Association of an Activity-Enhancing Variant of IRAK1 and an MECP2–IRAK1 Haplotype With Increased Susceptibility to Rheumatoid Arthritis

Tae-Un Han,1 Soo-Kyung Cho,2 Taehyeung Kim,1 Young Bin Joo,2 Sang-Cheol Bae,2 and Changwon Kang1

Objective. To investigate whether a human X chromosome locus of IRAK1 and MECP2 is associated with susceptibility to rheumatoid arthritis (RA), an autoimmune disease that predominantly affects women.

Methods. A total of 2,334 unrelated Korean participants (including 1,318 patients with RA) were genotyped for 5 tag single-nucleotide polymorphisms (SNPs) and 3 additional SNPs in an Xq28 region harboring MECP2 and IRAK1. Twenty-nine additional neighboring SNPs were imputed using the Korean HapMap Project data. All 37 SNPs were statistically tested for association with RA susceptibility, and 2 SNPs associated with RA were examined for their functional effects.

Results. RA susceptibility was associated with multiple SNPs in a 79-kb linkage disequilibrium block harboring both MECP2 and IRAK1. The most significant association was for MECP2 SNP rs1734792 (P = 0.00089), but 2 nonsynonymous IRAK1 SNPs, rs1059702 (P = 0.0034) and rs1059703 (P = 0.0042), which were in strong linkage disequilibrium with the MECP2 SNP (D' = 0.87 and 0.91, respectively) affected IRAK1 protein activity. The major haplotype of the 2 nonsynonymous SNPs was associated with a 1.7-fold increase in RA susceptibility versus the minor haplotype (P = 0.0082), and with increased IRAK1 activity, which was demonstrated by a 1.7-fold increase in the intracellular activity of transcription factor NF-κB.

Conclusion. Our findings indicate that RA susceptibility is associated with multiple SNPs in MECP2 and IRAK1, but high linkage disequilibrium between them does not allow for further localization. Therefore, both genes remain candidates. Nevertheless, the major haplotype of the 2 nonsynonymous IRAK1 SNPs encoding for pPhe196Ser and pSer532Leu confers enhanced IRAK1 activity and, consequently, enhanced susceptibility to RA, as compared to the minor haplotype.

The genetic contribution to rheumatoid arthritis (RA), a chronic autoimmune disease, has been estimated to be 53–60% (1). A number of genes have been identified as being associated with RA susceptibility in diverse populations (2,3). Among them, several genes, such as HLA–DRB1, PTPN22, STAT4, and IRF5, have additionally been shown to be associated with susceptibility to another autoimmune disease, systemic lupus erythematosus (SLE), in several populations (4–6). These results support the notion that multiple diseases may be associated with the same common genetic variants (7).

Several single-nucleotide polymorphisms (SNPs) at an X chromosome locus harboring MECP2, IRAK1, and TMEM187, from rs2239464 in MECP2 through rs2266890 in TMEM187, have previously been shown to be associated not only with susceptibility to SLE in Caucasian and Korean populations (8–11), but also with other systemic autoimmune diseases, such as primary Sjögren’s syndrome (rs17435 in MECP2) (12) and sys-

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**Supported by grants from the National Research Foundation of Korea (2011-0020334 and 2011-0017999 to Dr. Kang) and the Korea Healthcare Technology R&D Project (A111218-11-GM01 to Dr. Bae).**

1Tae-Un Han, PhD, Taehyeung Kim, BS, Changwon Kang, PhD: Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea. 2Soo-Kyung Cho, MD, Young Bin Joo, MD, Sang-Cheol Bae, MD, PhD, MPH: Hanyang University Hospital for Rheumatic Diseases, Seoul, Republic of Korea.

Drs. Han and Cho contributed equally to this work. Drs. Bae and Kang contributed equally to this work.

Address correspondence to Changwon Kang, PhD, Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea (e-mail: ckang@kaist.ac.kr); or to Sang-Cheol Bae, MD, PhD, MPH, Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul 133-792, Republic of Korea (e-mail: scbae@hanyang.ac.kr).

Submitted for publication January 6, 2012; accepted in revised form November 15, 2012.
temic sclerosis (rs1059702 in IRAK1) (13) in Caucasian populations.

Recently, a SNP (rs3027898) located downstream of IRAK1 was found to be associated with RA in Greek (14) and Chinese (15) populations, but those studies were both too small to be affirmative. Two other SNPs, rs17435 in MECP2 and rs1059703 in IRAK1, were found not to be associated with RA in a Spanish population (16) and a Greek population (14), respectively, although an extended region of Xq24 through Xq28 has been reported as a suggestive RA linkage locus in Japanese families (17). In this case-control study, the Xq28 locus was comprehensively examined for association with RA susceptibility using a large Korean population for confirmation.

PATIENTS AND METHODS

Study subjects. A total of 2,334 unrelated participants (1,318 Korean patients with RA and 1,016 ethnicity- and sex-matched controls) were recruited at Hanyang University Hospital for Rheumatic Diseases. The study was approved by the institutional review board of the hospital, and written informed consent was obtained from all subjects. All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA (18). The ethnically matched healthy control subjects had no personal or family history of autoimmune disease, as determined by a questionnaire administered by trained interviewers. The clinical and demographic characteristics of the cases and control subjects are presented in Table 1. Age and sex were known for all cases and controls. Information on age at disease onset, disease duration, and whether patients had erosive RA was available for 1,315 (99.8%) of the patients, and anti-cyclic citrullinated peptide (anti-CCP) autoantibody and rheumatoid factor (RF) status were both too small to be affirmative. Two other SNPs, rs1059702, rs1059703, rs3027898, rs6571303, rs5945380, and rs2071128) using a MassArray iPLEX Gold assay according to the instructions of the manufacturer (Sequenom). Genotyping was performed at the Korea Advanced Institute of Science and Technology under the approval of its institutional review board. The SpectroDesigner program (Sequenom) was used for designing the amplification and extension primers, and the SpectroTyper program (Sequenom) was used for calling genotypes. Genotypes of untyped and uncalled SNPs were all imputed in each sample using Mach-1 software (http://www.sph.umich.edu/csg/abecasis/MACH/index.html) with the Korean HapMap Project genotype data (http://www.khapmap.org/) as a template. All samples were additionally genotyped for HLA–DRB1 using a sequencing-based typing method as previously described (20).

Cell culture and complementary DNA (cDNA) synthesis. Epstein-Barr virus–transformed human B cell lines were grown in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (FBS; HyClone) and penicillin, and HEK 293T cells were maintained in Dulbecco’s modified Eagle’s medium (Gibco) supplemented with 10% FBS. RNAs were isolated using an RNasy Mini kit according to the instructions of the manufacturer (Qiagen). The cDNA was synthesized with 4 µl of messenger RNA (mRNA) using an ImProm II reverse transcription system according to the instructions of the manufacturer (Promega).

Construction of an IRAK1 expression vector. The full-length IRAK1-encoding cDNA sequence (2,138 bp) was amplified using a 5′-GAGGCGGCCGCCAGGTC-3′ and 5′-CAGAGTGCTGAGGACTCGTGCAC-3′, respectively. These restriction sites were used in cloning the IRAK1 cDNA into pFLAG CMV2 (Sigma-Aldrich).

Luciferase reporter assay. For the luciferase reporter assay, 293T cells (2 × 10⁵) were seeded in 12-well tissue culture plates and transiently transfected the next day with 0.65 µg of plasmids, including firefly luciferase–expressing vectors, and the products were used as templates in the secondary PCR with the primers 5′-AAGAGACCCGCGGACGCG-3′ and 5′-AAAATTCATGCGCCGCGGACGCG-3′ and 5′-AAAATTCATGCGCCGCGGACGCG-3′, which were designed to introduce Eco RI and Xba I restriction enzyme sites (underlined) at the 5′ and 3′ ends of the cDNA, respectively. These restriction sites were used in cloning the IRAK1 cDNA into pFLAG CMV2 (Sigma-Aldrich).

| Table 1. Characteristics of the RA cases and controls* |
|-----------------|-----------------|-----------------|
| Characteristic | RA cases (n = 1,318) | Controls (n = 1,016) |
| Sex, % women | 89.1 | 86.2 |
| Age, mean ± SD years | 51.8 ± 12.2 | 36.7 ± 12.5 |
| HLA–DRB1 risk allele positivity, % of subjects | 80.2 | 47.0 |
| Age at disease onset, mean ± SD years | 40.1 ± 12.4 | NA |
| Disease duration, mean ± SD years | 11.7 ± 8.5 | NA |
| Erosive RA, % of subjects | 81.6 | NA |
| RF positivity, % of subjects | 90.4 | Not tested |
| Anti-CCP antibody positivity, % of subjects | 84.8 | Not tested |

* RA = rheumatoid arthritis; NA = not applicable; RF = rheumatoid factor; anti-CCP = anti-cyclic citrullinated peptide.
0.15 μg of pRL-TK control vector (Promega), using Lipojectamine 2000 (Invitrogen) according to the manufacturer’s instructions. The cells were harvested after 24 hours, and luciferase activity was then measured using a Dual-Luciferase Reporter Assay system (Promega). All experiments were performed 4 times.

**Real-time quantitative PCR.** Quantitative PCR was carried out for both the quantification of cellular levels of IRAK1 and MECP2 mRNA isoforms and the detection of copy number variation in a genomic region of IRAK1 and MECP2, using iQ SYBR Green Supermix and an iCycler iQ Real-Time PCR Detection System (Bio-Rad). PCR conditions were 3 minutes at 94°C followed by 40 cycles of 15 seconds at 94°C and 30 seconds at 60°C. Samples were run in triplicate for mRNA measurements and in duplicate for genomic DNA measurements. The comparative ΔΔCt method was used to calculate relative amounts of mRNA or DNA. Primers were designed using the Primer BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A primer set for GAPDH was used for normalization in mRNA quantification, and another primer set for NXF5 at Xq22 was used for normalization in measurements of copy number variation.

Primer sets for mRNA measurement were as follows: for GAPDH cDNA, 5′-CTCCGTCCTCCTGCCTCC-3′ (forward) and 5′-CCCCATAGGACCAAACTCCGT-3′ (reverse); for IRAK1a cDNA, 5′-CTCTCATGACCCAGGTGTACGA-3′ (forward) and 5′-TGCTGGACAGCTTAGGGTTCTCCT-3′ (reverse); for IRAK1b cDNA, 5′-TCCTATGAGCCAGGAGAACCTCC-3′ (forward) and 5′-TAGGCTCTGTCAGCTCTCCA-3′ (reverse); and for MECP2A cDNA, 5′-GGAGGAAGTGGAGTGGAGGAG-3′ (forward) and 5′-ACCAGAAGCCGAAA-3′ (forward) and 5′-ACCAGGATGGAGTGGA-3′ (reverse) for NXF5, and 5′-TGCTGGATGAATA-ACCACAACAC-3′ (forward) and 5′-GAAGGTCTCCAGCCATCGAA-3′ (reverse) for MECP2-IRAK1.

**Table 2.** Association of the 8 genotyped SNPs of IRAK1 and MECP2 with RA susceptibility*

<table>
<thead>
<tr>
<th>SNP (position)†</th>
<th>Controls, AA/A/AB/BB/B</th>
<th>RA cases, AA/A/AB/BB/B</th>
<th>OR (95% CI)‡</th>
<th>P_{allele}§</th>
<th>P_{genotype}&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1734792 (153341060)</td>
<td>465/92/34/56/44</td>
<td>699/110/391/61/26</td>
<td>1.33 (1.09–1.61)</td>
<td>0.0040</td>
<td>0.00089</td>
</tr>
<tr>
<td>rs2075597 (153297636)#</td>
<td>677/119/176/7/15</td>
<td>936/138/204/14/5</td>
<td>1.21 (0.92–1.59)</td>
<td>0.17</td>
<td>0.031</td>
</tr>
<tr>
<td>rs1059702 (153284192)#**</td>
<td>465/94/336/56/44</td>
<td>707/112/393/62/20</td>
<td>1.31 (1.08–1.59)</td>
<td>0.0056</td>
<td>0.0034</td>
</tr>
<tr>
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* AA indicates 2 major alleles, AB indicates 1 major allele and 1 minor allele, and BB indicates 2 minor alleles in women; A indicates the major allele and B indicates the minor allele in men. The numbers of AA, AB, and BB genotypes in women were each equally split into 2 alleles, as previously described by Clayton (22,23). Values are the number of subjects with each genotype. RA = rheumatoid arthritis; OR = odds ratio; 95% CI = 95% confidence interval.
† The chromosomal position of each single-nucleotide polymorphism (SNP) corresponds to the GRCh37.p5 genomic build 37.3 in the NCBI Reference Assembly.
‡ For allele association, calculated for the estimated numbers of active major alleles (A) versus minor alleles (B).
§ By Pearson’s chi-square test.
# Tag SNPs.
** Nonsynonymous SNPs.

**RESULTS**

Association of a linkage disequilibrium block of IRAK1 and MECP2 with RA susceptibility. Seven genes, including IRAK1 and MECP2, were found to be enclosed in a 159-kb linkage disequilibrium block on human chromosome Xq28 (Figure 1A) when the SNP genotyping data from the Korean HapMap Project were analyzed using Haploview version 4.1 (with the upper...
confidence interval maximum for strong recombination at 0.85). All 34 SNPs with minor allele frequencies of $\geq 0.05$ (referred to herein as “common SNPs”) in the block could be tagged by only 5 SNPs (rs2075597, rs1059702, rs6571303, rs5945380, and rs2071128) with $r^2 \geq 0.75$. A total of 2,334 unrelated Korean female and male participants, including 1,318 RA cases and 1,016 sex-matched controls (Table 1), were genotyped for the 5 tag SNPs (Table 2), and their genotype distribution in the female control samples conformed to Hardy-Weinberg equilibrium.

Association with susceptibility to RA was examined for each tag SNP by counting each allele in female subjects as one-half, according to the method previously described by Clayton (22,23), reflecting a dosage compensation for X inactivation. Among the 5 tag SNPs, 1 SNP (rs1059702 T>C in IRAK1) showed a significant association with RA susceptibility ($P = 0.0056$) even when corrected for multiple testing (significance level $\alpha = 0.0098$), and subjects with the major T allele had 1.31-fold higher odds of having RA compared to subjects with the minor C allele (95% confidence interval [95% CI] 1.08–1.59).

Genotype association was additionally tested using Clayton’s method (22,23), in which X inactivation was taken into account by scoring genotypes as 0, 1, or 2 in women, and as 0 or 2 in men. After adjustment for age and HLA–DRB1 genotype (Table 2), of the 5 tag SNPs, only SNP rs1059702 showed a significant association with RA susceptibility ($P = 0.0034$), consistent with the allele association. When subjects were stratified by sex, this association remained significant in women ($P = 0.0043$), but not in men ($P = 0.50$), possibly due to the small size of the male subgroup.

We also genotyped 2 SNPs (rs3027898 and rs1734792) at the MECP2–IRAK1 locus that were previously reported to be associated with RA or SLE. Supporting the previously demonstrated associations with RA in Greek (14) and Chinese (15) populations, in this study rs3027898 was marginally associated with RA susceptibility among Koreans in an allelic model (odds ratio [OR] 1.26 [95% CI 1.04–1.53], $P = 0.019$) (Table 2). Interestingly, rs1734792, which was previously shown to be associated with SLE susceptibility in Korean (11) and European (10) populations, showed the most significant association with RA susceptibility in the present study (OR 1.33 [95% CI 1.09–1.61], $P = 0.0040$ in an allelic model, $P = 0.00089$ in a genotypic model).

Using the genotype data for these 7 SNPs, genotype data for a total of 36 SNPs spanning 211 kb were imputed for all subjects, using the Mach-1 program with the Korean HapMap Project data as a template. These 36 SNPs were tested for an association with RA susceptibility (Figure 1A). The associations between RA and the 2 SNPs mentioned above, rs1059702 and rs1734792, remained significant ($P = 0.0038$ and $P = 0.0041$, respectively), and 15 additional SNPs showed a marginal association with RA ($0.0048 \leq P \leq 0.014$) when corrected for multiple testing ($\alpha = 0.0042$).

The significant and marginal associations between RA and these 17 SNPs were not independent from each other in pairwise conditional regression analysis, likely because all of these SNPs were in high linkage disequilibrium and highly correlated with each
other \((0.85 \leq D' \leq 1.00, 0.73 \leq r^2 \leq 1.00)\). These 17 SNPs associated with RA were widely distributed in a 78.6-kb region encompassing a 61.2-kb portion (intron 2 and downstream) of the 75.9-kb MECP2, the 1.9-kb intergenic region between MECP2 and IRAK1, the entire length of the 9.4-kb IRAK1, and a 6.2-kb region downstream of IRAK1 (Figure 1B), suggesting genetic association of RA susceptibility with variations in both MECP2 and IRAK1.

Association of a functional haplotype with increased susceptibility to RA and increased IRAK1 activity. Since a significantly associated SNP in IRAK1 \((rs1059702)\) was nonsynonymous, we examined 10 additional nonsynonymous SNPs registered for IRAK1 in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP). When DNA samples from 1,000 randomly chosen RA patients and 700 randomly chosen control subjects were pooled and genotyped for the 10 SNPs, none of them were estimated to be polymorphic, except for \(rs1059703\). Since it had not been examined in the Korean HapMap Project to be used for imputation, the second polymorphic nonsynonymous SNP \((rs1059703)\) was genotyped for all samples in this study (Table 2). It showed a significant association with RA \((OR 1.31 [95\% CI 1.08–1.59], P = 0.0059\) in an allelic model, \(P = 0.0042\) in a genotypic model) and was in high linkage disequilibrium and highly correlated with \(rs1059702\) \((D' = 0.99, r^2 = 0.95)\).

These 2 nonsynonymous SNPs constituted the 2 common haplotypes found in a vast majority \((98.8\%)\) of the control group. The most frequent haplotype (referred to as the major haplotype) carried the major alleles \((rs1059702\ T\ \text{and}\ rs1059703\ C)\) in both SNPs \((72.6\%\ \text{in}\ \text{the}\ \text{controls})\), and the other common haplotype (the minor haplotype) carried the minor alleles \((rs1059702\ C\ \text{and}\ rs1059703\ T)\) in the SNPs \((26.2\%\ \text{in}\ \text{the}\ \text{controls})\). The remaining 2 haplotypes were too rare for their association to be tested.

When subjects who were homozygous for the major haplotype were compared to those who were homozygous for the minor haplotype (Table 3), those who were homozygous for the major haplotype had 1.7-fold higher odds of having RA than those who were homozygous for the minor haplotype \((95\%\ CI 1.2–2.5, P = 0.0082)\). When cases were stratified by anti-CCP or RF status, being homozygous for the major haplotype was still significantly associated with increased RA susceptibility in the RF-positive patients \((OR 1.98, P = 0.0013)\) and the anti-CCP-positive patients \((OR 1.92, P = 0.0048)\), as compared to controls. However, the association was not significant in the RF-negative patients \((P = 0.59)\) or anti-CCP–negative patients \((P = 0.54)\).

The 2 IRAK1 SNPs \(rs1059702\) and \(rs1059703\) encode the amino acid changes Phe196Ser and Ser532Leu, respectively. In order to examine the effects of these changes on IRAK1 activity, 2 forms of IRAK1 cDNA were cloned in separate expression vectors. These IRAK1 cDNA variants were cloned from total RNA preparations of Epstein-Barr virus–transformed B cells from 2 randomly chosen female subjects, one who was homozygous for the major haplotypes of the 2 SNPs and one who was homozygous for the minor haplotypes of the 2 SNPs. The full length of the cDNA was then sequenced in both directions. The 2 cDNA sequences were identical except for the positions corresponding to the 2 nonsynonymous SNPs and an additional synonymous SNP \((rs1059701)\), which was consistent with our finding that other reportedly nonsynonymous SNPs were not polymorphic in this population. Functional effects of these allele changes on IRAK1 protein activity

**Table 3. Association of nonsynonymous SNP haplotypes in IRAK1 with RA susceptibility**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Major haplotype</th>
<th>Minor haplotype</th>
<th>OR (95% CI)†</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>572 (85.4)</td>
<td>95 (14.6)</td>
<td>1 (reference)</td>
<td>–</td>
</tr>
<tr>
<td>All RA patients</td>
<td>827 (90.5)</td>
<td>86 (9.5)</td>
<td>1.70 (1.15–2.51)</td>
<td>0.0082</td>
</tr>
<tr>
<td>RF-positive RA patients</td>
<td>745 (91.2)</td>
<td>72 (8.8)</td>
<td>1.98 (1.31–3.00)</td>
<td>0.0013</td>
</tr>
<tr>
<td>RF-negative RA patients</td>
<td>67 (82.7)</td>
<td>14 (17.3)</td>
<td>0.83 (0.42–1.63)</td>
<td>0.59</td>
</tr>
<tr>
<td>Anti-CCP antibody–positive RA patients</td>
<td>507 (90.9)</td>
<td>51 (9.1)</td>
<td>1.92 (1.22–3.01)</td>
<td>0.0048</td>
</tr>
<tr>
<td>Anti-CCP antibody–negative RA patients</td>
<td>82 (82.8)</td>
<td>17 (17.2)</td>
<td>0.82 (0.44–1.54)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* Values are the number (%) of subjects. SNP = single-nucleotide polymorphism; RA = rheumatoid arthritis; RF = rheumatoid factor; anti-CCP = anti–cyclic citrullinated peptide.
† Odds ratios (ORs), 95% confidence intervals (95% CIs), and P values were calculated using logistic regression with adjustment for age and HLA–DRB1 genotype. Heterozygous female subjects and carriers of rare haplotypes were excluded from these analyses.
were examined by measurements of NF-κB activation as previously described (24,25).

The major and minor haplotype variants of IRAK1 cDNA were transiently overexpressed separately in human 293T cells, along with a luciferase gene under an NF-κB responsive promoter. We measured luciferase activity, which directly reflected the intracellular activity of NF-κB and indirectly reflected IRAK1 activity (Figure 2). Because exogenous IRAK1 was actually auto-phosphorylated to activate NF-κB (data not shown), luciferase activity was measured after 24 hours of cell culture without signal-inducing interleukin-1β (IL-1β). The luciferase activity differed significantly between the 2 variant-expressing cells (P = 1.3 × 10⁻¹⁰). The major haplotype showed 1.7-fold higher luciferase activity than the minor haplotype, suggesting that the major haplotype variant of IRAK1 had both higher IRAK1 activity and higher NF-κB activity than the minor haplotype variant.

**Lack of an association between local SNPs and IRAK1 and MECP2 mRNA levels.** Using the gene expression profiling data for 270 participants in the International HapMap Project available in the Gene Expression Variation database (ftp://ftp.sanger.ac.uk/pub/genevar) (26), we examined whether IRAK1 or MECP2 mRNA levels were associated with 12 local SNPs that had been typed for all 4 populations included in the study.

**Figure 2.** Functional tests of 2 nonsynonymous IRAK1 SNPs. Human 293T cells were cotransfected with 0.15 μg of a pFLAG CMV2 vector (pFC) expressing a variant of IRAK1 cDNA (which was absent in the control cells), and with 0.5 μg of a pGL4.32 vector expressing the luciferase gene under an NF-κB responsive promoter. Two variants of IRAK1 cDNA were constructed and separately tested for their influences on the intracellular activity of NF-κB, as measured by relative luciferase activity. One variant (pFC-R) carried the RA risk-associated major haplotype of IRAK1 referring to the Phe196Ser- and Ser532Leu-encoding SNPs. The other variant (pFC-N) carried the non-risk-associated, minor haplotype. The 2 IRAK1 variants were assayed in parallel in a 12-well culture plate. Values are the mean ± SD from 4 independent experiments. See Figure 1 for other definitions.

**Figure 3.** Tests for association of SNPs with levels of mRNA for MECP2 and IRAK1 and with copy number variation. A, Relative IRAK1 and MECP2 mRNA levels in subjects with the C allele or T allele of SNP rs1059702. All 270 participants in the International HapMap Project, except for heterozygous females, were divided into 2 groups according to their rs1059702 genotype (CC for women and C for men or TT for women and T for men), which was retrieved from the phase II project database (http://hapmap.ncbi.nlm.nih.gov). Data on MECP2 and IRAK1 mRNA levels in the individual subjects were retrieved from the Gene Expression Variation database (ftp://ftp.sanger.ac.uk/pub/genevar). Student’s t-test was used for comparison of the 2 groups. B, Cellular levels of IRAK1a, IRAK1b, and MECP2A in subjects with the C allele or T allele of rs1059702. Cellular amounts of IRAK1a, IRAK1b, IRAK1c, MECP2A, and MECP2B mRNA isoforms in Epstein-Barr virus–transformed B cells from 10 control subjects in this study (6 subjects who were homozygous for the major allele and 4 subjects who were homozygous for the minor allele of rs1059702) were determined by quantitative reverse transcription–polymerase chain reaction (PCR). Their association with rs1059702 was analyzed as described in A. Values in A and B are the mean ± SD. C, Copy numbers of a block of MECP2 and IRAK1 in 134 Korean male subjects (110 RA patients and 24 controls) in this study, determined by real-time quantitative PCR. Samples are ranked by copy number. See Figure 1 for other definitions.
in the HapMap Project (Japanese in Tokyo, Japan; Han Chinese in Beijing, China; Utah residents with ancestry from northern and western Europe [CEU]; and Yoruba in Ibadan, Nigeria). Heterozygous individuals were excluded from these analyses because of the X inactivation issue. No significant association was observed between the expression levels of IRAK1 or MECP2 and rs1059702 (Figure 3A) or any other SNP tested ($P = 0.01–0.99$), after correction for multiple testing.

Next, the levels of mRNA for IRAK1 and MECP2 were experimentally measured in 10 Epstein-Barr virus–transformed B cell lines derived from the female control subjects who were homozygous for the major alleles ($n = 6$) or homozygous for the minor alleles ($n = 4$) in the nonsynonymous IRAK1 SNPs (rs1059702 and rs1059703). Among the 3 alternative splicing isoforms of IRAK1 mRNA, IRAK1a, IRAK1b (24), and IRAK1c (25), and the 2 isoforms of MECP2 mRNA, MECP2A and MECP2B (27), IRAK1c and MECP2B mRNA were rarely detected by PCR in any of the 10 cell lines. The levels of mRNA for IRAK1a, IRAK1b, and MECP2A were measured using quantitative reverse transcription–PCR but were not significantly different between the 2 haplotypes of rs1059702 and rs1059703 (Figure 3B).

Absence of copy number variation. Because a copy number variation was previously reported in a genomic region harboring MECP2 and IRAK1 in a Caucasian population (28), samples from 134 male subjects (approximately one-half of the total number of male subjects [$n = 278$]), including 110 RA cases and 24 controls, were examined for copy number variation using quantitative PCR. Because the reported copy number variation block was large enough to cover both MECP2 and IRAK1, amplification of intron 2 of MECP2 alone was used for quantification of gene copies in the single X chromosome. The copy number measured ranged from 0.74 to 1.33 (Figure 3C), and was never close to 0 or 2. Accordingly, the previously reported copy number variation was not detected in Korean male subjects, suggesting an absence of copy number variation in this locus either among Koreans or among RA patients.

DISCUSSION

Multiple SNPs in a block including IRAK1 and MECP2 were associated with RA susceptibility in a large Korean population in this study. In particular, when the 2 common haplotypes of 2 highly correlated nonsynonymous SNPs in IRAK1 (rs1059702 and rs1059703) were compared, the major haplotype was associated with a 1.7-fold increase in the odds of having RA, and with increased cellular activity of IRAK1 as demonstrated by a 1.7-fold increase in the expression of a reporter gene under an NF-κB responsive promoter. Taken together, these findings indicate that IRAK1 contributes to RA pathogenesis.

IRAK1 is one of the serine/threonine protein kinases associated with signals downstream of the Toll/IL-1 receptor (TIR) family (29). Upon stimulation by TIR-specific ligands, IRAK1 is autophosphorylated at multiple residues to dissociate from a TIR complex, and subsequently activates the transcription factor NF-κB (29,30). Activated NF-κB is a crucial mediator of the expression of proinflammatory cytokines. In synovial fibroblasts from RA patients, stimulation of Toll-like receptor 2 increases the expression of proinflammatory cytokines such as IL-6, tissue-destructive matrix metalloproteinases (31), and chemokines (32). These previous findings are consistent with our finding that a variant of IRAK1 leading to increased intracellular activity of NF-κB is associated with increased susceptibility to RA, supporting the notion of involvement of the TIR complex downstream pathway in RA pathogenesis.

Increased nuclear levels of NF-κB have previously been shown to be associated with an IRAK1 haplotype in Caucasian patients with sepsis (33). This haplotype harbored the alleles of 8 SNPs: rs3027898, rs731642, rs2239673, rs5945174, rs7061789, rs1059701, rs1059702, and rs1059703. The 4 underlined SNPs, including the 2 above-mentioned nonsynonymous SNPs, were found to be associated with RA risk in this study (Figure 1B).

RA susceptibility is believed to be associated with at least the 18 SNPs listed in Figure 1B. They all constitute a large linkage disequilibrium block of 79 kb, which harbors both MECP2 and IRAK1. They were not independent from each other in pairwise conditional regression analysis and are all in strong linkage disequilibrium with each other ($0.85 \leq D' \leq 1.00$), which does not allow for further localization of RA susceptibility between MECP2 and IRAK1. Therefore, both genes should remain candidates. In fact, a recent study demonstrated that both MECP2 and IRAK1 were functionally affected by the SNPs in this locus that are associated with SLE, including the nonsynonymous IRAK1 SNP rs1059702, since the homozygous risk genotype increasing IRAK1-mediated NF-κB activity was associated with lower levels of mRNA for MECP2 (34).  

Among the 18 SNPs associated with RA (Figure 1B), a SNP (rs3027898) located just downstream of
IRAk1 has recently been shown to be associated with RA susceptibility in a small Greek population (n = 150) (14), but another SNP (rs1059703) did not show a significant association although it was highly correlated with rs3027898 in the CEU population (r² = 0.93) in the same study. In another study of a Spanish population, a SNP in MECP2 (rs17435) was not associated with RA susceptibility (16) although it was highly correlated with rs1059703 in the CEU population (r² = 0.86). Recent genome-wide association studies using Caucasian subjects did not find an association between RA and any SNP on the X chromosome (35–37). Accordingly, the association of MECP2–IRAk1 with RA susceptibility in Caucasians is a subject of controversy and needs to be re-evaluated in large studies.

Common variants in MECP2 or IRAk1 that have previously been shown to be associated with SLE, primary Sjögren’s syndrome, or systemic sclerosis included a SNP identified as being associated with RA in the present study, rs1059702 (13), and SNPs with which it is highly correlated, rs17435 (12), rs763737 (8), and rs1734792 (10,11). Thus, a functional polymorphism in IRAk1 or MECP2 can be associated with multiple autoimmune diseases. In addition, rare mutations in MECP2 have been reported in Rett syndrome (38), a neurodevelopmental disorder, but we did not examine any rare mutations in this study.

In summary, we identified a haplotype in a human chromosome Xq28 locus that is associated with susceptibility to RA, an autoimmune disease that predominantly affects women, and with increased cellular activity of IRAk1. This RA-associated haplotype appears to be largely overlapping with, if not the same as, a haplotype that has been shown to be associated with another autoimmune disease that predominantly affects women, SLE, by not only increasing IRAk1 activity but also decreasing MECP2 mRNA levels. Our findings specifically suggest that the IRAk1-related inflammatory pathway may be a common mechanism underlying the etiology of multiple autoimmune diseases.

ACKNOWLEDGMENTS

The authors are grateful to Drs. Kwangwoo Kim and Suk-Jo Kang for critical discussion, and to Jung-Ah Kim, Young-Hi Lee, and Eun-Kyoung Ju for assistance in sample preparation and data collection.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Bae and Kang had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Han, Bae, Kang.

Acquisition of data. Han, Cho, Kim, Joo, Bae.

Analysis and interpretation of data. Han, Cho, Kim, Joo, Bae, Kang.

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