Association of CD8+ T-cells with bone erosion in patients with rheumatoid arthritis

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Abstract

Aim: Bone erosion is a major problem worsening quality of rheumatoid arthritis (RA) patients’ lives. However, causal factors responsible for bone erosion in RA have remained unclear. We aimed to examine genetic variants conferring bone erosion in RA using a Korean genome-wide association study (GWAS) and to search for possible biological mechanisms underlying the development of bone erosion.

Method: We obtained genome-wide single nucleotide polymorphism (SNP) data for 711 Korean RA patients using Illumina HapMap 550v3/660W arrays. Associations between SNPs and bone erosion status based on the Steinbrocker staging system were examined using multivariate logistic regression. Cell-type-specific enrichment of the epigenomic chromatin annotation H3K4me3 at the bone erosion associated variants was further investigated using National Institute of Health Roadmap Epigenomics data.

Results: As we tested the associations between 439,289 SNPs and bone erosion in 385 patients with erosive RA and 326 with non-erosive RA, none of the tested SNPs reached the genome-wide significance threshold, although many loci showed modest genetic effect on bone erosion status with suggestive association (e.g., rs2741200 [P = 3.75 × 10^{-8}] in the SLA-TG locus and rs12422918 [P = 4.13 × 10^{-6}] in SRGAP1). However, the top-ranked SNPs and their linked proxies, which were mostly located in non-coding variants, were significantly co-localized with the highly tissue-specific regulatory marker H3K4me3 in CD8+ memory T-cells (P = 0.014).

Conclusion: Although, there was no large-effect variants associated with bone erosion in our GWAS, we have shown that CD8+ memory T-cells may have relevance with bone erosion in patients with RA through the analysis of ChiP-seq data.

Key words: bone erosion, CD8 T-cell, chromatin, genome-wide association study, rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is characterized by altered immune reaction, inflammation and cartilage or bone destruction in joints. The heritability of RA was estimated to be approximately 60% in two previous twin studies1,2 and numerous genome-wide association studies (GWAS) for RA susceptibility have been conducted to find causal variants. A recent GWAS meta-analysis reported a total of 101 RA risk loci.3 Radiographic RA joint damage is also heritable and a recent Icelandic study reported that heritability of the rate of joint damage was 45–58%.4 However, in contrast to RA susceptibility risk loci, there is little
information about which genetic factors influence radiographic joint damage. Most studies have reported risk loci of radiographic joint damage using a candidate gene approach, rather than a hypothesis-free approach, and the results of these studies showed inconsistencies. Recently, one GWAS for radiographic joint damage conducted in anti-citrullinated peptide antibody (ACPA)-positive RA patients reported that sperm-associated antigen 16 (SPAG16) was associated with progression of joint damage. Another study using immunochip genotyping data found that genetic variants in the region of matrix metalloproteinase 9 (MMP-9) were associated with the progression of joint damage. The contribution of genetic markers to progression of joint damage in RA was investigated by van Steenbergen et al. using a total of 17 genetic variants, including variants in the region of SPAG16 and MMP-9 as well as several genetic variants reported in candidate gene studies. These genetic factors explained only 12–18% of the variance in radiologic progression and additionally explained 3–7% of the variance when added to clinical factors. However, information about the genetic contribution to radiographic joint damage in RA remains limited as previously reported genetic loci were used. Further GWAS could be helpful to explain the genetic contribution to radiographic joint damage and might elucidate possible associated mechanisms of joint damage.

Here, we conducted a Korean GWAS for the association of genetic variants with bone erosion in RA patients. Then, enrichment tests of associated variants in GWAS were also conducted to search for possible mechanisms underlying the development of bone erosion using epigenetic information, chromatin immunoprecipitation-sequencing (ChiP-seq) data.

**MATERIALS AND METHODS**

**Patients**

The Hanyang Bae RA Cohort of Hanyang University Hospital for Rheumatic Diseases was used in this study. From this cohort, a total of 711 RA patients with more than 2 years after symptom onset who had available genotyping data and radiographs were enrolled. All patients were of Korean descent and satisfied the American College of Rheumatology 1987 classification criteria. This study protocol was approved by the Institutional Ethics Review Board of Hanyang University Hospital and all enrolled patients provided informed consent.

**Radiographic outcome**

All patients had a baseline radiograph taken at enrollment. Radiographic damage was assessed from hand radiographs using the Steinbrocker method, which has a four-point scale: I, the absence of destructive changes on radiographs; II, osteoporosis, with or without slight subchondral bone destruction; III, radiographic evidence of cartilage and bone destruction subluxation, or ulnar deviation; and IV, fibrous or bony ankylosis. Two expert radiologists independently scored the radiographs. Discordant scores were discussed and a consensus was reached. According to their erosion status based on the Steinbrocker stage, patients were classified as non-erosive RA (stages I and II) or erosive RA (stages III and IV).

**Genotyping**

We used the previously reported Korean RA GWAS data. In brief, Illumina HapMap 550v3 or 660W genotyping platforms were used for genotyping. Quality control of GWAS genotype data was done based on single nucleotide polymorphism (SNP) genotype call rates (> 90% completeness), minor allele frequency (> 1%) and Hardy–Weinberg equilibrium (P > 10^-6).

**Statistical analysis**

To identify genetic variants and biological mechanisms associated with bone erosion, a single-locus level test for GWAS and an enrichment test of top associated variants in GWAS were performed. For the single-locus level test, the association between 439,289 SNPs and bone erosion was tested for the GWAS. Additive model was selected and minor allele dosage represented as 0, 1 or 2 were analyzed using logistic regression analysis, adjusted for age at symptom onset, sex, disease duration from symptom onset to the time taking the radiograph, smoking status at symptom onset, and the top 10 principal components (PC) using the PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/).

For the enrichment test of top associated variants in GWAS, phenotypic cell-type-specificity was analyzed using sets of SNPs resulting from GWAS. Based on National Institutes of Health (NIH) Roadmap Epigenomics data, we observed overlap between SNPs associated with bone erosion and peaks for trimethylation of histone H3 at lysine 4 (H3K4me3) which were evaluated using the Epigwas program developed by Trynka et al. In brief, GWAS SNPs with P < 1 × 10^-4 (clumped at r^2 of 0.2) and their proxies in the data
from the 1000 Genomes Project \( (r^2 > 0.8 \text{ with the GWAS SNPs}) \) were scored on the height and distance of the nearest H3K4me3 peak in 34 cell types evaluated in the NIH Roadmap Epigenomics Project. Statistical significance of the observed scores for each cell type was calculated based on the proportion of phenotype-unassociated random SNP sets (sampled 10,000 times to have the same total number of chromatin mark peaks in the region in linkage disequilibrium (LD) across all cell types) with cell type specificity scores exceeding the actual scores.

**RESULTS**

**Baseline characteristics**

Among the 711 RA patients included in the GWAS, bone erosion was observed in 54% \( (n = 385) \) with mean disease duration of 11.6 years. Mean age of patients at onset was 39.8 ± 11.5 years and 90% were female (Table 1).

**Results of GWAS**

In the association test using 439,289 SNPs of the Korean GWAS dataset in 385 patients with erosive RA and 326 with non-erosive RA, the most significant SNP was rs2741200 in SLA-TG \( (P = 3.75 \times 10^{-9}) \), followed by rs12422918 in SRGAP1 \( (P = 4.13 \times 10^{-9}) \) (Table 2). However, none of the tested SNPs in GWAS reached the genome-wide significance threshold \( (P \leq 5 \times 10^{-8}) \), suggesting that bone erosion may not be associated with large-effect common variants.

**Biological mechanisms underlying the development of bone erosion**

We expanded the association analysis from the single-locus level to the enrichment of top associated variants in GWAS by investigating the phenotypic cell-type specificity using epigenetic chromatin marks. Chromatin marks tend to concentrate in and around genes, and the H3K4me3 peak is known to be the most cell-type-specific histone mark.\(^1\) Thus, we observed whether the significant SNPs in our GWAS dataset co-localized with cell-type-specific H3K4me3 peaks. We selected the top-ranked independent 26 SNPs with \( P < 1 \times 10^{-4} \) after clumping the set of SNPs \( (r^2 > 0.2) \) and their linked proxies, and found that these SNPs overlapped most significantly with the H3K4me3 peak for CD8\(^+\) memory T-cells \( (P = 0.0144) \) (Fig. 1). In particular, the first-ranked SNP in our GWAS dataset (rs2741200 located in TG-SLA, with \( P = 3.75 \times 10^{-6} \)) showed the greatest overlap with the H3K4me3 peak for CD8\(^+\) memory T-cells among the SNPs tested.

The second most significant cell type was chondrocytes from bone marrow (BM)-derived mesenchymal stem cells (MSCs) \( (P = 0.0365) \). The second-ranked SNP in our GWAS dataset (rs12422918 located in SRGAP1, with \( P = 4.13 \times 10^{-6} \)) overlapped closely with the H3K4me3 peak for BM-derived MSCs, only 187 base pairs away, and showed the greatest overlap among SNPs.

**DISCUSSION**

We conducted a Korean GWAS to identify genetic variants associated with bone erosion in patients with RA and found that bone erosion was not explained by a single powerful common variant. Instead, analysis of cell-type specificity using epigenetic chromatin marks revealed that CD8\(^+\) memory T-cells were most strongly associated with the candidate variants related to bone erosion in GWAS. According to the notion that genetic markers for disease susceptibility might affect disease phenotype, several candidate gene approaches were used to search for causal variants of radiographic severity. Among the RA susceptibility loci, HLA-DRB1, PTPN22, TNFAIP3-OLIG3, C5-TRAF1, CD40 and IL2RA were found to be genetic markers associated with radiographic severity.

### Table 1  Characteristics of rheumatoid arthritis patients included in the genome-wide association study

<table>
<thead>
<tr>
<th></th>
<th>Total  ( (n = 711) )</th>
<th>Non-erosive ( (n = 326) )</th>
<th>Erosive ( (n = 385) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at symptom onset, years</td>
<td>39.8 ± 11.5</td>
<td>41.6 ± 12.0</td>
<td>38.2 ± 5.7</td>
</tr>
<tr>
<td>Sex, female, %</td>
<td>640 (90)</td>
<td>287 (88)</td>
<td>353 (91.7)</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>11.6 ± 6.1</td>
<td>9.4 ± 5.7</td>
<td>13.5 ± 5.8</td>
</tr>
<tr>
<td>Anti-CCP antibody, %</td>
<td>569/646 (88.1)</td>
<td>260 (87.5)</td>
<td>309 (88.5)</td>
</tr>
<tr>
<td>Smoking at symptom onset, %</td>
<td>92 (12.9)</td>
<td>51 (15.6)</td>
<td>41 (10.6)</td>
</tr>
</tbody>
</table>

Anti-CCP, anti-cyclic citrullinated peptides.
in more than two populations. However, these results were inconsistent and sometimes showed negative results in meta-analyses of these studies. Our study also showed no statistical significance of these loci, even HLA-DRB1 which is a well-known risk factor of radiographic severity in RA. Previous reports which identified HLA-DRB1 as a risk factor of radiographic severity were analyzed using a classic allele, not GWAS data. Thus, to define whether HLA-DRB1 alleles affect bone erosion in a Korean RA population, further study using HLA-DRB1 classical alleles appears to be needed.

To search for possible genetic etiologies and mechanisms of bone erosion development, we hypothesized that the aggregation of genetic variants associated with bone erosion in our GWAS results would be enriched in...
specific cell types. Trynka et al.\textsuperscript{12} showed that trait-associated variants alter regulatory regions, and they fall within chromatic marks in relevant cell types, and H3K4me3 is the most useful in defining cell types associated with disease and finding mapping variants. That is, SNPs associated with the same trait likely overlap specific histone modification marks in the same cell type. For example, they demonstrated that significant variants from GWAS results were enriched in CD4\textsuperscript{+} regulatory T-cells for RA, pancreatic islet cells for diabetes mellitus, neuronal tissues for neuropsychiatric disease, and liver cells for plasma low-density lipoprotein concentration.\textsuperscript{12} Based on the above evidence, we assessed the enrichment of possible risk loci for bone erosion in epigenetic chromatin marks. Of 34 cell types investigated, CD8\textsuperscript{+} memory T-cells were the most significant cell type. T-cells are important in the pathogenesis of RA, and in particular, levels of CD8\textsuperscript{+} T-cells are elevated in the peripheral blood and synovial fluid of RA patients compared to healthy controls. In the active

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**Figure 1** Enrichment test for overlap of rheumatoid arthritis erosion risk single nucleotide polymorphisms (SNPs) with H3K4me3 peaks in different cell types.
disease stage of RA, synovial CD8+ T-cells express cytotoxic effector markers and secrete inflammatory cytokines such as interleukin 6 (IL-6), IL-17A and interferon gamma (IFNγ).18 CD8+ T-cells are important in maintaining synovial ectopic germinal centers and also play a crucial role in progression toward a more aggressive disease phenotype,19,20 although some subtypes of CD8+ T-cells induce immunomodulatory effects in RA patients.21 Overall, altered CD8+ memory T-cells in RA patients would affect progression of bone and cartilage damage in joints.

The most significant risk locus (rs2741200) overlapped with H3K4me3 peaks for CD8+ memory T-cells was located in SLA (Src-Like-Adaptor), a protein coding gene that is known to inhibit the T-cell receptor and reduce the activation of nuclear factor of activated T-cells (NFAT). In addition, this gene is involved in negative regulation of positive selection and mitosis of T-cells.22 Thus, it will be worth further investigating the functional contribution of this gene to CD8+ T-cell function and bone erosion development.

This study has some limitations. First, we used cross-sectional data rather than longitudinal data. Also, radiographs were scored by the Steinbrocker staging system, and not the Sharp/Van der Heijde modified score (SHS). As it maybe influence the phenotype, the study using a quantitative scale such as Lansen or Sharp score to assess radiographic damage is needed to confirm out results. Second, there were no replication data of the cell-specific enrichment test. Thus, it is necessary to replicate this result in another large ethnic population.

In summary, although we did not find any SNPs surpassing a genome-wide significance level in our study subjects, we showed that multiple associated SNPs in GWAS would affect bone erosion development. In particular, top-ranked SNPs in GWAS were mostly significantly co-localized with histone modification of CD8+ memory T-cells. These results could provide insight into the molecular mechanisms underlying bone erosion in RA.

CONFLICTS OF INTEREST
None.

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AUTHOR CONTRIBUTIONS
All authors made substantial contributions to conception and design, data acquisition/analysis, interpretation, drafting or critical revision of manuscript and approved the manuscript for publication. They have met the International Committee of Medical Journal Editors Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (ICMJE Recommendations) recommend criteria for authorship.

REFERENCES


