A Variant in RUNX3 Is Associated with the Risk of Ankylosing Spondylitis in Koreans

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Ankylosing spondylitis (AS) is a chronic autoinflammatory disease that affects the spine and sacroiliac joints. Regarding its etiology, although HLA-B27 is known to be the strongest genetic factor of AS, much evidence suggests the potential contribution of non-MHC genes to the susceptibility to AS. Most of these non-MHC genes have been discovered in non-Asian populations; however, just some of them have been validated in Koreans. In this study, we aimed to identify additional AS-associated single-nucleotide polymorphism (SNP) candidates by replicating the candidate SNPs in Korean AS patients and healthy controls. For this, we selected three SNPs (rs11249215 in RUNX3, rs6556416 in IL12B, and rs8070463 in TBKBP1), which were previously reported as risk factors of AS but have not been studied in Koreans, and performed genotyping assays using a total of 1138 Korean samples (572 AS patients and 566 healthy controls). Of the three SNP candidates, one SNP in RUNX3 (rs11249215) was significantly associated with the risk of AS (odds ratio, 1.31; 95% confidence interval, 1.02 to 1.68, p = 0.03). These results will be helpful in elucidating the pathogenesis of AS and may be useful for developing AS risk prediction models in Koreans.

Keywords: ankylosing spondylitis, HLA-B27, single nucleotide polymorphism

Introduction

Ankylosing spondylitis (AS) is a type of chronic autoinflammatory disease that affects the spine and sacroiliac joints. The pathogenesis of AS is not clear; however, it is believed to involve a combination of genetic and environmental factors. Factors that contribute to the development of AS include genetic factors, such as the human leukocyte antigen B27 (HLA-B27), and environmental factors, such as infection, stress, and smoking. HLA-B27 is the strongest genetic factor of AS, with a prevalence of 80% to 90% in patients with AS. However, only a small portion of HLA-B27-positive individuals (<5%) develop AS, suggesting a potential contribution of non-MHC genes to the susceptibility to AS [2].

A number of genome-wide association studies (GWASs) of single-nucleotide polymorphisms (SNPs) have identified non-MHC genes associated with AS, such as IL-12B, ERAP1, RUNX3, IL1R2, and ANTXR2 [3-6]. In addition to SNPs, through genomewide copy number variant (CNV) analysis, CNVs associated with AS have also been identified [7]. These AS-associated non-MHC genes support the contribution of non-MHC genes to the susceptibility to AS. They can also be valuable markers of the development of AS.

From a clinical point of view, the identification of early AS before the appearance of radiological changes in the patient’s spine or sacroiliac joint is important, because current treatment options, such as tumor necrosis factor-α (TNF-α) inhibitors and nonsteroidal anti-inflammatory drugs, after these changes have occurred cannot block or slow down disease progression [8]. Instead, many reports suggest that earlier treatment of TNF-α inhibitors may prevent the progression of AS more effectively [8-10].

In our previous work, to identify AS-associated SNP candidates in Koreans, we selected seven AS-associated SNP candidates, which had been identified in Caucasians by a
GWAS and consistently replicated in East Asians, and performed a replication study with 285 Korean AS cases and 363 healthy controls [11]. However, in contrast to our expectation, only one of them was replicated in Koreans. In this study, to identify other AS-associated SNP candidates, we selected three SNPs in non-MHC genes from previous GWASs [4, 5, 12, 13] that are associated with AS but have not been tested in Koreans—rs11249215 in RUNX3, rs6556416 in IL12B, and rs8070463 in TBKBP1—and examined them in a large cohort of Koreans with AS and healthy controls.

Statistical analysis

The differences in allele and genotype frequencies between cases and controls were assessed using chi-squared test and Fisher exact test. Three genetic models (dominant, recessive, and allele) were used to test the SNP associations. Hardy-Weinberg equilibrium had been tested for all three SNPs, and their genotypes were in Hardy-Weinberg equilibrium in our cohort. Statistical analyses were performed using PLINK [15], and p-values less than 0.05 were considered to be significant in all statistical analyses.

Results

To evaluate the candidate AS-associated genetic markers, we selected three SNPs for a replication study in Koreans (rs11249215, rs6556416, and rs8070463) that have been previously reported as risk factors of AS but have not been studied in Koreans. All of them were located in non-exonic regions: rs11249215 was located in the promoter of RUNX3, rs6556416 in IL12B, and rs8070463 in TBKBP1. General information on the three SNPs is summarized in Table 1.

We performed genotyping assays for the three SNPs using a total of 1,138 Koreans (572 AS patients and 566 healthy controls). Minor allele frequencies (MAFs) of the three SNPs in this study (0.57 for rs11249215, 0.91 for rs6556416, and 0.44 for rs8070463) were largely similar to those in East Asians from the 1000 Genomes Project, suggesting that our genotyping assays were reliable (Table 1). In the association analysis, one SNP in RUNX3 (rs11249215) was significantly associated with the risk of AS in the recessive model (Table 2). The MAF of rs11249215 was significantly higher in AS patients (MAF = 0.59) than in controls (MAF = 0.56) (odds ratio, 1.31; 95% confidence interval, 1.02 to 1.68; p = 0.03). However, significance was not detected in the allelic or dominant model for rs11249215. The other two SNPs in IL12B and TBKBP1 showed increased odds ratios, but they

Table 1. General information for the three SNPs

| SNP         | Genotype | Position* | Putative gene | All  | AFR  | AMR  | EAS  | EUR  | SAS  |
|-------------|----------|-----------|---------------|------|------|------|------|------|------|
| rs11249215  | G/A      | chr1:25297184 | RUNX3        | 0.48 | 0.23 | 0.62 | 0.53 | 0.52 | 0.63 |
| rs6556416   | A/C      | chr5:158818745 | IL12B        | 0.83 | 0.88 | 0.81 | 0.91 | 0.70 | 0.84 |
| rs8070463   | T/C      | chr17:45768836 | TBKBP1       | 0.53 | 0.68 | 0.42 | 0.44 | 0.50 | 0.51 |

SNP, single-nucleotide polymorphism; Genotype, reference allele/alternated allele; MAF, minor allele frequencies; All, average of entire population; AFR, African; AMR, American; EAS, East Asian; EUR, European; SAS, South Asian.

*UCSC GRCh37/hg19.
Table 2. Association results for the three SNPs

| SNP       | Risk allele | AF (case/control) | HoF (case/control) | Allelic model | Recessive model | Dominant model |
|-----------|-------------|-------------------|--------------------|---------------|-----------------|----------------|
| rs11249215| A           | 0.57 (0.59/0.56)  | 0.33 (0.36/0.30)  | 1.13 (0.96–1.34) | 1.31 (1.02–1.68) | 1.00 (0.74–1.36) |
| rs6556416 | C           | 0.91 (0.92/0.90)  | 0.83 (0.86/0.82)  | 1.33 (0.99–1.78) | 1.34 (0.98–1.84) | 1.58 (0.52–4.87) |
| rs8070463 | C           | 0.44 (0.46/0.42)  | 0.19 (0.21/0.18)  | 1.16 (0.98–1.36) | 1.23 (0.91–1.65) | 1.20 (0.94–1.55) |

SNP, single-nucleotide polymorphism; AF, allele frequency; HoF, homozygous risk allele frequencies; OR, odds ratio; CI, confidence interval.

were not statistically significant (Table 2).

**Discussion**

HLA-B27 is the strongest and most well-known genetic factor of AS. However, AS develops in less than 5% of HLA-B27-positive individuals, suggesting the existence of additional genetic factors. Indeed, a number of genetic markers in non-MHC genes have been identified by SNP GWASs and CNV GWASs [3-7]. In this study, we performed an SNP genotyping assay to evaluate three AS-associated SNP candidates (rs11249215 in RUNX3, rs6556416 in IL12B, and rs8070463 in TBKBP1) that were discovered in Caucasians but have not been studied in Koreans. To generate more reliable data, we used a large sample set in this study (572 AS patients and 566 healthy controls). To our knowledge, this is the first report of the association of these three SNPs in Koreans. As a result, rs11249215 in RUNX3 was found to be significantly associated with the risk of AS in Koreans. However, rs6556416 in IL12B and rs4389526 in ANTXR2 were not replicated in Koreans.

RUNX3 (Runt-related transcription factor 3) is a member of a family of transcription factors that are important regulators of lineage-specific gene expression. Woolf *et al.* [16] demonstrated that Runx3 is highly expressed in thymic medulla and that it promotes the differentiation of T cell to CD8+ T cells during thymopoiesis. More recently, RUNX3 was found to be linked to human autoimmune disease and inflammation. For example, Fainaru *et al.* [17] reported that Runx3 knockout mice develop spontaneous eosinophilic lung inflammation, which is attributed to dendritic cells becoming insensitive to transforming growth factor β-induced inhibition of maturation. RUNX3 polymorphisms are associated with the susceptibility to autoimmune diseases, such as systemic lupus erythematosus and psoriatic arthritis [18, 19]. Apel *et al.* [18] identified that RUNX3 is involved in CD8+ T lymphocyte differentiation and is related to psoriatic arthritis through a T cell-mediated mechanism. The association between the RUNX3 polymorphism rs11249215 and AS has also been reported in Caucasian [4] and Han Chinese populations [12]. Furthermore, Vecello *et al.* [20] demonstrated that rs4648889 in RUNX3 is associated with AS and that its risk allele reduces RUNX3 expression. Consistent with previous studies, in this study, we confirmed that rs11249215 in RUNX3 is significantly associated with the risk of AS in Koreans in the recessive model. Because the homozygous risk allele frequency is important when an SNP is a recessive marker, we examined the homozygous risk allele frequency for rs11249215 and found that it was significantly higher in AS patients (homozygous risk allele frequencies [HoF] = 0.33) than in controls (HoF = 0.33) (Table 2). Of note, the MAF of rs11249215 in controls was largely similar to that in East Asians from the 1000 Genomes Project, suggesting that our study group was not biased and that the genotyping was appropriate. Because rs11249215 is located in the promoter of RUNX3, further studies on its molecular effects will help elucidate the pathogenesis of AS. In addition, considering that new SNPs are being reported to be associated with AS, other replication studies of newly identified SNPs are also needed.

In conclusion, we performed a replication study of three SNPs with a relatively large cohort of samples and found that rs11249215 in RUNX3 is significantly associated with the risk of AS in Koreans. These results will be helpful in elucidating the pathogenesis of AS and may be useful for developing risk prediction models for AS in Koreans.

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