Supportive evidence for a genetic association of the \textit{FCRL3} promoter polymorphism with rheumatoid arthritis

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Running title: Association between \textit{FCRL3} and RA susceptibility

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ABSTRACT

Objective. Recently, an association between susceptibility to rheumatoid arthritis and the Fc receptor-like 3 gene (FCRL3) was reported in a Japanese population. FCRL3 is a member of a new gene family that has a high structural homology with the Fc gamma receptor genes, which are suggested to be involved in the pathogenesis of arthritis. A case-control study showed that the strongest evidence of the association was derived from a polymorphism in the promoter region of FCRL3 (-169C->T, $P = 0.00000085$, OR = 2.15, 95% CI = 1.58 – 2.93), which has a regulatory effect on the expression of the gene. The objective of this study was to validate the findings of this previous report by examining the -169C->T SNP in a large cohort.

Methods. Seven hundred and fifty-two unrelated cases and 940 controls were genotyped. All the samples were from the same ethnic background as the original study. Genotyping was performed using 5’ allelic discrimination assays. Association between susceptibility to rheumatoid arthritis and -169C->T SNP was examined by Chi-square test.

Results. Identical to the result of the previous study, the SNP was found to show significant differences between the cases and the controls ($P = 0.022$, OR = 1.18, 95% CI = 1.02 - 1.35).

Conclusion. Our result supports a genetic association of the FCRL3 promoter polymorphism with rheumatoid arthritis.
INTRODUCTION
Rheumatoid arthritis (RA [MIM 180300]) is believed to be a complex disease that is influenced by genetic and environmental factors. The HLA locus has a great impact on RA 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 RA 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 locate nearby, the Fc gamma receptor genes [2]. The Fc gamma receptors are the receptors for the Fc portion of the IgG molecules, and they are suggested to be 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 RA, and most of the RA patients are RF positive. Although the function of FCRL3 is yet unknown, it also can be a candidate gene for the susceptibility to RA because it is a homolog of the Fc gamma 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, \(P = 0.00000085\), OR = 2.15, 95% CI = 1.58 – 2.93). Moreover, they clarified that the -169C->T 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 on association studies [6]: large sample size; small \(P\) values; FCRL3 possibly makes biological sense; and the most disease-associated polymorphism affected 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 (\(P = 0.21\)). Therefore, we 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, hoping to replicate the previous result.

PATIENTS AND METHODS
Disease criteria and subjects
Tokyo Women’s Medical University Genome Ethics Committee granted approval of this study. This study is a part of a RA cohort project of approximately 4,000 patients established in 2000 by Institute of Rheumatology, Tokyo Women's Medical University [7]. Out of 4000 Japanese RA patients registered, DNA of 1284 subjects were extracted. Of which, 754 samples were randomly selected for this study. Each individual signed an informed consent form after receiving a verbal explanation of the study. Diagnoses of RA followed the American College of Rheumatology
Statistical power

This study was designed to have $> 99.9\%$ power at the $5\%$ significance level to detect the odds ratio of $2.15$ conferred by homozygosity for 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/).

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 performed using the TaqMan fluorogenic 5’ nuclease assay (Applied Biosystems, Tokyo, Japan). The final volume of polymerase chain reaction (PCR) was 5 $\mu$l, containing 2 ng of genomic DNA and 2.5 $\mu$l of TaqMan Universal PCR Master Mix (2X), with 0.25 $\mu$l of 20X Assay Mix. Thermal cycle conditions were as follows: 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 92°C for 15 seconds and 60°C for 1 minute. All PCR and endpoint fluorescent readings were performed 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 2000-04.

Statistical analysis

Association between RA susceptibility or RF positivities and -169C->T SNP was estimated by the Chi-square test. Differences of serum RF levels among genotypes of -169C->T SNP were analyzed with a 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. Identical to the previous result, the SNP was found to show significant differences between the cases and the controls not only by allele ($P = 0.022$, OR = 1.18, 95% CI = 1.02 - 1.35) but also under the genetic model (susceptible homozygotes versus others: $P = 0.026$, OR = 1.35, 95% CI = 1.03 - 1.77; Table 1a).
Table 1a
Distribution of the FCRL3 polymorphism in RA patients and controls.

<table>
<thead>
<tr>
<th>Genotype of -169C-&gt;T *</th>
<th>Allele T vs. C</th>
<th>Genotype TT+CT vs. CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>CT</td>
<td>CC</td>
</tr>
<tr>
<td>Case</td>
<td>238</td>
<td>377</td>
</tr>
<tr>
<td>Control</td>
<td>333</td>
<td>472</td>
</tr>
<tr>
<td>Initial study by Kochi et al.</td>
<td>Case</td>
<td>291</td>
</tr>
<tr>
<td>Control</td>
<td>266</td>
<td>318</td>
</tr>
<tr>
<td>Replication study</td>
<td>Case</td>
<td>182</td>
</tr>
<tr>
<td>by Kochi et al.</td>
<td>Control</td>
<td>251</td>
</tr>
</tbody>
</table>

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

On the other hand, the promoter polymorphism of FCRL3, -169C->T SNP, was not associated with RF positivities in RA patients under any genetic model (P = ~ 0.18; Table 1b).

Table 1b
Positivities of rheumatoid factor according to -169C->T genotype in RA patients.

<table>
<thead>
<tr>
<th>Genotype of -169C-&gt;T SNP*</th>
<th>Genotype TT vs. CT+CC</th>
<th>Genotype TT+CT vs. CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>CT</td>
<td>CC</td>
</tr>
<tr>
<td>Seropositive 214 (89.9)</td>
<td>325 (86.2)</td>
<td>122 (91.7)</td>
</tr>
<tr>
<td>Seronegative 24 (10.1)</td>
<td>52 (13.8)</td>
<td>11 (8.3)</td>
</tr>
</tbody>
</table>

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

Moreover, serum RF level in individuals with RA 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 1c).

Table 1c
Serum rheumatoid factor level according to -169C->T genotype in RA patients.

<table>
<thead>
<tr>
<th>Genotype of -169C-&gt;T SNP*</th>
<th>Regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (n=238)</td>
<td>CT (n=377)</td>
</tr>
<tr>
<td>Serum level (IU/ml)</td>
<td>116.5 (49.0–116.5)</td>
</tr>
</tbody>
</table>

* Values are median (interquartile range).
DISCUSSION

In recent years, several RA-susceptible genes were identified with 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, independent validation study with ethnically and geographically matched population is meaningful since it can test the association without considering the difference of ethnicity as a confounding factor: population specific differences in linkage disequilibrium, gene-gene or gene-environment interactions between two studies.

We found supportive evidence of an association between susceptibility of RA and the $FCRL3$ promoter polymorphism, which Kochi et al. had recently reported. 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 RA.

Even though the susceptibility has been confirmed by the current study, the size of OR was inconsistent with the previous result (1.35 versus 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 much more replication studies would be required.

Despite the successful replication of the association between RA susceptibility and $FCRL3$, we could not validate the positive correlation between serum RF level and the genotypes of $FCRL3$, which Kochi et al. reported. Lack of this association could not be from a lack of statistical power because our sample size was fivefold larger than the original study (n=752 versus n=148, respectively). 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, the association of $FCRL3$ and susceptibility to RA was validated in a Japanese population. Further independent studies using other ethnic samples are helpful to know if the association is attributed to a common variance, irrespective of ethnicity.

ACKNOWLEDGEMENTS

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Competing Interests
There are no competing interests.

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