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Genome-wide association study in a Korean population identifies six novel susceptibility loci for rheumatoid arthritis

Young-Chang Kwon,¹ Jiwoo Lim,² So-Young Bang,^{1,3} Eunji Ha,² Mi Yeong Hwang,⁴ Kyungheon Yoon,⁴ Jung-Yoon Choe,⁵ Dae-Hyun Yoo ,^{1,3} Shin-Seok Lee,⁶ Jisoo Lee,⁷ Won Tae Chung,⁸ Tae-Hwan Kim,^{1,3} Yoon-Kyoung Sung ,^{1,3} Seung-Cheol Shim,⁹ Chan-Bum Choi,^{1,3} Jae-Bum Jun ,^{1,3} Young Mo Kang,¹⁰ Jung-Min Shin,³ Yeon-Kyung Lee,³ Soo-Kyung Cho ,^{1,3} Bong-Jo Kim,⁴ Hye-Soon Lee,^{1,3} Kwangwoo Kim ,² Sang-Cheol Bae ^{1,3}

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For numbered affiliations see end of article.

Correspondence to

Professor Sang-Cheol Bae, Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, Republic of Korea; scbae@hanyang.ac.kr and Professor Kwangwoo Kim, Department of Biology and Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul, Republic of Korea; kkim@khu.ac.kr

Y-CK, JL and S-YB contributed equally.

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ABSTRACT

Objective Genome-wide association studies (GWAS) in rheumatoid arthritis (RA) have discovered over 100 RA loci, explaining patient-relevant RA pathogenesis but showing a large fraction of missing heritability. As a continuous effort, we conducted GWAS in a large Korean RA case–control population.

Methods We newly generated genome-wide variant data in two independent Korean cohorts comprising 4068 RA cases and 36 487 controls, followed by a whole-genome imputation and a meta-analysis of the disease association results in the two cohorts. By integrating publicly available omics data with the GWAS results, a series of bioinformatic analyses were conducted to prioritise the RA-risk genes in RA loci and to dissect biological mechanisms underlying disease associations.

Results We identified six new RA-risk loci (*SLAMF6*, *CXCL13*, *SWAP70*, *NFKB1A*, *ZFP36L1* and *LINC00158*) with $p_{\text{meta}} < 5 \times 10^{-8}$ and consistent disease effect sizes in the two cohorts. A total of 122 genes were prioritised from the 6 novel and 13 replicated RA loci based on physical distance, regulatory variants and chromatin interaction. Bioinformatic analyses highlighted potentially RA-relevant tissues (including immune tissues, lung and small intestine) with tissue-specific expression of RA-associated genes and suggested the immune-related gene sets (such as CD40 pathway, IL-21-mediated pathway and citrullination) and the risk-allele sharing with other diseases.

Conclusion This study identified six new RA-associated loci that contributed to better understanding of the genetic aetiology and biology in RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex autoimmune disorder characterised by chronically inflamed joints and autoantibody production. A combination between genetic background and environmental triggers confers an increased risk for RA. The overall heritability of RA has been estimated to be about 50%–65% in twin studies.^{1,2} Genome-wide association studies (GWAS) have discovered over 100 RA-associated genetic variants in multiple ancestries.^{3,4} However, these loci collectively

Key messages

What is already known about this subject?

- Genome-wide association studies (GWAS) have identified >100 susceptibility loci for rheumatoid arthritis (RA).
- Although the heritability of RA was estimated at 50%–65% in twin studies, previously reported loci were able to explain only about 15% of the total phenotypic variance for RA.

What does this study add?

- We identified six new RA-risk loci (*SLAMF6*, *CXCL13*, *SWAP70*, *NFKB1A*, *ZFP36L1* and *LINC00158*) that reached the genome-wide significance threshold ($p=5.0 \times 10^{-8}$) through a meta-analysis of newly generated GWAS results for 4068 RA cases and 36 487 healthy controls in a Korean population.
- A series of bioinformatic analyses using the GWAS results identified 122 RA-relevant gene candidates from novel and known RA loci and suggested important roles of several tissues and gene sets in RA development.

How might this impact on clinical practice or future developments?

- Our finding about novel RA loci refines the known genetic architecture of RA and provides genetic biomarkers for RA.

explained only a small portion (about 15%) of the phenotypic variance for RA.^{4,5} Thus, continuous effort in identifying additional RA variants is necessary to better understand the disease aetiology. Despite the substantial missing fraction of heritability, the reported RA loci have provided insights into the pathogenesis of RA by the so-called post-GWAS analysis that integrates GWAS data with multiple biological resources.⁴

Here, we performed GWAS to identify novel loci exceeding the genome-wide significance threshold ($p=5 \times 10^{-8}$) in Korean cohorts comprising 4068 RA cases and 36 487 controls, followed by

subsequent post-GWAS analyses for prioritising RA-relevant genes and identifying variant-highlighted biology.

METHODS

GWAS participants

A total of 4068 RA cases and 36487 healthy controls from two independent case-control cohorts were analysed (3177 RA cases and 32820 controls in cohort #1; 891 RA cases and 3667 controls in cohort #2). All the cases were recruited from eight participating university hospitals in Korea and diagnosed through the 1987 revised American College of Rheumatology RA classification criteria.⁶ Anti-citrullinated protein antibodies (ACPAs) were positive in 83.2%, negative in 14.6% and not examined in 2.2% of participants. The controls were recruited through the KoGES and Hanyang University Hospital for Rheumatic Diseases. The genomic DNAs from the KoGES samples were stored in the National Biobank of Korea. All participants provided written informed consent for the study and the Institutional Review Board of Hanyang University approved this study.

Genotyping and whole genome imputation

Cohort #1 was newly genotyped with a customised genotyping array, Korea Biobank Array (KoreanChip).⁷ Genotyping of the RA cases of cohort #2 was performed on an Illumina HumanOmni2.5Exome-8 BeadChip, while the control genotype data were produced using Illumina Human Omni1-Quad BeadChip. The overlapping variants between cases and controls in cohort #2 were merged and used in the downstream analyses. The genotyping data for each cohort were filtered based on the general criteria to retain good-quality genetic data (~465 K variants in set #1 of 3177 cases and 32820 controls; ~559 K variants in set #2 of 891 cases and 3667 controls) that showed a high call rate per individual and variant (≥ 0.99), Hardy-Weinberg equilibrium ($p_{HWE} \geq 5 \times 10^{-6}$), no excessive heterozygosity, no difference in call rates per variant between cases and controls ($p \geq 5 \times 10^{-4}$), no cryptic first-degree relatedness among individuals, homogeneous genetic background among individuals and minor allele frequency (MAF) ≥ 0.005 .

Imputation for autosomal variants was performed by Eagle2⁸ and IMPUTE4⁹ using the reference panel constructed from the 1000 Genomes Project (1KGP) phase 3¹⁰ reference panel and whole genome sequencing data for 397 Koreans (Korean Personal Genome Project).¹¹ Each imputation chunk included 10000 variants with a 2Mb buffer region. Imputation for the non-pseudo-autosomal region of the X chromosome was performed by Shapeit2¹² and Minimac3¹³ with the 1KGP phase 3¹⁰ reference panel, making imputation chunks of 5 Mb regions with 1 Mb buffer. The numbers of the imputed variants with good quality (showing imputation quality index ≥ 0.3 , MAF $\geq 0.5\%$ and p_{HWE} in control $\geq 1 \times 10^{-6}$) were 10934705 in cohort #1 and 10868813 in cohort #2. These variants were analysed in the subsequent analyses.

Association analysis

The genetic association between RA and each autosomal variant was tested by logistic regression adjusting for gender and the top four principal components using EPACTS.¹⁴ The association analysis for the X chromosome variants was performed separately for females and males by the same model excluding the gender variable. An inverse-variance-weighted fixed-effects meta-analysis for the genomic-control association results in the two independent cohorts were conducted using METAL.¹⁵ To investigate statistically independent association signals within

each RA locus, we performed a conditional analysis by GCTA-COJO¹⁶ using the association summary statistics based on the reference linkage disequilibrium (LD) calculated in cohort #1.

Gene set and tissue-specific expression analysis

Gene-level association p values for 19840 protein-coding genes were calculated by MAGMA¹⁷ using a variant-wide mean model based on variant-level association summary statistics within and around genes. Statistical significance for the association of the gene-level Z scores (converted from gene-level p values) with MSigDB¹⁸ gene sets including curated gene sets and gene ontology terms and tissue-specific gene expression in 54 tissues in Genotype-Tissue Expression (GTEx) v8 RNA Sequencing (RNA-Seq) data¹⁹ were tested according to the MAGMA¹⁷ regression models.

Enrichment analysis for tissue-specific histone modifications

GREGOR²⁰ was deployed to calculate the enrichment estimates of RA variants within four tissue-specific histone marks (H3K4me1, H3K4me3, H3K27ac and H3K27me3) from Roadmap Epigenomics Project.²¹

Gene prioritisation in RA loci

For each RA locus, we identified a lead variant with the lowest p_{meta} value within a physically (± 300 kb) or genetically ($r^2 > 0.1$) defined region. FUMA²² was then employed to identify the most likely RA-relevant genes based on the lead RA-risk variants and the meta-analysis association summary statistics according to the following three mapping strategies—(1) Positional mapping: this strategy found genes within and 10 kb around the region containing a lead variant and its proxy variants in each RA locus. (2) Expression quantitative trait locus (eQTL) mapping: this mapping collected the genes regulated by known eQTL in LD with lead RA-risk variants in blood, spleen, lung and small intestine. The known eQTL was retrieved from GTEx,¹⁹ single-cell RNA-seq data in peripheral blood mononuclear cells,²³ and DICE immune-cell data.²⁴ (3) Chromatin interaction mapping: this approach found genes making promoter-involved or enhancer-involved chromatin interactions with the region with RA-risk variants in blood, spleen, lung and small intestine based on the GSE87112 (Hi-C) data²⁵ and FANTOM5²⁶ annotations.

Variant-based heritability

An LD score regression method²⁷ was performed to estimate the genome-wide and partitioned heritability²⁸ using East Asian LD scores from the 1KGP phase 3¹⁰ and the association summary statistics, assuming an RA prevalence of 0.01.

Ancestral genetic correlation of RA associations between Koreans and Europeans

A correlation of RA GWAS between Korean and European⁵ populations was examined by the Popcorn²⁹ programme accounting for the ancestry-specific LD scores and cross-covariance scores in two ancestries in the 1KGP.

Correlation analysis of genetic architectures among diseases

We assessed the correlation of genome-wide disease associations between RA and other diseases ($n=28$; from Biobank Japan Project^{30,31} and Bentham *et al*³²) using the LD score regression method.²⁷ The association summary statistics in 27 diseases excluding systemic lupus erythematosus were estimated from Japanese populations, whose LD structure is highly consistent with Korean ancestry.³³ The data for systemic lupus

erythematosus were generated from European case-control populations. Each set of genome-wide association summary statistics was generated using >1000 cases and displayed at least one disease locus with $p < 5 \times 10^{-8}$.

RESULTS

Identification of six novel RA loci

To identify new RA risk loci in a Korean population, we performed a fixed-effects meta-analysis of the RA association statistics of the newly generated, imputed variants in two independent Korean cohorts comprising 4068 RA cases and 36487 controls. There were 10404202 intersecting variants between the two cohorts. The genomic control inflation factors (λ_{GC}) of the meta-analysis results including and excluding the major histocompatibility complex (MHC) region were estimated to be 1.035 and 1.020, respectively, showing no substantial population stratification in a quantile-quantile plot (online supplementary figure 1).

We identified 19 loci surpassing the genome-wide significance threshold (figure 1 and table 1). Of these loci, six (*SLAMF6*, *CXCL13*, *SWAP70*, *NFKB1A*, *ZFP36L1* and *LINC00158*; figure 2) were novel loci that showed highly consistent effect sizes on the risk of RA in the two cohorts (table 1). The other 13 loci including the MHC region with the most significant association in the human genome had been reported in other RA genetic studies^{3,4} (figure 1 and table 1). No secondary association signals in novel RA loci were detected in conditional analyses.

The overall variant-based heritability for RA on the liability scale was estimated to be 43.04%. The contribution of the non-MHC regions to heritability was 18.60%. When partitioned by the known non-MHC risk loci and the six novel loci, the heritability for RA was 6.65% from the known loci and 0.65% from the novel loci. The genetic correlation of RA associations between Korean and European⁵ populations was strong in a genome-wide level (correlation coefficient $r=0.76$; $p=1.3 \times 10^{-5}$) and in 100 known and six novel RA variants ($r=0.76$; online supplementary table 3 and figure 4).

Asian-specific RA-risk missense variant in the *SH2B3* loci

Among the replicated known loci in this study, the RA association of the *SH2B3* locus was previously explained by the intergenic variant rs10774624 in European populations⁵ that were neither common (MAF=0.56%) nor associated with RA ($p_{meta}=0.64$) in Korean populations (online supplementary table 3). However, we discovered that the *SH2B3* missense variant (rs78894077, c.724C>T of exon 2; p.Phe242Ser of the PH domain) was associated with RA in the same locus ($p_{meta}=3.89 \times 10^{-9}$). Interestingly, the RA-risk allele T (p.242Ser) of rs78894077 was frequent in Korean populations (MAF=7.43%) but absent in other non-Asian ancestries according to the 1KGP phase 3 data. The RA-risk allele of the same variant in *SH2B3* was also reported to associate with the increased risk of myeloproliferative neoplasms in East Asian populations.^{34,35}

Tissues and gene sets implicated by genome-wide association results

We calculated gene-level disease association p values from the variant-level association summary statistics using MAGMA¹⁷ and tested for the association between gene-level Z scores (converted from the gene-level p values) and tissue-specific expression level to provide deeper insights into the underlying disease-driving tissues in RA development. The analysis revealed five significant tissues with tissue-specific expression of

Figure 1 Genome-wide association analysis results for RA in a Korean population. A meta-analysis of genome-wide association results in two independent Korean cohorts identified 19 RA loci including 6 novel and 13 known loci. The minus $\log_{10}$ -transformed association p values for the variants were plotted against their chromosomal positions. Novel and known RA-associated loci exceeding the genome-wide significance threshold (indicated by a red vertical line) are displayed with locus names in red and black, respectively. RA, rheumatoid arthritis.

RA-associated genes, including spleen ($p=1.50 \times 10^{-9}$), Epstein-Barr virus (EBV)-transformed lymphocytes ($p=5.32 \times 10^{-9}$), whole blood ($p=1.12 \times 10^{-6}$), lung ($p=2.99 \times 10^{-4}$) and small intestine ($p=7.36 \times 10^{-4}$), among 54 different tissues based on GTEx v8 RNA-Seq data¹⁹ (figure 3A and online supplementary table 1). Consistent with the association between the tissue specificity of gene expression and gene-level associations, we identified a significant enrichment of the identified lead and proxy variants within three transcription-activating histone marks (H3K4me1, H3K4me3 and H3K27ac) in the same tissues in a GREGOR²⁰ enrichment analysis using Roadmap Epigenomic data²¹ at a false discovery rate (FDR) threshold of 5%

Table 1 RA associations of 19 loci in a meta-analysis

Variant	Chr:Pos*	Gene†	Cohort #1		Cohort #2		Meta-analysis		
			OR (95% CI)‡	P value	OR (95% CI)‡	P value	OR (95% CI)‡	p _{meta}	p _{Het} §
rs11464953	1:17645185	<i>PADI4</i>	1.23 (1.16–1.29)	1.5×10 ⁻¹³	1.25 (1.12–1.40)	8.9×10 ⁻⁵	1.23 (1.17–1.29)	7.0×10 ⁻¹⁶	0.73
rs148363003	1:160443973	<i>SLAMF6</i>	1.63 (1.39–1.92)	3.5×10 ⁻⁹	1.59 (1.12–2.27)	1.0×10 ⁻²	1.62 (1.39–1.89)	5.1×10 ⁻¹⁰	0.91
rs11889341	2:191943742	<i>STAT4</i>	1.21 (1.14–1.28)	6.3×10 ⁻¹¹	1.26 (1.12–1.41)	1.3×10 ⁻⁴	1.22 (1.15–1.28)	2.9×10 ⁻¹³	0.57
rs117605225	4:78508197	<i>CXCL13</i>	0.66 (0.57–0.76)	1.6×10 ⁻⁸	0.59 (0.40–0.87)	7.8×10 ⁻³	0.65 (0.56–0.75)	1.9×10 ⁻⁹	0.63
rs112062732	6:32453936	<i>HLA-DRB1</i>	2.38 (2.25–2.52)	1.1×10 ⁻¹⁹³	2.79 (2.45–3.19)	1.6×10 ⁻⁵²	2.44 (2.31–2.58)	5.5×10 ⁻²²⁷	0.034
rs1044690	6:44245241	<i>NFKBIE</i>	1.19 (1.12–1.27)	1.4×10 ⁻⁸	1.20 (1.06–1.37)	4.6×10 ⁻³	1.20 (1.13–1.27)	9.0×10 ⁻¹⁰	0.92
rs9494893	6:138223490	<i>TNFAIP3</i>	1.40 (1.27–1.55)	4.7×10 ⁻¹¹	1.15 (0.92–1.45)	0.23	1.36 (1.23–1.49)	3.5×10 ⁻¹⁰	0.13
rs3093019	6:167539655	<i>CCR6</i>	0.83 (0.78–0.87)	2.3×10 ⁻¹²	0.84 (0.75–0.94)	2.0×10 ⁻³	0.83 (0.79–0.87)	1.4×10 ⁻¹³	0.85
rs113066392	7:74026152	<i>GTF2IRD1</i>	1.46 (1.35–1.57)	6.3×10 ⁻²²	1.22 (0.95–1.57)	0.12	1.43 (1.33–1.55)	1.3×10 ⁻²⁰	0.20
rs71508903	10:63779871	<i>ARID5B</i>	1.27 (1.19–1.36)	2.9×10 ⁻¹³	1.12 (0.98–1.28)	0.11	1.24 (1.17–1.32)	2.4×10 ⁻¹²	0.092
rs360136	11:9773517	<i>SWAP70</i>	1.14 (1.08–1.20)	1.4×10 ⁻⁶	1.31 (1.18–1.46)	1.2×10 ⁻⁶	1.17 (1.11–1.23)	4.5×10 ⁻¹⁰	0.026
rs78894077	12:111856673	<i>SH2B3</i>	1.30 (1.19–1.43)	3.6×10 ⁻⁸	1.42 (1.10–1.83)	7.2×10 ⁻³	1.32 (1.20–1.45)	3.9×10 ⁻⁹	0.56
rs111597524	14:35861800	<i>NFKBIA</i>	1.18 (1.12–1.26)	1.3×10 ⁻⁸	1.15 (1.01–1.31)	3.5×10 ⁻²	1.18 (1.12–1.24)	5.3×10 ⁻⁹	0.68
rs194757	14:69213851	<i>ZFP36L1</i>	0.87 (0.83–0.92)	2.9×10 ⁻⁶	0.81 (0.72–0.91)	3.4×10 ⁻⁴	0.86 (0.82–0.91)	2.5×10 ⁻⁸	0.24
rs2841269	14:105386149	<i>PLD4</i>	0.80 (0.76–0.85)	5.9×10 ⁻¹⁵	0.82 (0.72–0.92)	7.5×10 ⁻⁴	0.80 (0.76–0.85)	2.4×10 ⁻¹⁶	0.77
rs8032939	15:38834033	<i>RASGRP1</i>	0.84 (0.80–0.89)	1.6×10 ⁻¹⁰	0.93 (0.83–1.04)	0.18	0.86 (0.81–0.90)	9.5×10 ⁻¹⁰	0.13
rs1547233	21:26769507	<i>LINC00158</i>	1.17 (1.10–1.24)	8.4×10 ⁻⁸	1.18 (1.05–1.33)	6.3×10 ⁻³	1.17 (1.11–1.24)	6.4×10 ⁻⁹	0.92
rs2049985	22:39756650	<i>SYNGR1</i>	0.78 (0.72–0.85)	1.2×10 ⁻⁹	0.89 (0.75–1.05)	0.17	0.80 (0.74–0.86)	4.1×10 ⁻⁹	0.20
rs1059702	X:153284192	<i>IRAK1</i>	0.86 (0.81–0.91)	8.2×10 ⁻⁷	0.74 (0.65–0.84)	3.3×10 ⁻⁶	0.84 (0.79–0.89)	4.9×10 ⁻¹⁰	0.038

*Based on hg19.

†Novel RA loci are indicated in bold.

‡Frequencies, ORs and CIs for EAs.

§P values for cross-cohort association heterogeneity (p_{Het}) were calculated by Cochrane's Q tests.

Chr, chromosome; EAs, effect alleles; Freq, frequency; NEA, non-effect allele; Pos, Position; RA, rheumatoid arthritis.

(figure 3B). These results are supported by the well-documented knowledge about the aetiological roles of immune tissues and non-immune tissues in RA (eg, the increased risk of RA by EBV

infection to B cells,³⁶ the early production of RA autoantibody in lung³⁷ and the enhanced inflammation in small intestine in RA³⁸).

Figure 2 Regional association plots for novel RA-associated loci. Regional association plots for the six new RA loci: (A) *SLAMF6*, (B) *CXCL13*, (C) *SWAP70*, (D) *NFKBIA*, (E) *ZFP36L1* and (F) *LINC00158*, represent the minus log₁₀-transformed p values for variants in each locus according to their genomic positions. The lead variants are indicated by purple points and the other variants are coloured according to r² values with the lead variant in each locus. The names of lead variants and their closest genes are given on the top of the plots. RA, rheumatoid arthritis.

Figure 3 Tissue-specific gene expression in RA loci including the *SLAMF6* locus. (A) Tissue-specific gene expression levels in 54 GTEx tissues were analysed by MAGMA to identify tissue-specific expression of RA-associated genes. Five significant tissues (spleen, EBV-transformed lymphocytes, whole blood, lung and small intestine) are highlighted in red bars. The dashed line represents the Bonferroni-corrected significance threshold ($=0.05/54$). (B) Tissue-specific enrichment of RA variants within histone marks was analysed by GREGOR using Roadmap Epigenomics Project data. FDR-corrected p values for the overlap between RA-risk variants in 19 RA loci and 4 tissue-specific histone marks in 9 tissues were plotted with asterisks indicating significant enrichments with three transcription-activating histone marks in 5 tissues at the FDR threshold of 5% (represented by a dashed line). (C) The Circos plot for the *SLAMF6* locus summarises the physical location (blue) with RA-risk variants and chromatin interaction (orange loops) involving both the RA-risk variant and the tissue-specific regulatory regions (including promoters and enhancers) in the five identified tissues. The outer layer in the Circos plot represents the minus $\log_{10}$ -transformed RA association p values of variants according to the chromosomal position. The *SLAMF6* variant (rs148363003) had chromatin interactions with 12 other coding genes (*ARHGAP30*, *CD48*, *CD84*, *IGSF8*, *ITLN1*, *KCNJ10*, *LY9*, *PEA15*, *SLAMF1*, *SLAMF7*, *TSTD1* and *USF1*) on chromosome 1. (D) The heat map of gene expression levels represents the predominant expression levels of 11 prioritised RA-relevant RA genes in the 5 identified tissues (indicated by red tissue names and black box lines) among 54 tissues. The 11 RA genes that clustered in a gene expression analysis include known RA-associated genes and *SLAMF* genes (*SLAMF6*, *LY9*, *SLAMF1*, *SLAMF7*, *CD48* and *CD84*). EBV, Epstein-Barr virus; RA, rheumatoid arthritis.

In addition, a MAGMA¹⁷ gene set analysis was performed using the gene-level Z scores and the predefined gene sets in MSigDB.¹⁸ The results suggested important roles of several immune-related gene sets such as the CD40 pathway ($p=1.62 \times 10^{-14}$), citrullination ($p=8.60 \times 10^{-13}$), IL-21-mediated signalling pathway ($p=3.66 \times 10^{-9}$) and lymphocyte activation ($p=4.53 \times 10^{-8}$) in developing RA (online supplementary table 2).

Prioritisation of RA-relevant genes in RA loci

Using the genome-wide association results, we further identified 122 potentially RA-relevant genes within 18 non-MHC RA loci by mapping the genes positioned on, cis-regulated by, and chromatin-interacted with RA-risk variants in blood, spleen, lung and small intestine (online supplementary table 3). Of the novel loci, the RA-risk variant in *SLAMF6* (rs148363003) showed multiple chromatin interactions with several genes including five signalling lymphocytic activation molecule family (*SLAMF*) genes: *SLAMF1*, *CD48* (also known as *SLAMF2*), *CD84* (also known as *SLAMF5*), *LY9* (also known as *SLAMF3*) and *SLAMF7*

(figure 3C). Interestingly, the expression levels of *SLAMF6* and other interacting *SLAMF* genes were tissue-specifically over-expressed mostly in spleen, EBV-transformed B lymphocytes, whole blood, lung and small intestine terminal ileum. Similar tissue-specific expression patterns were observed in other well-known RA genes including *PADI4*, *CCR6* and *NCF1* (figure 3D).

Genetic correlation with other diseases

To assess the shared genetic risk between RA and other complex diseases using GWAS summary statistics, we calculated the genetic correlation of genome-wide disease associations between RA (in our Korean cohorts) and other diseases (27 diseases in Japanese populations^{30 31} and systemic lupus erythematosus in a European population³²). The genetic architecture of RA was positively correlated with that of RA (in Japanese populations), Graves' disease, systemic lupus erythematosus and atrial fibrillation, while a negative correlation was found with chronic hepatitis B infection and gastric cancer (online supplementary figure 2). The degrees of genetic correlations with RA were high in autoimmune

diseases such as Graves' disease ($r=0.39$) and systemic lupus erythematosus ($r=0.33$) (online supplementary figure 2).

Consistently, the prioritised RA-relevant genes in non-MHC RA loci in this study were significantly enriched in the susceptibility loci of inflammatory diseases such as systemic lupus erythematosus and inflammatory bowel disease at an FDR threshold of 0.05 ($p \leq 2.43 \times 10^{-7}$ in hypergeometric tests), according to GWAS catalog³⁹ (online supplementary figure 3).

DISCUSSION

In this study, we employed a new genome-wide array, KoreanChip, for the majority of the study subjects. The customised array KoreanChip has an extensive genomic coverage and powerful imputation performance for low-frequency variants in Korean ancestry compared with other commercial GWAS arrays with a similar number of probes. A high-density variant association result and a meta-analysis with another independent cohort helped us perform a powerful analysis to assess the genomic boundaries, the reliability (or cross-cohort heterogeneity) of disease association signals and functional annotations of RA loci.

The meta-analysis of GWAS data in Korean cohorts identified 19 susceptibility loci for RA, of which 6 loci have not been reported before, although there was considerably large missing heritability⁴⁰ and a lack of functional studies. Of these new loci for RA, previous functional studies on the products of *SLAMF6*, *CXCL13* and *SWAP70* have been associated with RA or other inflammatory diseases. *SLAMF6* is a member of superfamily immunoglobulin (Ig) domain-containing molecules that are involved in haemophilic interactions between naive B and T cells.⁴¹ *SLAMF6* acts as a costimulatory molecule on the surface of T lymphocytes to increase T-cell adhesiveness by activating the small GTPase Rap1, promoting the TCR downstream signal transduction and the differentiation to Th17 cells expressing IL-17A.⁴² *SLAMF* members including *SLAMF6* have been proposed as potential therapeutic targets for inflammatory and autoimmune diseases.⁴³ In this study, RA-risk variants in *SLAMF6* including rs148363003 had chromatin interaction with five *SLAMF* genes and the expression level of these six *SLAMF* genes in the locus was high in most of the potentially RA-relevant tissues. In other studies on rheumatic diseases, a *SLAMF6* mutation (p.Phe238Cys) was found in T cells in RA⁴⁴ and the increase in disease activity index in patients with systemic lupus erythematosus was associated with the enhanced levels of *SLAMF3* and *SLAMF6* on the surface of T lymphocytes and the high IL-17 level in serum.⁴⁵

C-X-C motif chemokine ligand 13 (*CXCL13*), a B-cell chemoattractant, binds to its receptor *CXCR5* to promote B-cell migration.⁴⁶ The RA-risk variant (rs117605225) was located in a highly conserved region in the intron of *CXCL13* according to two conservation scores (called GERP⁴⁷ and SiPhy⁴⁸). The RA-risk *CXCL13* variant showed consistent effect sizes in the two independent cohorts and had no proxy variants based on the LD indices in the Korean cohorts and the 1KGP populations. Circulating *CXCL13* levels had positive correlations with joint destruction rates and disease activity scores.⁴⁹ Similarly, a transcriptome analysis displayed the enriched *CXCL13* transcript levels in T cells in synovial fluid of patients with RA.⁵⁰

SWAP70 is a Rho GTPase-regulatory protein that controls both cytoskeletal dynamics and the induction of interferon regulatory factor 4 (*IRF4*).^{51 52} *SWAP70* is necessary for normal B-cell migration and immobilises F-actin filaments on phagosomes in human dendritic cells.^{51 53} Although a further investigation would be required to pinpoint and confirm *SWAP70* as an

RA-driving gene in the *SWAP70* locus, an experimental murine model showed that genetic defects of *SWAP70* resulted in lupus-like symptoms and the accumulation of T_{reg} cells.^{53 54}

This study emphasised the crucial role of the immune and non-immune tissues (lung and small intestine) for the development of RA by analysing the tissue-specific expression of RA-relevant genes, especially *SLAMF* member genes in the *SLAMF6* locus and several known RA-associated genes including *PADI4*, *CCR6* and *NCF1*. Among the RA-relevant tissues, lung has long been suggested as an early site of inflammation and ACPA in developing RA in an interaction of genetic factors (eg, *HLA-DRB1*,⁵⁵ *PADI4*^{56 57}) with cigarette smoking⁵⁸ and air pollution.⁵⁹ Our previous bioinformatic study⁶⁰ also identified the significant enrichment of RA-risk variants within lung-specific enhancer regions, indicating that non-coding RA-variants may regulate gene expression in allele-specific manner in lung.

Small intestine has large surfaces that are exposed to the gut environment and affects the human immune response with intestinal immune cells. As IgA-producing plasma cells of gut-associated lymphoid tissue are rich in lamina propria, the immune response by ACPA and rheumatoid factor of IgA isotype can be initiated in the intestinal mucosal tissue in RA.⁶¹ Interestingly, IL-21, highlighted by our gene set analysis, is primarily produced in the small intestine and is important in IgA responses to gut atypical commensals⁶² and T helper cell responses in intestinal inflammation in lamina propria in mice.⁶³ In gut microbiota-induced arthritis in a murine model, T follicular helper cells were induced in lamina propria of gut before the onset of experimental arthritis and migrated to systemic lymphoid tissues, resulting in autoantibody production and arthritis.⁶⁴

EBV infection has been associated with an increased risk of RA and other inflammatory diseases.^{36 65} An in silico study by Harley *et al*⁶⁶ analysed the binding sites of EBV-encoded EBNA2 proteins on the human genome in EBV-transformed B cells. The authors identified the significant enrichment of RA variants within the EBNA2-binding elements, which allowed the expression of RA-relevant genes to be highly allele-specific.

Functional studies on novel RA variants are needed in the future. Our bioinformatics approaches provide a list of 122 potentially RA-relevant genes in RA loci that may facilitate follow-up functional studies of candidate genes, patient stratification and drug target validation. The gene set analysis highlighted several immune-related pathways such as the CD40 signalling and IL-21-mediated pathways that had showed altered activities in patients with RA.^{67 68}

In summary, this study discovered six novel RA loci in Korean populations and demonstrated variant-driven pathological features, evoking the importance of the identification of new RA loci and the integration of disease association results with other omics data.

Author affiliations

¹Hanyang University Institute for Rheumatology Research, Seoul, Republic of Korea

²Department of Biology and Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul, Republic of Korea

³Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, Republic of Korea

⁴Division of Genome Research, Center for Genome Science, National Institute of Health, Osong Health Technology Administration Complex, Cheongju, Republic of Korea

⁵Department of Rheumatology, Catholic University of Daegu School of Medicine, Daegu, Republic of Korea

⁶Division of Rheumatology, Department of Internal Medicine, Chonnam National University Medical School and Hospital, Gwangju, Republic of Korea

⁷Division of Rheumatology, Ewha Womans University School of Medicine, Seoul, Republic of Korea

⁸Department of Internal Medicine, Dong-A University Hospital, Busan, Republic of Korea

⁹Division of Rheumatology, Department of Internal Medicine, Chungnam National University Hospital, Daejeon, Republic of Korea

¹⁰Division of Rheumatology, Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Republic of Korea

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Contributors KK and S-CB designed the study. Y-CK, JLim, EH, MYH, KY, KK and S-CB analysed the data and interpreted the results. S-YB, J-YC, D-HY, S-SL, JLee, WTC, T-HK, Y-KS, S-CS, C-BC, J-BJ, YMK, J-MS, Y-KL, S-KC, B-JK, H-SL and S-CB recruited and characterised patients with RA and controls. KK, S-YB, B-JK and S-CB generated genomic data. YCK, JLim, KK and S-CB wrote the manuscript. All authors reviewed and approved the manuscript.

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ORCID iDs

Dae-Hyun Yoo <http://orcid.org/0000-0002-0643-4008>

Yoon-Kyoung Sung <http://orcid.org/0000-0001-6691-8939>

Jae-Bum Jun <http://orcid.org/0000-0002-0208-0505>

Soo-Kyung Cho <http://orcid.org/0000-0003-4493-8837>

Kwangwoo Kim <http://orcid.org/0000-0001-8926-6216>

Sang-Cheol Bae <http://orcid.org/0000-0003-4658-1093>

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