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*Original Article*

# Genetic polymorphisms of interleukin-16 are associated with susceptibility to primary knee osteoarthritis

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**Abstract:** Interleukin-16 (IL-16) polymorphisms have been associated with various disease states, and its activity is dysregulated in synovial fibroblasts of individuals with rheumatoid arthritis. Here, the association between genetic polymorphisms in the gene encoding IL-16 and susceptibility to primary knee osteoarthritis was investigated in the Chinese Han population. The study included 228 unrelated patients, half of whom presented with primary knee osteoarthritis (OA); the remainder was healthy individuals. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to examine single nucleotide polymorphisms (SNPs) in *IL16* in these patients. Statistical analysis was performed using the chi-square goodness-of-fit test, Hardy-Weinberg (H-W) equilibrium, linkage disequilibrium analysis, and logistic regression analysis. The genotype distributions of three *IL16* SNPs, rs11556218, rs4778889, and rs4072111, were found to be in line with Hardy-Weinberg equilibrium criteria ($P > 0.05$). The single-factor logistic regression analysis showed that, compared with the T/T genotype, the T/G genotype decreased the risk of primary knee OA in rs11556218 (*OR* = 0.37, 95% CI = 0.18~0.82) and, compared with the C/C genotype, the C/T genotype increased the risk of primary knee OA in rs4072111 (*OR* = 1.83, 95% CI = 1.07~3.59). There was linkage disequilibrium between rs4778889 and rs11556218 (D= 0.592, $r^2 = 0.213$). Finally, logistic regression analysis showed that compared to haplotype TTC, the TTT haplotype was associated with an increased risk of primary knee OA (*OR* = 2.10, 95% CI = 1.09-4.98); however, the GCC haplotype was associated with a reduced risk of primary knee OA (*OR* = 0.36, 95% *CI* = 0.12-0.93). Thus, the genetic polymorphisms rs11556218, rs4778889, and rs4072111 in the gene encoding IL-16 are associated with primary knee OA in Chinese Han population.

**Keywords:** Knee osteoarthritis, IL-16, single-nucleotide polymorphism

## Introduction

Osteoarthritis (OA) as a degenerative lesion is a common disease in middle-aged and elderly populations [1]. The clinical manifestations of OA include slowly developing joint pain, joint swelling, limited movement, rigidity, and joint deformity. Primary knee osteoarthritis refers to a chronic joint disorder caused by cartilage degeneration and osteoarthritis of the knee joint [2-4]. This disorder occurs primarily in middle-aged and elderly populations, but it can also occur in young patients [5, 6]. Because of the high incidence of knee osteoarthritis and the high disability rate, it is important to understand the genetic causes of this disease.

The pathogenesis of knee osteoarthritis has not yet been fully elucidated, but it is believed to result from the interaction between environmental and genetic factors [7, 8]. Many susceptibility genes and single nucleotide polymorphisms are associated with primary knee osteoarthritis [9-13]. Interleukin-16 (IL-16) is a proinflammatory cytokine, playing a key role in inflammatory diseases by promoting the secretion of cytokines [14-16]. IL-16 dysregulation is closely correlated with rheumatoid arthritis [17]. Thus, it has been speculated that IL-16 is correlated with primary knee osteoarthritis.

In this study, we explored the correlation between IL-16 polymorphisms and primary knee osteoarthritis by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and statistical analyses. The aim of this work was to providing a scientific basis for

further studies of hereditary susceptibility to primary knee osteoarthritis.

## Materials and methods

### *Study population*

Between March and September 2014, 114 patients of Han nationality who were examined in Shandong Provincial Hospital were selected as a case group. All patients had been confirmed by clinical examinations to meet the diagnostic criteria for primary knee osteoarthritis [18]. To be included in the study, examinations involving imaging and physical examination had to show that patients met the following diagnostic criteria for primary knee osteoarthritis: a body mass index (BMI) ≤ 27 and a Kellgren-Lawrence (KL) classification [19] score ≥ 2 points for knee X-rays. Patients having a past history involving knee infections, knee surgery, knee tumors, knee deformity before adulthood, and metabolic bone disease were excluded from the study, as well as cases with incomplete clinical data. An additional 114 healthy patients of Han nationality who received physical examination in our hospital during the same time period were selected as the control group. The differences between the case group and control group regarding age and sex were not statistically significant ($P > 0.05$), and all subjects had no mutual kinships. This study was approved by the Hospital Ethics Committee and informed consent was obtained from all subjects.

### *DNA extraction and measurement*

For DNA analysis, 2 mL of peripheral venous blood were drawn from each fasting patient, anticoagulated with EDTA-Na2 (Sigma-Aldrich Trading Co., Ltd, Shanghai, China), and stored at -20°C. Phenol-chloroform was used to extract genomic DNA from leukocytes of each subject, and a spectrophotometer was used to measure the OD 260/280 ratio. An OD 260/280 ratio between 116-210 was considered to have good DNA purity, and the sample was included in the study. The DNA was then dissolved in Tris-EDTA buffer (TE buffer) and stored at -70°C.

### *Primer design and PCR amplification*

PCR primers were designed as previously described [20] and synthesized by TaKaRa Biotech (Dalian, China). Primer sequences for each SNP were as follows:

rs4778889T/C: 5'-CCATGTCAAAACGGTAGCCTCAAGC-3' and 5'-CTCCACA CTCAAAGCCTTTTGTTCCTATGA-3' rs4072111C/T: 5'-TTCAGGTACAAACC CAGCCAGC-3' and 5'-CAC TGTG ATC CCGGTCCAGTC-3' rs11556218T/G: 5'-TGTGACAATCACAGCTTGCCTG-3' and 5'-GCTCAGGTTCACAGAGTGTTT CCATA-3'

PCR amplification reactions were carried out in a total of volume of 25 μL, containing 2.0 μL of template DNA, 2.0 μL of dNTP (2.5 mM, TaKaRa Biotech, Dalian, China), 2.5 μL of 10 x PCR buffer, 1.5 μL of upstream primers (20 μM), 1.5 μL of downstream primers (20 μM), 0.2 μL of 5 U/μL Taq (TaKaRa Biotech Co. Led,, Dalian, China), and ionized water. PCR reaction conditions at rs11556218T/G were as follows: samples were denatured at 95°C for 5 min, then processed for 30 cycles of denaturation at 95°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 1 min, and ending with a final extension cycle at 72°C for 5 min. The annealing temperatures for rs4072111C/T and rs4778889T/C were 67°C and 63°C, respectively.

### *Restriction digestion and gel electrophoresis*

To digest the DNA, restriction endonucleases *Ahd* I, *Nde* I, and *BsmA* I (Merck, Darmstadt, Germany) were used for rs4778889T/C, rs11556218T/G, and rs4072111C/T, respectively. 10 μL of PCR amplification product were digested with 1.2 μL of the corresponding restriction endonuclease, and each digestion product was treated in a water bath at 37°C for 16 hours. The final product was run on a 2% agarose gel for electrophoresis and imaged. To verify genotypes, Generay Biotech (Shanghai, China) sequenced the amplified and digested DNA products.

### *Statistical analysis*

Statistical software included SAS 9.2 (SAS Institute, Cary, NC, USA), Haploview 4.2 (Broad Institute, Cambridge, MA, USA), and SNPStats (R package; http://bioinfo.iconcologia.net/SNPstats). SAS 9.2 was used to perform a chi-square test and perform unconditional logistic regression analysis. The Haploview software was used to analyze linkage disequilibrium (LD). SNPStats was used to test Hardy-Weinberg equilibrium and to evaluate the haplotypes at various SNP loci as well as the risks for the occurrence of primary knee osteoarthritis.

**Table 1.** Chi-square test of Hardy-Weinberg equilibrium for rs11556218

| Group | T/T | T/G | G/G | Total alleles | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| Control | 57 (53.47) | 32 (38.95) | 11 (7.47) | 400 | 2.623 | 0.269 |
| OA | 39 (43.23) | 53 (44.65) | 8 (12.23) | 400 | | |

Note: n (estimated n).

**Table 2.** Chi-square test of Hardy-Weinberg equilibrium for rs4778889

| Group | T/T | T/C | C/C | Total alleles | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| Control | 68 (67.64) | 27 (27.82) | 5 (4.72) | 400 | 2.020 | 0.364 |
| OA | 58 (57.76) | 35 (35.38) | 7 (6.68) | 400 | | |

Note: n (estimated n).

**Table 3.** Chi-square test of Hardy-Weinberg equilibrium for rs4072111

| Group | T/T | T/C | C/C | Total alleles | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| Control | 51 (54.22) | 45 (38.15) | 4 (7.23) | 400 | 0.702 | 0.704 |
| OA | 63 (60.53) | 28 (33.36) | 9 (6.51) | 400 | | |

Note: n (estimated n).

**Table 4.** Single-factor logistic regression analysis

| Genotype | OA (n, %) | Control (n, %) | *P* | *OR* (95% CI) |
|---|---|---|---|---|
| rs11556218 | | | 0.011 | |
| T/T | 39 (39.00) | 57 (57.00) | | 1.00 |
| T/G | 53 (53.00) | 32 (32.00) | | 0.37 (0.18~0.82) |
| G/G | 8 (8.00) | 11 (11.00) | | 0.13 (0.42~2.94) |
| rs4778889 | | | 0.340 | |
| T/T | 58 (58.00) | 68 (68.00) | | 1.00 |
| T/C | 35 (35.00) | 27 (27.00) | | 0.71 (0.45~1.36) |
| C/C | 7 (7.00) | 5 (5.00) | | 0.62 (0.20~2.47) |
| rs4072111 | | | 0.028 | |
| C/C | 63 (63.00) | 51 (51.00) | | 1.00 |
| C/T | 28 (28.00) | 45 (45.00) | | 1.83 (1.07~3.59) |
| T/T | 9 (9.00) | 4 (4.00) | | 0.78 (0.35~2.28) |

## Results

*Hardy-Weinberg equilibrium test for the distribution of genotypes*

*IL16* SNP sequencing identified genotypes T/T, T/G, and G/G at rs11556218; T/T, T/C, and C/C at rs4778889; and C/C, C/T, and T/T at rs4072111. In the OA group and the control group, the frequencies of all genotypes at rs11556218, rs4778889, and rs4072111 met the Hardy-Weinberg equilibrium expectations according to chi-square tests (*P* value > 0.05: **Tables 1**-**3**).

*Multivariate unconditional logistic regression analysis of each genotype of three IL16 SNPs*

Unconditional logistic regression analysis of various genotypes at each locus showed that at rs11556218, compared with the T/T genotype, the T/G genotype showed a reduced risk of occurrence of primary knee osteoarthritis (*OR* = 0.37, 95% CI = 0.18-0.82; **Table 4**). At rs4778889 the differences among various genotypes were not statistically significant (*P* > 0.05). At rs4072111, when compared with the C/C genotype, the C/T genotype showed an increased risk of occurrence of primary knee osteoarthritis (*OR* = 1.83, 95% CI = 1.07-3.59; **Table 4**).

*Linkage disequilibrium and haplotype analysis at three IL16 SNP loci*

Linkage disequilibrium was detected at rs4778889 and rs11556218 (D = 0.592, $r^2$ = 0.213). Unconditional logistic regression analysis of various SNP loci revealed that, compared with the TTC haplotype, the TTT haplotype was associated with increased risk of occurrence of primary knee osteoarthritis (*OR* = 2.10, 95% CI = 1.09-4.98; **Table 5**), while the GCC haplotype was associated with reduced risk of occurrence of primary knee osteoarthritis (*OR* = 0.36, 95% CI = 0.12-0.93; **Table 5**). Because the total frequencies of the other 3 haplotypes (GTT, GCT, and TCT) were lower, meaningful statistical analyses were not possible.

## Discussion

The pathogenesis of knee osteoarthritis is not yet fully understood; therefore, finding biologi-

**Table 5.** Three SNP loci haplotypes of *IL16* constructed from non-conditional logistic regression analysis

| Haplotype | rs11556218 | rs4778889 | rs4072111 | *P* | *OR* (95% CI) |
|---|---|---|---|---|---|
| Haplotype1 | T | T | C | - | 1.00 |
| Haplotype2 | T | T | T | 0.018 | 2.10 (1.09~4.98) |
| Haplotype3 | G | T | C | 0.531 | 0.98 (0.34~2.17) |
| Haplotype4 | G | C | C | 0.041 | 0.36 (0.12~0.93) |
| Haplotype5 | T | C | C | 0.695 | 2.01 (0.45~5.32) |

cal treatments to effectively reduce or even eliminate the effects of proinflammatory cytokines before the occurrence of knee osteoarthritis is an important area of study. In recent years, many studies have attempted to determine the related genes that cause knee osteoarthritis [9-13]. *IL16* is one such gene, as a proinflammatory cytokine that has been shown to be closely correlated with rheumatoid arthritis [18]. Using PCR-RFLP technology, this study revealed a correlation between *IL16* polymorphisms and primary knee osteoarthritis.

In particular, two SNP sites were associated with knee OA susceptibility. The TC genotype at rs11556218 was associated with reduced risk of primary knee osteoarthritis compared to the TT genotype. In contrast, at rs4072111 the CT genotype was associated with increased risk of primary knee osteoarthritis compared to the CC genotype. Further, the rs4778889 SNP was in linkage disequilibrium with rs11556218. Thus, unconditional logistic regression analysis revealed that IL16 haplotypes were also important in knee OA: the TTT haplotype was associated with increased risk of knee OA compared to the TTC haplotype, while the GCC haplotype was associated with reduced risk of knee OA. These findings suggest that the genotypes and haplotypes of *IL16* are closely correlated with primary knee osteoarthritis. Some studies suggest that variations in *IL16* produce altered protein products with varying cytokine activity [21]. Thus, the contribution of IL-16 to OA may arise from altered levels of IL-16 production or activity in the serum.

In summary, polymorphisms of *IL16* are associated with susceptibility to OA. This suggests that the hereditary variation at these three SNP loci of *IL16* may contribute to primary knee osteoarthritis in the Han population. However, there are some limitations to this study including the small sample size and that no multivariate analysis was performed for the patients with primary knee osteoarthritis. Therefore, in follow-up studies we will collect more detailed data from a larger number of subjects to more systematically explore the correlation between *IL-16* polymorphism and genetic susceptibility for primary knee osteoarthritis.

## Disclosure of conflict of interest

None.

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