Genome-wide Association Study Identified TIMP2 Genetic Variant with Susceptibility to Osteoarthritis

Bhumsuk Keam2¶, Joo-Yeon Hwang 1¶, Min Jin Go1, Jee Yeon Heo1, Mi Sun Park1, Ji Young Lee3, Nam Hee Kim1, Miey Park1, Ji Hee Oh1, Dong-Hyun Kim3, Jin-Young Jeong4, Jong-Young Lee1, Bok-Ghee Han1 and Juyoung Lee1*

1Center for Genome Science, National Institute of Health, Osong Health Technology Administration Complex, Chungcheongbuk-do 363-951, Korea, 2Department of Internal Medicine, Seoul National University Hospital, 101 Daehak-ro, Seoul 110-744, Korea, 3Department of Social and Preventive Medicine, College of Medicine, Hallym University, 1, Ok-cheon, Chuncheon, Gangwon-do 200-702, Korea, 4Institute of Aging, Hallym University, 1, Ok-cheon, Chuncheon, Gangwon-do 200-702, Korea

Abstract

Osteoarthritis (OA) is the most common degenerative joint disorder in the elderly population. To identify OA-associated genetic variants and candidate genes, we conducted a genome-wide association study (GWAS). A total of 3,793 samples (476 cases: wrist + knee and 3317 controls) from a community-based epidemiological study were genotyped using the Affymetrix SNP 5.0. An intronic SNP (rs4789934) in the TIMP2 (tissue inhibitor of metalloproteinase-2) showed the most significance with OA (odds ratio [OR] = 2.06, 95% confidence interval [CI] = 1.52-2.81, p = 4.01 × 10^{-6}). Furthermore, a polymorphism (rs1352677) in the NKAIN2 (Na^{+}/K^{+} transporting ATPase interacting 2) was suggestively associated with OA (OR = 1.43, CI = 1.22-1.66, p = 7.01 × 10^{-6}). The present study provides new insights into the identification of genetic predisposing factors for OA.

Keywords: genome-wide association study, osteoarthritis, polymorphism, TIMP2

Introduction

Osteoarthritis (OA) is the most common joint disorder characterized by progressive cartilage loss, and consequently increases serious social/economic problems in the elderly population worldwide (Du et al., 2005; Ikegawa, 2007). As with any joint affected by osteoarthritis, the primary symptom for wrist and knee OA as predominant weight-bearing joints is intractable pain with increased stiffness. Specifically, wrist OA can occur secondary to an intra-articular fracture of the distal radius or an extra-articular fracture resulting in malunion (Weiss and Rodner, 2007). Several risk factors such as age, gender, obesity, and trauma have been confirmed as environmental confounding factors (Haq et al., 2003).

So far, genetic factors contribute to the risk of OA have been identified through candidate gene approaches (Loughlin, 2002; MacGregor and Spector, 1999). However, candidate gene-based studies (Miyamoto et al., 2007; Mototani et al., 2005; Rodriguez-Lopez et al., 2008) have only a small fraction of genetic interactions involved in the pathogenesis, and thereby provide little chance of discovering novel candidate genes.

As a solution to the problem, OA susceptibility genes by recent GWAS have been reported across different groups (Abel et al., 2006; Dai and Ikegawa, 2010; Loughlin, 2002; Spector et al., 2006). However, most of the previously reported findings have produced conflicting results with differential genetic effects and limited replication between ethnic populations (Jiang et al., 2008; Kerkhof et al., 2008; Shi et al., 2008; Snelling et al., 2007; Valdes et al., 2007). The ethnic-specific variants remain to be identified in East Asian population to overcome ethnic difference and population structure. We therefore undertook a genome-wide association study to identify genetic polymorphisms influencing OA.

Methods

Study population

The study samples were drawn from the Korean Genome and Epidemiology Study (KoGES) which is an ongoing prospective community-based epidemiological study in the communities of Ansung (rural) and Ansan (urban) (Table 1). Details of the KoGES and the methods are described in our previous report (Cho et al., 2006; Cho et al., 2009; Kim et al., 2005; Lim et al., 2006). In brief, eligible subjects (age 40-69 years) were examined in 2001-2002 for demography and epidemiology and then follow up biannually. A total of 3,588 men and
3,927 women agreed to participate in a baseline examination which included an interview, blood tests, X-rays, and measurement of the body. Informed consents were obtained from all of the participants, and Declaration of Helsinki for biomedical research involving human subjects was also followed. The study protocol was approved by the Institutional Review Board of the Korea Centers for Disease Control and Prevention.

Diagnostic criteria

X-rays of both wrists and knees of subjects were taken. Radiographic OA was assessed using the Kellgren/Lawrence (K/L) grading system (Kellgren and Lawrence, 1957). Patients with K/L grades of 2 or higher in wrists or knees were diagnosed with OA. Among the 7,515 subjects of the study, 3,793 subjects consented to the X-ray examination. Of these, 476 patients were diagnosed with OA (387 women, 89 men, median age = 68 years [range 44-74]), and 3,317 subjects were classified as normal controls (women = 1,773, men = 1,544, median age = 57 years [range 43-74]).

Genome-wide association

Genomic DNA from peripheral blood mononuclear cells was used for this study. We performed a pilot GWAS, typing cases and controls on a single platform using the Affymetrix Genome-Wide Human SNP 5.0 (Affymetrix, Inc., Santa Clara, CA, USA). Genotype calls were determined by Bayesian Robust Linear Modeling using the Mahalanobis Distance algorithm (Rabbee and Speed, 2006). Details of the genotyping process are presented in our previous report (Cho et al., 2009). Of the SNPs assayed on the chip, 179,626 SNPs were excluded because they showed: 1) a call rate lower than 96.0% in cases or controls; or 2) a minor allele frequency (MAF) < 1% in the population; or 3) a significant distortion from Hardy-Weinberg equilibrium (p < 0.05), A total of 320,942 SNPs passed all the quality control filters (mean call rate 98.0%). To identify the effect of population stratification, the genomic inflation factor (λ) (Clayton et al., 2005) was calculated for all individuals. The two study cohorts (Ansung and Ansan) have shown very
similar MAFs for the SNPs on the array, and quantile-quantile (Q-Q) analysis for the comparison of genotype frequencies in both cohorts confirmed the genetic homogeneity of these two components of the KoGES study population (Cho et al., 2009).

**Statistical analysis**

The allele frequencies in OA cases were compared to those in controls using the Chi-square test and logistic regression analysis. The associations between the case-control status and each individual SNP were measured by the odds ratio (OR). Covariates used for multi-variable-adjustment were age, sex, and body mass index (BMI), because these variables were significantly associated with OA in our cohort.

Pair-wise linkage disequilibrium (LD) among the polymorphisms was determined by the expectation maximization algorithm. When determining LD between SNPs, we used either of two measures of LD: an $r^2$ value $\geq 0.8$ or a disequilibrium coefficient ($D'$) $\geq 0.95$.

**Results**

The Q-Q plot indicated lack of inflation due to stratification, and no differences between case and control populations were detected. The estimated genomic control inflation factor ($\lambda$) was 1.009, indicating limited evidence of population stratification in the KARE study samples - are described in Fig. 1.

Table 2 lists the most significant genetic variants from GWAS analysis. We identified two intronic SNPs with P-values $< 1 \times 10^{-5}$. A SNP (rs4789934) in the TIMP2 (tissue inhibitor of metalloproteinase-2) gene showed the most significant association with OA. The adjusted ORs were 2.06 (95% confidence interval (CI) = 1.52-2.81), and the adjusted P-values were $4.01 \times 10^{-6}$. In addition, our study also identified suggestive association in the NKAIN2 (Na+/K+ transporting ATPase interacting 2) (OR = 1.43, CI = 1.22-1.66, p = 7.01$\times 10^{-6}$).

To support the biological relevance in silico, we provide graphically summarized information on preliminary association by confidence values of textual evidence (Fig. 1, D). Nodes represent biological entities with different colors for different classes. Edges represent strong associations between two entities (i.e., TIMP2 effects to tissue inhibition, and functional interactions with TIMP4).

**Discussion**

In this study, we identified two novel polymorphisms associated with OA. Intronic SNPs, rs4789934 in TIMP2 and rs1352677 in NKAIN2, showed significant associations with OA. To date, OA susceptibility genes from genome-wide association studies have been reported primarily in samples of European descent.

The function of TIMP2 is inhibition of MMP. Osteoarthritic cartilage is characterized by the imbalance between MMPs and TIMPs. The level of lytic enzymes such as MMP-1, MMP-3, and MMP-13 was increased, and the level of TIMP-1 was decreased in OA cartilage (Krane and Inada, 2008; Martel-Pelletier et al., 1994). With the decreasing level of TIMP, MMP activity increases and leads to further destruction of cartilage, the key process in OA (Cawston, 1998; Krane and Inada, 2008). Estrogen may regulate the balance between MMP and TIMP activity (Lee et al., 2003), which would explain the gender and menopausal correlations with OA. Although prior reports focused primarily on TIMP-1, levels of TIMP2, a member of the same gene family, have also been shown to increase in osteoarthritic cartilage (Martel-Pelletier et al., 1994). Lee et al., reported that TIMP-4 polymorphism (rs17035945) was associated with the risk of OA in the Korean population as a result
of altering the folding of the mRNA (Lee et al., 2008).

The Na⁺/K⁺ ATPase is an important regulator of ion homeostasis in many cell types (Mobasheri et al., 2000), but its role in OA is not clear. In osteoblasts, which synthesize collagen and other extracellular matrix (ECM) components, the Na⁺/K⁺ ATPase appears to maintain the transmembrane gradient of Na⁺ that drives intracellular calcium homeostasis and ECM calcification (Francis et al., 2002). Hence, the role of NKAIN2 in the cellular calcium homeostasis and ECM calcification are interesting to us.


Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee, *Arthritis, Rheum.*, 56, 137-146.
