New Sequence Variants in HLA Class II/III Region Associated with Susceptibility to Knee Osteoarthritis Identified by Genome-Wide Association Study

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Abstract

Osteoarthritis (OA) is a common disease that has a definite genetic component. Only a few OA susceptibility genes that have definite functional evidence and replication of association have been reported, however. Through a genome-wide association study and a replication using a total of ~4,800 Japanese subjects, we identified two single nucleotide polymorphisms (SNPs) (rs7775228 and rs10947262) associated with susceptibility to knee OA. The two SNPs were in a region containing HLA class II/III genes and their association reached genome-wide significance (combined \( P = 2.43 \times 10^{-8} \) for rs7775228 and 6.73 \( \times 10^{-8} \) for rs10947262). Our results suggest that immunologic mechanism is implicated in the etiology of OA.

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Introduction

We are living in the “Bone and Joint Decade” (http://www.boneandjointdecade.org/). As the WHO initiative shows, bone and joint diseases are serious problems all over the world, putting us under severe medical, economical and social burden. Osteoarthritis (OA; MIM 165720) is one of the most common diseases among them. OA affects synovial joints of all over the world, mainly knee, hip, hand and spine. OA is characterized by progressive loss of articular cartilage and, often, proliferation of synovium and bone, which lead to pain, loss of joint function and disability. More than tens of millions patients in the world are suffering from this non-lethal, but intractable disease, and the number is relentlessly increasing; however, its etiologic picture remains unclear and we have no fundamental treatment for it.

OA is a polygenic disease. Both environmental and genetic factors contribute to its etiology and pathogenesis [1]. To understand its genetic factor, identification of its susceptibility gene(s) must be the first step. Many OA susceptibility genes identified by candidate-gene association studies have been reported, but only a few have supporting functional evidence and replication of the results in different populations [1,2]. Large-scale association studies including the genome-wide association study (GWAS) using high-density single nucleotide polymorphisms (SNPs) have been reported by a few groups in Asia and Europe [3–6], but only one gene fulfilled genome-wide significance level [2]. The genetic basis of OA susceptibility remains largely uncharacterized. To identify OA susceptibility gene(s), we conducted a GWAS for knee OA and identified two SNPs with genome-wide significance level.

Methods

Samples

Characteristics of each cohort group are shown in Table 1. Case samples of GWAS for the Japanese population were obtained from
from a resident cohort. SNPs with minor allele frequency of ≤0.1 in both case and control samples were excluded from the further analysis. In the replication analysis, we genotyped SNPs using the multiplex PCR-based invader assay (Third Wave Technologies) or by direct sequencing of PCR products using ABI 3700 DNA analyzers [Applied Biosystems], or by SNaPshot Multiplex System [Applied Biosystems] according to manufacturers’ protocols.

Statistical analysis
In the GWAS and replication analyses, we applied Fisher’s exact test to two-by-two contingency table in three genetic models: an allele frequency model, a dominant-effect model, and a recessive-effect model. We conducted the meta-analysis using the Mantel-Haenszel method. We examined heterogeneity among studies by using the Breslow-Day test. Significance levels after the Bonferroni correction for multiple testing were $P=1.09 \times 10^{-7}$ (0.05/459,393). Age, gender- and BMI-adjusted odds ratios were obtained by logistic regression analysis [11]. Odds ratios and confidence intervals were calculated using the risk allele as a reference. We analyzed the haplotype association using Haplovie software [12]. We conducted a principal component analysis to detect population stratification [13].

Software
For general statistical analysis, we used R statistical environment version 2.6.1 or Microsoft Excel. Drawing the LD map, estimation of haplotype frequencies and analysis of haplotype association were performed by Haplovie software.

Results
To identify genetic variants that determine OA susceptibility, we conducted a GWAS in Japanese knee OA. We examined 906 individuals with knee OA and 3,396 control individuals (Table 1) using Illumina HumanHap550v3 Genotyping BeadChip. After confirming the data quality, we compared the results of 459,393 SNPs between cases and controls by Fisher’s exact test for three genetic models: allelic, dominant or recessive (Figure 1). Fifteen
SNPs selected for the replication study that had the smallest $P$ values (minimum $P = 1.610^{-5}$) were next genotyped in an independent set of 167 Japanese knee OA individuals and 347 Japanese controls from a resident cohort study. Through these studies, only two SNPs, rs7775228 (combined $P = 2.43 \times 10^{-8}$; OR = 1.34; 95% CI = 1.21–1.49) and rs10947262 (combined...
\( P = 6.73 \times 10^{-6}; \) OR = 1.32; 95% CI = 1.19–1.46) were significant even after the Bonferroni correction for multiple testing (\( P = 1.09 \times 10^{-7} \)) (Table 2). The two SNPs showing significant associations are located within a 340-kb region within the HLA locus, including BTNL2, HLA-DRA, HLA-DRB5, HLA-DRB1, HLA-DQA1 and HLA-DQB1 (Figure 2). Although the HLA region is known to show extensive linkage disequilibrium (LD) spanning over 7 Mb, only SNPs in the 340-kb region showed strong associations with OA (Figure 2), and SNPs outside of this region did not have significant association.

Application of the Cochrane-Armitage test to all the tested SNPs indicated that the genetic inflation factor lambda was 1.08 for GWAS (Figure 3), implying a low possibility of false positive associations due to population stratification. We also carried out age, gender- and BMI-adjusted analysis using a logistic regression model, and confirmed similar association after adjustment (data not shown). The principal component analysis [13] revealed that there was no evidence for population stratification between the two control groups used for the GWAS (Figure S1).

To check the association of rs7775228 and rs10947262 in different ethnic populations, we examined the association of the SNPs with knee OA in two European Caucasian populations from Greece and Spain. We genotyped a total of 813 OA and 1,071 control subjects (Table 1). We conducted the meta-analysis using the Mantel-Haenszel method. The combined European results for rs7775228 were not significant with OR (95% CI) of 0.93 (0.76–1.13) (Table 2), while those for rs10947262 were supportive with OR (95% CI) of 1.29 (1.03–1.61). rs10947262 showed replication in the Greek population and the same trend in the Spanish population (Table 2). A meta-analysis of the Japanese and two European studies gave more significant association (combined \( P = 5.10 \times 10^{-6} \)).

We estimated the pairwise LD indexes (\( D' \) and \( r^2 \)) between rs7775228 and rs10947262 using the genotype data of Japanese populations (GWAS and the replication study), and found that they were in strong LD with each other (\( D' = 0.82, r^2 = 0.56 \)). They formed two frequent haplotypes (Haplotype I and II; Table 3) accounting for about 90% of all observed chromosomes. The haplotypes were also significantly associated with knee OA; Haplotype I, the most frequent haplotype was a risk haplotype (\( P = 1.48 \times 10^{-6}; \) OR = 1.33; 95% CI = 1.20–1.46).

**Discussion**

We performed a GWAS followed by a replication in an independent population using a total of \( \sim4,800 \) Japanese subjects, and identified two SNPs (rs7775228 and rs10947262) in the HLA class II/III locus associated with susceptibility to knee OA. To our knowledge, this study represents the first GWAS of OA with extensive coverage (\( \sim550,000 \) markers) and definite genome-wide significance even after Bonferroni’s correction, which is very

![Figure 2. Case-control association analysis and linkage disequilibrium (LD) map of the HLA class II/III region of chromosome 6.](https://www.plosone.org/doi/10.1371/journal.pone.0009723.g002)

(A) The LD map based on \( D' \) was drawn using HapMap data release 24 for the JPT population. (B) Genomic structure within the extended HLA-II/III region. (C) Results of GWAS for osteoarthritis in Japanese population. The log_{10}-transformed \( P \) values are plotted on the y axis.
The HLA-DQA system by presenting peptides derived from extracellular proteins (cells and macrophages) and play a central role in the immune response. HLA class II molecules are expressed in antigen presenting cells (B lymphocytes, dendritic cells, and macrophages) and play a central role in the immune response. An association of sarcoidosis with rs2076530, a coding SNP on exon 5 of the BTNL2 gene has been reported [16], but the SNP was not in LD with rs10947262 (D' = 0.11, r² = 0).

The 340-kb region of HLA loci, where the two SNPs are located also includes HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5 and HLA-DQA1. HLA-DRA, HLA-DRB1/3/4/5 and HLA-DQA1 encode HLA-DR α, β and HLA-DQ α chains, which could also belong to the HLA class II molecules. HLA-DRB1 is present in all individuals. Allelic variants of HLA-DRB1 are linked with either none or one of the genes HLA-DRB3, HLA-DRB4, and HLA-DRB5 [17]. Among these genes, HLA-DRB1 is strongly associated with RA. Some subtypes of HLA-DRB1 alleles, such as *0101, *0401, and *0405, is associated with RA [20], but not with generalized OA [21].

Although OA has generally been considered a non-inflammatory disease, accumulating evidences suggest that this is not the case. Inflammation involving activated T cells in the synovial membrane of OA patients is well documented [22]. Recently, we identified a genetic variant of EDG2 gene encoding lysophosphatidic acid receptor associated with knee OA [23]. A GWAS has identified a genetic variant of the PTGS2 gene encoding cyclooxygenase-2 involved in risk for knee OA [6]. These genetic associations of genes such as EDG2 and PTGS2 underscore the potential role of inflammatory pathways in the pathogenesis of knee OA.

Several studies have suggested associations of OA with HLA class I and II alleles. Study on generalized OA revealed association with HLA A1-B8 in Caucasian [24] and with HLA-Cw4 in Japanese [21]. An association of the HLA-DRB1*0402 alleles with knee and hip OA was identified in a cohort of 106 patients [25]. Interestingly, chondrocyte, which are normally HLA-DRB1 negative, become positive for them in OA patients [25]. Among these genes, HLA-DRB1 is strongly associated with RA. Some subtypes of HLA-DRB1 alleles, such as *0101, *0401, and *0405, is associated with RA [20], but not with generalized OA [21].

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Author Contributions
Wrote the paper: MN SI. Planned and supervised the whole project: SI. Performed the Japanese association study: MN IK TF JD. Managed the European association study: A. Tsezou AG. Helped with statistic analysis: MK YN.

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