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The −169C/T Polymorphism in *FCRL3* Is Not Associated With Susceptibility to Rheumatoid Arthritis or Systemic Lupus Erythematosus in a Case–Control Study of Koreans

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Objective. **In Japanese individuals, the −169C/T single-nucleotide polymorphism (SNP) in *FCRL3* has been reported to be associated with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and autoimmune thyroid diseases. The objective of this study was to test the association of this SNP with RA and SLE, in a case–control study of Korean individuals.**

Methods. **The −169C/T SNP in *FCRL3* was genotyped in 1,060 patients with RA, 457 patients with SLE, and 697 unaffected control subjects, using the MassARRAY SNP genotyping system. Associations were tested by multivariate logistic regression, with adjustments for age and sex.**

Results. **No association was detected between the −169C/T SNP and RA (odds ratio [OR] 1.11, 95% confidence interval [95% CI] 0.83–1.48, $P = 0.50$) or SLE (OR 1.00, 95% CI 0.73–1.37, $P = 0.99$). This SNP was not associated with rheumatoid factor status, shared epitope status, radiographic severity in patients with RA, or disease manifestations in patients with SLE.**

Conclusion. **The association of the −169C/T SNP in *FCRL3* with RA and SLE that was observed in Japanese patients was not replicated in a Korean population.**

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by synovial inflammation and joint destruction that affects up to 1% of the population. Although multiple genetic and environmental factors are believed to be involved in the pathogenesis of RA, its precise etiology has not been clarified. Genetic factors account for 60% of the population variance in RA. The HLA region, especially the HLA–DRB1 allele, which is commonly referred to as the shared epitope (SE), consistently shows the strongest association, although it accounts for less than one-third of the genetic contribution (1). Systemic lupus erythematosus (SLE) is an autoimmune disease with diverse chronic systemic manifestations. SLE undoubtedly has a genetic predisposition, with involvement of other etiologic factors (2).

Identifying affiliated genes is challenging, but genome-wide linkage analyses have identified several non-HLA susceptibility genes along with nonrandom clustering in a common set of susceptibility genes for clinically distinct autoimmune diseases, and a single candidate gene has been proposed to contribute to multiple autoimmune diseases (3). These autoimmune diseases with complex traits are believed to be caused by a common allele with low penetrance rather than by multiple rare alleles with high penetrance (4). Linkage disequilibrium–based association analyses have eluci-

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Table 1. Association of the *FCRL3* −169C/T SNP in patients and controls*

	Genotype†			C allele frequency	CC vs. CT + TT		CC + CT vs. TT	
	CC	CT	TT		OR (95% CI)	*P*	OR (95% CI)	*P*
RA patients	196	521	343	0.43	1.11 (0.83–1.48)	0.50	1.15 (0.91–1.46)	0.25
SLE patients	83	220	154	0.42	1.00 (0.73–1.37)	0.99	1.16 (0.90–1.50)	0.25
Controls	125	317	255	0.41	1	–	1	–

* *P* values were calculated by age- and sex-adjusted multivariate logistic regression. SNP = single-nucleotide polymorphism; OR = odds ratio; 95% CI = 95% confidence interval; RA = rheumatoid arthritis, SLE = systemic lupus erythematosus.
† Values are the number of patients or controls.

dated several susceptibility genes in autoimmune disease, although the results of such analyses have not been universally replicated in different ethnic groups.

A functional single-nucleotide polymorphism (SNP), −169C/T, in the *FCRL3* promoter region was recently shown to confer susceptibility to RA in a Japanese population (5). That study showed that SNP −169C/T alters the expression of *FCRL3* by affecting the binding affinity of NF-κB to the *FCRL3* promoter. Therefore, an increased level of *FCRL3*, which is correlated with the −169C susceptibility allele, may result in B cell abnormalities and a higher level of autoantibodies such as rheumatoid factor (RF) and anti–cyclic citrullinated peptides. The investigators also showed the association of SNP −169C/T with SLE and autoimmune thyroid diseases. These associations were recently replicated in another Japanese population (6).

The aim of the present study was to determine whether the −169C/T polymorphism in *FCRL3* is associated with susceptibility to RA and SLE in a Korean population.

PATIENTS AND METHODS

Patients. For this study, 1,060 unrelated patients with RA satisfying the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for RA (7), 457 unrelated patients with SLE satisfying the ACR 1997 revised criteria for the classification of SLE (8), and 697 nonpatient controls of Korean ethnic origin were recruited from the Hospital for Rheumatic Diseases at Hanyang University Medical Center in Seoul, Korea. The study was approved by the institutional review board of Hanyang University Hospital, and all subjects provided written informed consent. The patients with RA were stratified according to their RF status and their SE status. They were also stratified according to radiographic severity and by the staging system described by Steinbrocker et al (9), in order to assess the association of the polymorphism with disease severity. The patients with SLE were categorized according to the ACR criteria. In all subjects, genomic DNA was extracted from blood leukocytes, using a standard protocol.

Among the patients with RA, the mean ± SD age was 52.1 ± 12.1 years, the mean ± SD age at disease onset was 39.8 ± 12.5 years, 89.7% were female, 35.8% were positive for the SE, 17.3% showed no erosive changes (Steinbrocker stage I), and 82.7% demonstrated erosions (Steinbrocker stages II, III, and IV). Among patients with SLE, the mean ± SD age was 32.2 ± 11.4 years, the mean ± SD age at disease onset was 25.4 ± 10.6 years, and 93.5% were female. The mean ± SD age of control subjects was 36.5 ± 13.2 years, and 86.7% were female.

Genotyping. All of the subjects were genotyped for the HLA–DRB1 alleles, using polymerase chain reaction and sequence-specific oligonucleotide probe hybridization according to the reference protocol set forth by the 12th International Histocompatibility Workshop, followed by direct DNA sequencing (10). The SE was defined by the following alleles: DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0413, *1001, and *1402 (1).

An SNP in *FCRL3*, fcrl3_3, located at nucleotide −169 relative to the transcription initiation site (referred to as −169C/T), was genotyped using the MassARRAY system (Sequenom, San Diego, CA), according to the manufacturer's protocol. The genotyping success rates for patients with RA, patients with SLE, and controls were 98.4%, 96.4%, and 91.7%, respectively.

Statistical analysis. Genotype frequencies of the SNP were in Hardy-Weinberg equilibrium in all groups, according to chi-square tests. Associations were tested by calculating the odds ratios (ORs) and 95% confidence intervals (95% CIs), using multivariate logistic regression with adjustments for age, sex, and disease duration. All analyses were performed using SPSS software (version 11.5; SPSS, Chicago, IL).

RESULTS

The SNP was successfully genotyped in all subjects, including 1,060 patients with RA, 457 patients with SLE, and 697 control subjects. The SNP failed to show significant association with RA in Koreans, because the group of patients with RA was not significantly different from the control group in terms of genotype distributions according to either recessive-model (OR 1.11, 95% CI 0.83–1.48, $P = 0.50$) or dominant-model (OR 1.15, 95% CI 0.91–1.46, $P = 0.25$) association tests (Table 1).

Table 2. Association of the *FCRL3* −169C/T SNP with RF status in patients with RA*

RF status†	Genotype‡			C allele frequency	CC vs. CT + TT	
	CC	CT	TT		OR (95% CI)	*P*
Positive	177	482	315	0.43	1.32 (0.78–2.23)	0.30
Negative	19	39	28	0.45	–	–

* *P* values were calculated by age- and sex-adjusted multivariate logistic regression. SNP = single-nucleotide polymorphism; RF = rheumatoid factor; RA = rheumatoid arthritis; OR = odds ratio; 95% CI = 95% confidence interval.
† Cutoff value = 20 IU/ml.
‡ Values are the number of patients.

In addition, the SNP was not associated with SLE in Koreans. The differences in genotype distributions between the group of patients with SLE and the control group were not statistically significant in either recessive-model (OR 1.00, 95% CI 0.73–1.37, $P = 0.99$) or dominant-model (OR 1.16, 95% CI 0.90–1.50, $P = 0.25$) association tests (Table 1).

Furthermore, SNP −169C/T was not associated with the presence of RF. The genotype frequencies in the RF-positive subgroup were not statistically significantly different from those in the RF-negative subgroup (OR 1.32, 95% CI 0.78–2.23, $P = 0.30$), as shown in Table 2. When both the group of patients with RA and the control group were separately divided into 2 subgroups depending on carriage of the SE (Table 3), no genotype association was detected in the subgroup carrying at least 1 copy of the SE (OR 1.20, 95% CI 0.19–1.76, $P = 0.35$). Similarly, genotype association was not detected in the subgroup with no copies of the SE (OR 0.94, 95% CI 0.19–1.36, $P = 0.75$).

When the 184 patients with severe RA (Steinbrocker stages II, III, and IV) were compared with the 897 patients with mild RA (Steinbrocker stage I), only marginal association was observed between the SNP genotypes and the radiographic severity of RA (OR 0.68, 95% CI 0.47–1.00, $P = 0.051$). This finding needs to be confirmed or replicated in another study.

DISCUSSION

Although the −169C/T SNP in *FCRL3* was associated with susceptibility to RA and SLE in 2 independent studies in the Japanese population (5,6), it was not associated with either susceptibility to or the severity of RA and SLE in this study in Koreans. In addition, the SNP was not associated with the RF status or the SE status in patients with RA.

The reason for the discrepancy regarding association of the SNP with RA and SLE between the present study of Korean individuals and previous studies in the Japanese population is unclear, but several possibilities are conceivable. The discrepancy could be attributable to different characteristics of the cohorts, such as age or the sex ratio. However, the mean ± SD age of patients with RA in the Korean cohort (52.1 ± 12.1 years) was not different from that of patients in the first Japanese cohort (59.0 ± 12.3 years) (5); such information for the second Japanese cohort (6) is not available. The prevalence of female patients with RA in the Korean cohort (89.7%) was also very similar to that in the second

Table 3. Association of the *FCRL3* −169C/T SNP with SE status in patients with RA and controls*

	Genotype†			C allele frequency	Genotype CC vs. CT + TT	
	CC	CT	TT		OR (95% CI)	*P*
SE positive						
RA patients	133	351	223	0.44	1.20 (0.19–1.76)	0.35
Controls	43	125	95	0.40	–	–
SE negative						
RA patients	63	170	120	0.42	0.94 (0.19–1.36)	0.75
Controls	82	192	160	0.41	–	–

* The shared epitope (SE) was defined by the following alleles: DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0413, *1001, and *1402. *P* values were calculated by age- and sex-adjusted multivariate logistic regression. SNP = single-nucleotide polymorphism; SE = shared epitope; RA = rheumatoid arthritis; OR = odds ratio; 95% CI = 95% confidence interval.
† Values are the number of patients or controls.

Japanese cohort (88%) (6) and was not much different from that in the first Japanese cohort (84.2%) (5). Other information, such as the age at disease onset and the percentage of patients with severe disease, was not available for the Japanese cohorts being compared with this cohort of Koreans. Thus, it is unlikely that such factors contributed to the discrepancy.

The discrepancy could be attributable to true variation between the ethnic groups. The susceptibility to various diseases does vary with race and ethnicity, and in complex diseases such as RA and SLE, reports of linkage to specific genetic regions are only rarely replicated in different racial and ethnic groups. Koreans and Japanese have been considered to be genetically similar, and results of a recent study demonstrated that a functional haplotype of 4 exonic SNPs of the *PADI4* gene was associated with susceptibility to RA in Japanese individuals (11); this association was not replicated in Caucasian patients with RA but was replicated in Koreans (12). In a recent study of SNPs of disease-candidate genes (13), Koreans and Japanese also showed the least estimated genetic difference among ethnic groups. Although this difference is substantially smaller than the average worldwide difference in general, it still may be sufficient to cause contradictory results in an association study, as demonstrated by the difference in the allele frequency of the *FCRL3* −169C/T polymorphism in Korean and Japanese control subjects.

The difference between results in a Korean population and results in 2 Japanese populations may also be attributable to false-negative errors. Replication studies tend to produce smaller effect sizes (14). However, our study was designed to have >90% power to detect the relative risk of the SNP in the Japanese study (OR 2.15) (5) at a significance level of 5%. In a replication study of another Japanese population, however, the relative risk was 1.35, which compromises the power substantially (6). Moreover, the true relative risk can be even smaller, and the number of subjects in our study might have been insufficient to investigate the association.

In the Japanese study (5), genotyping was carried out using Invader and TaqMan assays, while the MassARRAY system was used in our study. The difference in genotyping methods used, together with the amplification of DNA, may have contributed to the discrepant results obtained in the Korean population. Nonetheless, both methods are deemed reliable, and the possibility of different assays being the cause of such a discrepancy would be remote.

Other confounding environmental factors may affect the genetic contributions to susceptibility. Even though Korea and Japan are geographically adjacent, the populations of these countries have their own distinct diets, lifestyles, and cultures that may (together with other environmental factors) account for the different results obtained in these 2 populations.

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