Table 2 Case-control series for systemic lupus erythematosus[14]

Series	Patients: No. of samples	Patients: Mean age, years	Patients: Female (%)	No. of control samples
TWMU	238	NA	95.0	752*
RIKEN	188	42.2	91.6	940†
Tokushima/Fukuoka	165	NA	100	507††
Total	591			2199

*Genotype information was obtained from the controls in the IORRA rheumatoid arthritis series.
†Genotype information was obtained from the controls in the RIKEN/BioBank rheumatoid arthritis series.
‡Genotype information was obtained from the controls in the Tokushima rheumatoid arthritis series.
NA, not available; TWMU, Tokyo Women's Medical University.

Stratified analyses on autoantibody status in patients with RA

Most of the patients with RA from the IORRA and RIKEN/BioBank cohorts were positive for anti-CCP (assessed by ELISA) and RF (ELISA) (n = 2671) and were combined for stratified analyses on autoantibodies (see Supplementary material; patients from the RIKEN and Tokushima cohorts were without anti-CCP status). Although studies in populations of European descent indicated that rs10818488 predisposed patients to autoantibody-positive disease,[2–4 6] we did not observe a significant difference in allele frequencies among groups of individuals with different autoantibody status (anti-CCP and RF).

Association with radiographic severity with the genotypes

Of the 625 patients with proper anteroposterior radiographs of the hands at 5-year disease duration, 450 were risk allele (G) carriers of rs10818488 and 169 were risk allele non-carriers (6 patients were not genotyped). Neither anti-CCP status (G carriers 87% positive, non-G carriers 92% positive) nor gender (G carriers 85% female, non-G carriers 80% female) was significantly different between the two groups. Though Kurreeman *et al* reported positive effect of the risk allele of the SNP on radiographic severity in Caucasian patients with RA,[2] we could not find any effect on SHS (hands) in the Japanese RA population (fig 1, p = 0.28).

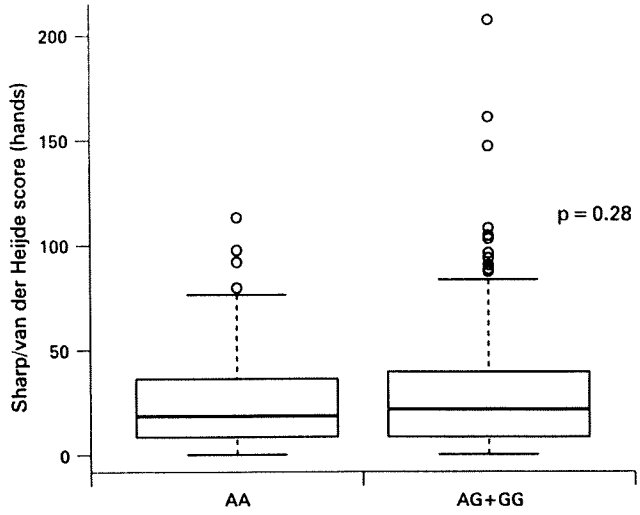

Figure 1 Each box plot shows the different rs10818488 genotypes (risk allele non-carriers (AA) and risk allele carriers (AG and GG)) for Sharp/van der Heijde score (hands) (n = 625). Each box represents the interquartile range of values, with the bold line showing the median value. The vertical lines show maximum and minimum value that fall within 1.5 box lengths, the open circles show extreme values >1.5 box plot lengths.

Association of *TRAF1-C5* polymorphisms with SLE

Though significant difference in allele frequencies between SLE cases and controls was observed in the Tokushima/Fukuoka series (OR 1.36, 95% CI 1.05 to 1.76 for rs3761847; OR 1.29, 95% CI 1.00 to 1.67 for rs10818488), a combined analysis including 3 case-control series comprising 591 cases and 2199 control subjects did not show any association between the both SNPs and susceptibility to SLE (table 4).

Table 3 Distribution of *TRAF1-C5* alleles and genotypes in rheumatoid arthritis (RA)

	IORRA		RIKEN		RIKEN/BioBank		Tokushima		
	RA	Controls	RA	Controls	RA	Controls	RA	Controls	Combined
rs3761847:									
Genotype									
GG	387	191	211	193	289	266	274	152	
GA	747	388	418	322	573	475	442	264	
AA	331	162	201	137	248	198	227	85	
Total	1465	741	830	652	1110	939	943	501	
Allele									
MAF	0.48	0.48	0.49	0.46	0.48	0.46	0.48	0.43	
p Value	0.98		0.046		0.26		0.031		0.013
OR (95% CI)	1.00 (0.88 to 1.14)		1.16 (1.00 to 1.34)		1.07 (0.95 to 1.22)		1.18 (1.01 to 1.39)		1.09 (1.02 to 1.17)
rs10818488:									
Genotype									
AA	405	199	217	202	298	271	277	154	
AG	757	389	422	320	575	476	448	263	
GG	320	152	190	132	238	191	217	82	
Total	1482	740	829	654	1111	938	942	499	
Allele									
MAF	0.47	0.47	0.48	0.45	0.47	0.46	0.47	0.43	
p Value	0.85		0.044		0.32		0.039		0.013
OR (95% CI)	1.01 (0.89 to 1.15)		1.16 (1.00 to 1.35)		1.06 (0.94 to 1.21)		1.18 (1.01 to 1.38)		1.09 (1.02 to 1.17)

IORRA, Institute of Rheumatology Rheumatoid Arthritis; MAF, minor allele frequency.

Table 4 Distribution of *TRAF1-C5* alleles and genotypes in systemic lupus erythematosus (SLE)

	TWMU		RIKEN/BioBank		Tokushima/Fukuoka		
	SLE	Controls	SLE	Controls	SLE	Controls	Combined
rs3761847:							
Genotype							
GG	57	191	55	266	42	152	
GA	126	388	93	475	77	264	
AA	47	162	40	198	45	85	
Total	230	741	188	939	164	501	
Allele							
MAF	0.48	0.48	0.46	0.46	0.51	0.43	
p Value	0.94		0.90		0.016		0.28
OR (95% CI)	0.99 (0.80 to 1.23)		0.99 (0.78 to 1.24)		1.36 (1.05 to 1.76)		1.08 (0.95 to 1.23)
rs10818488:							
Genotype							
AA	60	199	57	271	46	154	
AG	130	389	93	476	76	263	
GG	44	152	38	191	43	82	
Total	234	740	188	938	165	499	
Allele							
MAF	0.47	0.47	0.45	0.46	0.49	0.43	
p Value	0.93		0.78		0.046		0.43
OR (95% CI)	0.99 (0.80 to 1.23)		0.97 (0.77 to 1.22)		1.29 (1.00 to 1.67)		1.06 (0.93 to 1.20)

MAF, minor allele frequency; Tokyo Women's Medical University.

TRAF1 and *C5* expression in relation to genotypes

Though there was no genotypic effect on gene expression level of *TRAF1* in non-stimulated lymphoblastoid cell lines from healthy donors (fig 2A), we found a significant difference between risk allele (G) carriers (n = 31) and risk allele non-carriers (n = 9) on the *TRAF1* transcript level in phorbol myristate acetate (PMA)-stimulated human lymphoblastoid cell lines (fig 2B; p = 0.04). However, there was no genotypic effect on gene expression level of *C5* (fig 2C,D). Since the expression of *C5* was at lower levels (the mean cycle threshold (Ct) values for non-stimulated and PMA-stimulated cells were 35.2 and 35.5, respectively), the results of *C5* expression in relation to genotypes may not be very meaningful.

These results were consistent with the other internal controls, glucuronidase (GUS) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

DISCUSSION

In the current study, the association of the *TRAF1-C5* locus with RA susceptibility was detected in 4397 Japanese RA cases and 2857 control subjects from a combination of 4 studies. The results indicate that the locus is a risk factor for RA susceptibility in at least two major racial groups. Though a discrepancy in the magnitude of genetic risk of the *TRAF1-C5* locus was observed between the Japanese (1.09) and the Caucasian populations in first two reports,[1 2] it was quite similar to UK populations (1.08–1.09).[3] Thus, the discrepancy in the magnitude of genetic risk may be explainable with the "winner's curse": the overestimation of the genetic effect in the first positive genetic association study.[17] Otherwise, it could be explainable by population difference, which has been observed for *PADI4* or *PTPN22*.[16 17]

Despite the positive results of the combined analysis, two out of the four case-control studies (IORRA and RIKEN/BioBank) showed no association. This may be because of limitations in statistical power. Since the present study indicated that the allelic OR for the *TRAF1-C5* SNPs was modest, the statistical power for each case-control series was at most 0.28 ($\alpha = 0.05$, risk allele frequency in controls = 0.46), while the statistical power for the combined analysis was 0.69.

Contradictory patterns of association of the *TRAF1-C5* locus with RA were observed between Japanese and Caucasian populations, such that the risk alleles in the previous studies decreased the risk for RA in the present populations.[1 2] The association of opposite alleles has been reported previously, especially in different ethnic populations.[25]

One possible reason is that the positive association detected in the current study was a false positive. In fact, we cannot entirely rule out the possibility of a false positive result. However, though some of the case-control studies showed no significant association, the risk allele frequency was always higher in the case groups than in the control groups in all four case-control studies. This result reduces the possibility of a false positive.

Another possible explanation is that a single-locus association could be complicated by interaction between an investigated locus and other risk factors when multiple factors act in concert to cause a disease.[25] Though the pathogenesis of RA is not fully determined, RA is believed to be a complex multifarious disease that is influenced by genetic and environmental factors, and the genetic and environmental background of patients might be different among different ethnic populations.

Since the SNPs tested are only genetic markers, the ethnic differences in haplotype structure may also explain the discrepancy. The disease-associated allele and the disease-causing allele in linkage disequilibrium might be flipped in different populations when they are located across the historical recombination.[26] However, we investigated the difference in recombination blocks using HapMap data of Japanese and European ancestry, but could not find any major difference in haplotype structures nor identify a putative common variant that is in linkage disequilibrium with the flip-flopped alleles. Since the HapMap SNPs do not cover all the genetic variability, revealing the whole picture of genetic variations by resequencing the locus with Japanese and Caucasian samples could help to identify genuine functional variants, which would solve the flip-flop phenomenon.

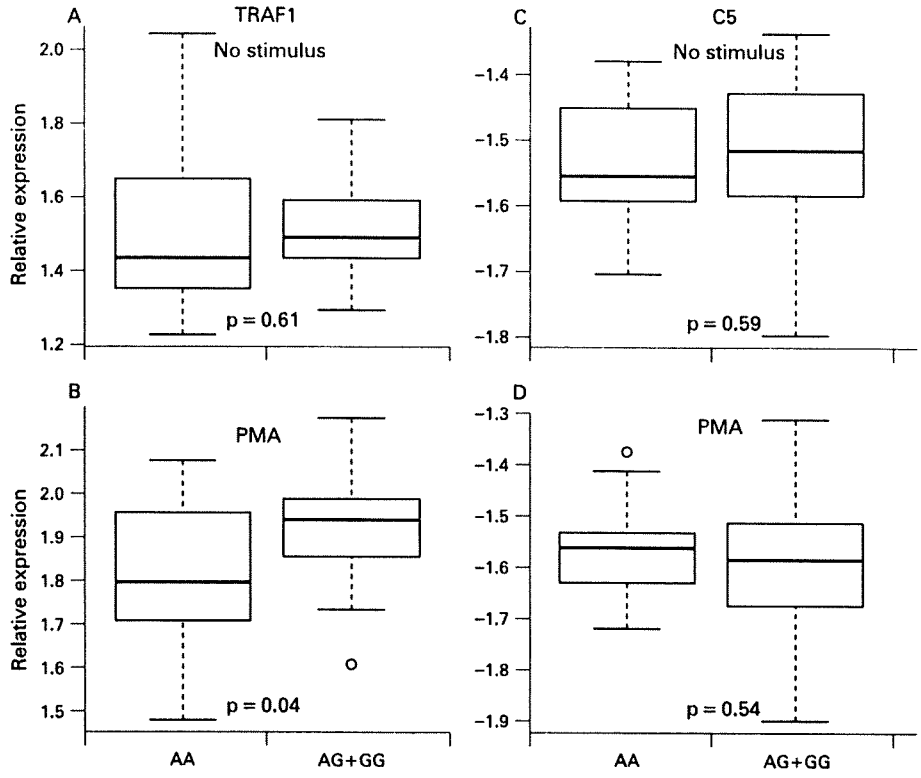

Figure 2 Each box plot shows the different rs10818488 genotypes (risk allele non-carriers (AA, n = 9) and risk allele carriers (AG and GG, n = 31)) for the log10-transformed expression level of *TRAF1* or *C5*, measured by TaqMan assay and calculated relative to the reference gene 18S rRNA. A. Expression of *TRAF1* in non-stimulated cell lines. B. Expression of *TRAF1* in phorbol myristate acetate (PMA)-stimulated cell lines. C. Expression of *C5* in non-stimulated cell lines. D. Expression of *C5* in PMA-stimulated cell lines. Each box represents the interquartile range of values, with the bold line showing the median value. The vertical lines show maximum and minimum value that fall within 1.5 box lengths, the open circles show extreme values >1.5 box plot lengths.

Autoimmune diseases sometimes share common susceptibility genes, which is demonstrated by the association of the *TRAF1-C5* locus and polyarthritis in juvenile idiopathic arthritis.[27] However, association of the *TRAF1-C5* locus with SLE could not be confirmed in the present study. A previous study in a Colombian population also revealed no association of *TRAF1-C5* with SLE.[28] These results may suggest that the contribution of the *TRAF1-C5* locus to the disease pathway may be different between RA and SLE.

Another consideration for the negative association with SLE is a lack of statistical power. Since this study is a multicentre study of 591 cases and 2199 shared controls, the statistical power achieved is 0.79 if the OR for the *TRAF1-C5* SNPs in SLE is 1.2. Nevertheless, this could be underpowered to detect a lower OR. A much larger sample size might be needed to observe a small risk.

The proportions of female samples were different in the patients with SLE and control groups. This might be a limitation of the study, although a specific analysis restricted to women showed negative association as well (data not shown).

Evidence for the genotypic effect of the *TRAF1-C5* polymorphism on *TRAF1* gene expression supports the genetic association between *TRAF1* and RA. Although the biological roles of TRAF1 are controversial, in the studies of *TRAF1*-deficient mice and B cell lines, TRAF1 enhances CD40 mediated activation signals in cooperation with TRAF2, resulting in nuclear factor (NF)κB or c-Jun N-terminal kinase (JNK) activation, which is considered to be a key component of the pathogenesis of RA.[29 30] The positive genetic association in different ethnic populations with the flip-flop phenomenon and the correlation of gene expression with genotypes might indicate that a genuine functional variant responsible for *TRAF1* expression, possibly located across the historical recombination from the intergenic polymorphisms, may exist. Investigating the genuine functional variant is necessary to reveal a whole picture of pathogenesis of RA involving *TRAF1*.

In conclusion, association of *TRAF1-C5* locus with RA was detected in the Japanese population, while association between this locus and SLE could not be observed. The putative functional variant, which is considered to be in strong linkage equilibrium with the intergenic polymorphism, might influence *TRAF1* expression that could impact RA susceptibility. Identification of the disease-causal variant of RA related to the *TRAF1-C5* locus and replication of the variant in other populations is necessary to reach a definitive conclusion regarding the association.

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