CONCISE REPORT

Supportive evidence for a genetic association of the *FCRL3* promoter polymorphism with rheumatoid arthritis

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Background: An association between susceptibility to rheumatoid arthritis and the Fc receptor-like 3 gene (*FCRL3*) has been reported in a Japanese population. A case–control study showed that the strongest evidence of the association was derived from a polymorphism in the promoter region of *FCRL3*, which has a regulatory effect on the expression of the gene.

Objective: To validate the findings of this previous report by examining the −169C→T single nucleotide polymorphism (SNP) in a large cohort.

Methods: 752 unrelated cases and 940 controls were genotyped. All the samples were from the same ethnic background as the original study. Genotyping was done using 5′ allelic discrimination assays. Association between susceptibility to rheumatoid arthritis and −169C→T SNP was examined by χ^2 testing.

Results: As in the previous study, the SNP showed significant differences between cases and controls (p = 0.022, odds ratio = 1.18, 95% confidence interval 1.02 to 1.35).

Conclusions: This result supports a genetic association of the *FCRL3* promoter polymorphism with rheumatoid arthritis.

Rheumatoid arthritis (MIM 180300) is a complex disease that is influenced by genetic and environmental factors. The HLA locus has a major impact on rheumatoid arthritis susceptibility, which has been estimated to account for one third of the genetic component.[1] Many other potential susceptibility genes have been investigated using genome-wide scanning and candidate approaches. Recently, Kochi and colleagues conducted a linkage disequilibrium mapping using 830 cases and 658 controls. They identified an association between susceptibility to rheumatoid arthritis and the Fc receptor-like 3 gene (*FCRL3*), which is a member of a new gene family that has a high structural homology with, and is locate near to, the Fcγ receptor genes.[2] The Fcγ receptors are the receptors for the Fc portion of the IgG molecules, and it is suggested that they are involved in the pathogenesis of arthritis.[3–5] Autoantibody against the Fc portion of IgG is known as rheumatoid factor (RF). RF is a well established disease marker for rheumatoid arthritis, and most rheumatoid patients are RF positive. Although the function of *FCRL3* is yet unknown, it also can be a candidate gene for the susceptibility to rheumatoid arthritis because it is a homologue of the Fcγ receptor genes.

Kochi *et al* reported that the strongest evidence of the association was derived from a polymorphism in the promoter region of *FCRL3* (−169C→T; p = 0.00000085, odds ratio (OR) = 2.15, 95% confidence interval (CI) = 1.58 to 2.93). Moreover, they determined that the −169C→T single nucleotide polymorphism (SNP) had a regulatory effect on *FCRL3* expression. A significant association between *FCRL3* genotypes and serum RF level was also reported. The reported association seemed to satisfy most of the proposed criteria for association studies[6]: large sample size; small p values; *FCRL3* making possible biological sense; and the most disease associated polymorphism affecting expression of the gene. In addition, they also validated the association by allele in another independent sample set (p = 0.041). Although the most significant association in the initial study had been observed under a certain genetic model (susceptible homozygotes versus others), they failed to confirm the association by the genotype in their replication study (p = 0.21). We therefore sought to replicate the findings of this previous report by examining the −169C→T SNP in a large scaled association study. Furthermore, we tested the differences between distribution of RF positivities and concentrations according to the genotypes of −169C→T SNP, with the aim of replicating the previous result.

METHODS

Disease criteria and subjects

Tokyo Women's Medical University genome ethics committee granted approval of this study. The study is part of a rheumatoid arthritis cohort project of approximately 4000 patients established in the year 2000 by the Institute of Rheumatology, Tokyo Women's Medical University.[7] Of the 4000 Japanese rheumatoid patients registered, DNA samples were available from 1284. Of these, 754 were randomly selected for this study. Each individual signed an informed consent form after receiving a verbal explanation of the study. The diagnosis of rheumatoid arthritis followed the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria.[8] Eighty eight per cent of the rheumatoid patients were RF positive and they were mostly female (88%). Nine hundred and forty population based control DNA samples were obtained from the Pharma SNP consortium (http://www.jpma.or.jp/psc/index.html). All control subjects were matched for ethnic origin and geographical area with the patients. Of the 940 population controls, 40% were female.

Statistical power

This study was designed to have >99.9% power at the 5% significance level to detect the odds ratio (OR) of 2.15 conferred by homozygosity for the risk allele of −169C→T SNP (12.25% frequency in the controls estimated by the moment method), as reported in the original study. Statistical power was calculated using a web power calculator (http://calculators.stat.ucla.edu/powercalc/).

Abbreviations: RF, rheumatoid factor; SNP, single nucleotide polymorphism

Table 1 Distribution of the *FCRL3* polymorphism in rheumatoid arthritis patients and controls

		Genotype of −169C→T*				Allele T *v* C		Genotype TT+CT *v* CC	
		TT	CT	CC	MAF	p Value	OR (95% CI)	p Value	OR (95% CI)
Current study	Case	238	377	133	0.430	0.022	1.18 (1.02 to 1.35)	0.026	1.35 (1.03 to 1.77)
	Control	333	472	129	0.391				
Initial study by Kochi *et al*[2]	Case	291	374	159	0.420	0.000035	1.37 (1.18 to 1.60)	0.00000085	2.15 (1.56 to 2.97)
	Control	266	318	65	0.345				
Replication study by Kochi *et al*[2]	Case	182	281	77	0.403	0.041	1.19 (1.00 to 1.41)	0.21	1.24 (0.87 to 1.78)
	Control	251	310	75	0.362				

*Values are n except for MAF.
CI, confidence interval; MAF, minor allele frequency; OR, odds ratio.

Genotyping

The polymorphism −169C→T SNP [rs7528684] was selected for investigation because it gave the best evidence for the association and was suggested to be crucial for the regulation of *FCRL3* expression in the original study.

Genotyping were carried out using the TaqMan fluorogenic 5′ nuclease assay (Applied Biosystems, Tokyo, Japan). The final volume of polymerase chain reaction (PCR) was 5 μl, containing 2 ng of genomic DNA and 2.5 μl of TaqMan Universal PCR Master Mix (2×), with 0.25 μl of 20× assay mix. Thermal cycle conditions were as follows: 50℃ for two minutes and 95℃ for 10 minutes, followed by 40 cycles of 92℃ for 15 seconds and 60℃ for one minute. All PCR and end point fluorescent readings were undertaken on an ABI PRISM 7900 HT sequence detection system (Applied Biosystems).

Rheumatoid factor

Serum RF concentration was determined by a latex agglutination turbidimetric immunoassay method. For each individual, we used the maximum value of RF measured in the cohort project during 2000–2004.

Statistical analysis

The association between rheumatoid arthritis susceptibility or RF positivities and −169C→T SNP was estimated by the χ^2 testing. Differences in serum RF levels among genotypes of −169C→T SNP were analysed by regression analysis. These tests were implemented in the R software package, version 2.0.1 (http://www.r-project.org/).

RESULTS

The observed genotype frequencies of the SNP were in Hardy–Weinberg equilibrium, and allele frequencies were similar to the original report. As in the previous result, the SNP was found to show significant differences between the cases and controls, not only by allele (p = 0.022, OR = 1.18 (95% CI, 1.02 to 1.35)) but also under the genetic model (susceptible homozygotes *v* others: p = 0.026, OR = 1.35 (95% CI, 1.03 to 1.77; table 1).

On the other hand, the promoter polymorphism of *FCRL3*, −169C→T SNP, was not associated with RF positivity in rheumatoid patients under any genetic model (p = ~0.18; table 2). Moreover, serum RF level in individuals with rheumatoid arthritis did not differ among genotypes of −169C→T SNP and did not correlate with the number of susceptible alleles ($R^2 = 0.0002$, p = 0.68), unlike the reported result (table 3).

DISCUSSION

In recent years, several rheumatoid arthritis susceptibility genes have been identified using powerful association studies in a Japanese population.[9 10] However, replication studies with samples from other ethnic populations failed to support the evidence of these associations.[11–14] Ethnic differences may explain the lack of replication.[15] In such cases, an independent validation study with an ethnically and geographically matched population is important, as it can test the association without ethnicity becoming a confounding factor: Ethnic specific differences in linkage disequilibrium and gene–gene or gene–environment interactions can then be tested between two studies.

We found supportive evidence of the association between susceptibility to rheumatoid arthritis and the *FCRL3* promoter polymorphism recently reported by Kochi *et al*. The association was tested using a large cohort with the same ethnic background as the previous study. Allele frequencies in the population we used were similar to those reported in the original study, and showed significant differences between cases and controls. Furthermore, the association was also validated by genotype, unlike the replication study conducted by Kochi *et al* themselves. The result confirmed the finding that the functional polymorphism in the promoter region of *FCRL3* plays an independent role in the susceptibility to rheumatoid arthritis.

Even though the susceptibility has been confirmed by the current study, the size of the odds ratio was inconsistent with the previous result (1.35 *v* 2.15). However, it is known that the first association study often overestimates a genetic effect.[16] To provide the accurate estimation of population-wide effect of a genetic risk factor, the meta-analysis with many more replication studies would be required.

Despite the successful replication of the association between rheumatoid arthritis susceptibility and *FCRL3*, we could not validate the positive correlation between serum RF level and the genotypes of *FCRL3* which Kochi *et al* reported. This was not because of a lack of statistical power because

Table 2 Positivities of rheumatoid factor according to −169C→T genotype in rheumatoid patients

	Genotype of −169C→T SNP*			Genotype TT *v* CT+CC		Genotype TT+CT *v* CC	
RF†	TT	CT	CC	p Value	OR (95% CI)	p Value	OR (95% CI)
Seropositive	214 (89.9%)	325 (86.2%)	122 (91.7%)	0.37	1.26 (0.75 to 2.16)	0.18	0.64 (0.30 to 1.26)
Seronegative	24 (10.1%)	52 (13.8%)	11 (8.3%)				

*Values are n (% in each genotype).
†Cut off, 15.0 IU/ml.
CI, confidence interval; OR, odds ratio; RF, rheumatoid factor.

Table 3 Serum rheumatoid factor level according to −169C→T genotype in rheumatoid patients

	Genotype of −169C→T SNP*			Regression analysis	
	TT (n = 238)	CT (n = 377)	CC (n = 133)	R^2	p Value
Serum level (IU/ml)	116.5 (49.0 to 116.5)	83.0 (29.0 to 210.0)	109.0 (55.0 to 246.0)	0.0002	0.68

*Values are median (interquartile range).

our sample size was fivefold larger than the original study (n = 752 *v* n = 148). Our result casts doubt on the reported association between RF level and a polymorphism in *FCRL3*. However, because of the potential importance of the original findings, further study of the genetic effect on the level of RF will be needed to resolve the inconsistent results.

In conclusion, an association of *FCRL3* and susceptibility to rheumatoid arthritis was validated in a Japanese population. Further independent studies using other ethnic samples would be helpful to determine whether the association is attributed to a common variable, irrespective of ethnicity.

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