD226* Gly307Ser variant has also been associated with susceptibility to several ADs across different racial groups, including RA in Caucasian, Colombian, and Chinese populations [6–8].

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Genetic risks may differ among different populations and sometimes even among groups in the Asian ethnicities [9, 10]. Therefore, replicating previously reported genetic associations in other populations is essential in order to establish the associations as well as to reveal the magnitude of the genetic risk in each population. We undertook a case–control study in a Japanese RA cohort to support the interethnic consistency of the association of the *CD226* variant with disease susceptibility in Japanese AD patients diagnosed with RA, SLE, and SSc.

The Tokyo Women's Medical University Genome Ethics Committee approved the study, and each participant signed an informed consent form following a verbal explanation of the study. The case–control study was performed using Japanese DNA donors: 1504 RA patients, 243 SLE patients, 189 SSc patients, and 752 ethnically matched population controls (Table 1). The American College of Rheumatology criteria for the diagnosis of RA, SLE, and SSc were used to identify patients for the study [11–13].

The SNP (rs763361) in *CD226* was selected based on evidence for an association in RA patients [4, 6]. TaqMan SNP genotyping was performed according to the manufacturer's instructions (Applied Biosystems, Tokyo, Japan). Duplicate samples and negative controls were included to monitor accuracy. The chi-square test was performed to compare allelic frequencies of the variant and to test for Hardy–Weinberg equilibrium (HWE). Stratified analysis using rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA) status was performed to test whether the putative genetic risk factor is predominant in the autoantibody-positive subset of RA patients. These analyses were performed using the R software package (http://www.r-project.org/).

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The genotyping success rate was greater than 99% and the genotype concordance rate was 100% as assessed by duplicate samples. The genotypic distribution of the Gly307Ser variant was in HWE. There was no gender difference in the allelic distribution of the polymorphism in controls ($P = 0.94$). Allele frequencies are shown in Table 2. The 307Ser allele was significantly associated with RA in the Japanese population [$P = 0.01$, odds ratio (OR) = 1.17 (1.03–1.33)]. The allele showed a trend for association with SSc [$P = 0.08$, OR = 1.23 (0.97–1.55)], but no association was found with SLE [$P = 0.44$, OR = 1.08 (0.88–1.34)]. Stratification analysis revealed that 307Ser is a risk factor for RA in autoantibody-positive patients in the presence of RF [$P = 0.007$, OR = 1.19 (1.04–1.36)] and ACPA [$P = 0.009$, OR = 1.19 (1.05–1.36)].

Table 1 Demographics of AD patients

RA	
Age, years (median)	60
Sex, female (%)	84
RF positive (%)	88
ACPA positive (%)	87
SLE	
Age, years (median)	34
Sex, female (%)	94
SSc	
Age, years (median)	41
Sex, female (%)	90
Controls	
Age, years (median)	35
Sex, female (%)	50

RF rheumatoid factor, *ACPA* anti-cyclic citrullinated peptide antibody, *SSc* systemic sclerosis

Recent studies have indicated that the genetic background of RA might vary among ethnic groups. While the genetic association between HLA–DRB1 and RA susceptibility is well established in most populations, other reported associations with genes such as *PTPN22* and *PADI4* have been difficult to replicate in different populations [14, 15]. The results of this report support previous studies indicating that a variant on *CD226* is a genetic risk factor for RA across different racial groups. The overall OR for the variant on RA susceptibility was 1.2 in non-European populations; slightly higher than previously reported for Europeans (1.09) [6].

This was the first attempt to test the association between Gly307Ser, the putative disease causal variant for a variety of autoimmune diseases, and SSc in Japanese. Though we found a trend for an association with SSc that had an OR similar to that of RA, it was not significant. We also observed no association between the variant and SLE. One possible reason for the negative associations is the lack of statistical power. While the sample size of RA provided a statistical power of 0.94 with an OR = 1.25 [7] and a T allele frequency = 0.477 (Japanese HapMap Japanese Project), the sample sizes of SLE and SSc could not provide enough power (<0.8). Further large-scale study is needed to verify the association of 307Ser and SSc, since the population we used was relatively small ($n = 189$). Another possible reason for the negative associations is that the contribution of *CD226* to the disease pathway may differ between RA and SLE or SSc. Other independent association studies would help to improve the hypothesis.

Thus, replication studies using other ethnic populations are useful to establish genetic association and to define the genetic impact in each ethnic population. We conclude that we have successfully validated the association of *CD226* Gly307Ser with RA susceptibility in a Japanese population.

Table 2 Genotype distributions of Gly307Ser in AD patients and controls

Phenotype	Genotype			Total	MAF	OR (95% CI)	*P*
	CC	CT	TT				
RA	417	727	355	1479	0.47	1.17 (1.03–1.33)	0.01
RF positive	365	636	304	1305	0.48	1.19 (1.05–1.36)	0.007
RF negative	50	91	31	172	0.45	1.05 (0.82–1.34)	0.69
ACPA positive	355	602	294	1251	0.48	1.19 (1.04–1.36)	0.009
ACPA negative	45	107	31	183	0.46	1.12 (0.89–1.42)	0.32
SLE	76	114	53	243	0.45	1.08 (0.88–1.34)	0.44
SSc	48	94	42	184	0.48	1.23 (0.97–1.55)	0.08
Control	236	372	136	744	0.43		

Gly glycine, *Ser* serine, *RA* rheumatoid arthritis, *MAF* minor allele frequency, *OR* odds ratio, *CI* confidence interval, *RF* rheumatoid factor, *ACPA* anti-cyclic citrullinated peptide antibody, *SSc* systemic sclerosis

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Conflict of interest The authors declare that there is no conflict of interest.

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The Influence of Individual Joint Impairment on Functional Disability in Rheumatoid Arthritis Using a Large Observational Database of Japanese Patients

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ABSTRACT. ***Objective.*** To clarify the influence of individual joint impairment on functional capacity through a retrospective study with a 3-year interval, using a large cohort of Japanese patients with rheumatoid arthritis (RA).

Methods. Subjects included 3457 patients with RA who participated in a large observational cohort study in both April 2004 and April 2007; 43 joints were assessed and classified into 10 joint areas. Impairment of each joint area was scored based on the presence of swelling or tenderness: score 0 (no swelling or tenderness in either joint), score 1 (swelling or tenderness in a unilateral joint), and score 2 (swelling or tenderness in bilateral joints). Score change was defined as the difference between scores from 2004 and 2007. The Japanese validated version of the Health Assessment Questionnaire is the J-HAQ; ΔJ-HAQ score was determined by subtracting J-HAQ score in 2007 from that in 2004. The relationship between score change and ΔJ-HAQ score, and the effect of joint impairment on ΔJ-HAQ score were assessed.

Results. Major joint areas that contributed to ΔJ-HAQ score included the wrist (31%), shoulder (21%), knee (13%), and ankle (10%). The shoulder, wrist, knee, and ankle in the worsening group were associated with a J-HAQ score increase of 0.13 to 0.18 compared to the improvement group.

Conclusion. Our study demonstrated that impairment of the shoulder, wrist, knee, and ankle significantly affects functional capacity in patients with RA. Care of these joints is suggested to be especially important for better functional outcomes. (First Release Jan 15 2012; J Rheumatol 2012;39:476–80; doi:10.3899/ jrheum.110770)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
JOINT INVOLVEMENT
FUNCTIONAL DISABILITY
FUNCTIONAL OUTCOME

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Rheumatoid arthritis (RA) is characterized by persistent polyarthritis and progressive joint damage that lead to functional disability. Suppression or improvement of functional disability is one of the major goals of RA treatment. Previous studies showed that Health Assessment Questionnaire (HAQ) score is associated with disease activity, joint distraction, disease duration, age, sex, muscle strength, work disability, and mortality[1,2,3,4,5,6]. RA disease activity has been shown to be significantly associated with decreased HAQ scores throughout the course of RA[1,2].

Functional disability in patients with RA has both reversible and irreversible components[7]. The reversible component involves inflammation, indicating that it can be improved by medical intervention. The irreversible component is associated with joint destruction and deformity; this can be ameliorated by surgical treatment or physical therapy. Therefore, care of individual joints is as important as systemic treatment to avoid worse functional outcomes. The influence of joint impairment on functional disability may differ among individual joints. However, only a few studies with relatively small samples have been conducted on the effect of individual joint impairment on functional disability.