論文の内容の要旨

論文題目 A Genome-Wide Association Study for Identifying the Susceptibility Genes to Interstitial Lung Disorder in Rheumatoid Arthritis Patients

（ゲノムワイド関連解析を用いた関節リウマチに併発する間質性肺病変における感受性遺伝子の探索）

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Rheumatoid arthritis (RA) is a chronic disorder characterized by systemic inflammation caused by autoimmune dysfunction in the synovial joints, leading to the destruction of articular cartilage and joint deformity. Patients afflicted with RA develop various signs and symptoms including articular and extra-articular manifestations accompanying disease progression. Interstitial lung disorder (ILD) is one of the most common clinical manifestations of the lung among RA patients.

The prevalence of ILD in RA patients (RA-ILD) is about 30% in RA patient demonstrated evidence of abnormalities consistent with ILD by high-resolution computed tomography (HRCT) in various reports. RA-ILD demonstrates a poor prognosis and one of the main causes of death in RA patients both in Japan.

Advanced age, male gender, increased severity of joint involvements and drugs (i.e. leflunomide and methotrexate) have been shown to be risk factors for RA-ILD. Human leukocyte antigen (HLA) is known to be associated with RA. *HLA-DRB1*0101*, *HLA-DRB1*0401*, *HLA-DRB1*0404*, *HLA-DRB1*0405* and

shared epitope (SE) are well known risk alleles among RA. *HLA-DRB1*04*, SE and *HLA-DQB1*04* were significantly associated with a decreased risk of RA-ILD. *HLA-DRB1*16*, DR2 serological group and *HLA-DQB1*06* showed an increased risk of RA-ILD.

Genome-wide association study (GWAS) has emerged as an effective genetic screening tool to reveal associations between specific polymorphisms and corresponding phenotypes utilizing a large number of single nucleotide polymorphisms (SNPs). However, a comprehensive study using GWAS has yet to be conducted for RA-ILD. The present study performed GWAS to identify the susceptibility genes or SNPs that are associated with RA-ILD among Japanese.

This study was a collaborative effort between our laboratory and the Sagamihara National Hospital in Kanagawa, Japan. 620 RA patients were recruited from 10 hospitals. All patients fulfilled the revised American College of Rheumatology 1987 criteria for the classification of RA. RA patients were examined for the diagnosis of ILD, based on the findings by chest radiography or HRCT. To avoid interaction between the therapeutic drug and RA-ILD, drug induced interstitial lung disorder (DI-ILD) patients were excluded from this study.

Genome-wide SNP genotyping was conducted using the Affymetrix Axiom Genome-Wide ASI 1 Array containing more than 600,000 SNP loci that were optimized to maximize the coverage for Asian populations.

After SNP quality control (QC), sample QC, principal component analysis and identical by descend ascertainment, genome-wide association tests were conducted using 169 patients that have RA with ILD and 294 patients with RA without ILD.

The association test between RA with ILD and RA without ILD identified 11 SNPs with minimum p-values of less than 5×10^{-6} according to the five models (allelic, dominant, recessive, genotypic model and Cochran-Armitage trend test). No SNP showed a significant *p*-value as calculated by Bonferroni correction. The region 19q13.11 and 12q24.11 showed p-values of less than 1×10^{-6}. Therefore, SNPs within these regions were applied to the subsequent analysis, including regional imputation analysis, eQTL analysis by GENEVAR and assessment of functional annotations of SNPs in the region by Haploreg v2.

The SNP in the region of 19q13.11, showed the lowest p-value (*p*-value: 5.98×10^{-7} calculated by Cochran-Armitage trend test). The nearest gene, which encodes a zinc finger protein, was located 150 kb upstream of the SNP. From a regional imputation analysis, 13 SNPs showed lower p-values than that of the genotyped SNP and the lowest p-value was 1.87×10^{-7}. One SNP, which was in high LD (r^2= 0.82) with the SNP, was shown to exhibit a promoter chromatin state and was located in DNase hypersensitivity site. The other SNP in the region 12q24.11 showed an association (*p*-value: 6.86×10^{-7} and odds ratios (OR): 0.37 under dominant model). The gene containing this SNP in 11th intron encodes a transporter-like protein that is expressed in the entire brain.

To exclude the effect of HLA class II region, a sub-group analysis was performed. By focusing on *HLA-DRB1*04* positive patients among those having RA with ILD, association tests were conducted for 85 *HLA-DRB1*04* positive RA patients with ILD and 197 *HLA-DRB1*04* positive RA patients without ILD. The SNP in the region 7p21.3 showed an association (*p*-value: 4.2×10^{-7} and OR: 2.71), which could not be detected before division. One of the SNP in the region of 7p21.3

was reported to show an association with protein expression level of TGF-β1. Next, association tests were conducted for 84 *HLA-DRB1*04* negative RA patients with ILD and 97 *HLA-DRB1*04* negative RA patients without ILD. The SNP in the region 10q23.1 located on the second intron showed an association (p-value: 2.72×10^{-6} and OR: 5.77). The gene containing the SNP is a member of the neuregulin (*NRG*) gene family, which encodes ligands for the transmembrane tyrosine kinase receptors.

To reveal possible overlap of susceptibility SNPs to RA or pulmonary fibrosis (PF) with those of RA-ILD, reported RA and PF susceptibility SNPs were selected and compared with results derived from the present study. Firstly, association results of RA-ILD and reported RA susceptibility SNPs were compared. The susceptibility SNPs of the present study did not overlap with RA susceptibility SNPs. Secondly, 12 PF susceptibility SNPs from previous reports were selected to compare to the association results of the present study. The susceptibility SNPs of the present study did not overlap with PF susceptibility SNPs.

This study detected novel SNPs which might cause RA-ILD and subgroup analysis divided by presence/absence of *HLA-DRB1*04* revealed those two groups had different predispositions for RA-ILD. From the comparison of the previously reported associations of RA and PF with the results of RA-ILD GWAS, sharing susceptibility SNPs were not observed.