**IL1F7 Gene Polymorphism Is not Associated with Rheumatoid Arthritis Susceptibility in the Northern Chinese Han Population: A Case–Control Study**

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**Abstract**

**Background:** Interleukin (IL)-37, also called IL1F7, is a natural inhibitor of inflammatory and immune responses. It is involved in the pathogenesis of rheumatoid arthritis (RA). This study aimed to investigate the role of *IL1F7* gene polymorphism in RA susceptibility in a large cohort of patients.

**Methods:** Five selected single-nucleotide polymorphisms in *IL1F7* genes (rs2723186, rs3811046, rs4241122, rs4364030, and rs4392270) were genotyped by TaqMan Allelic Discrimination in Northern Chinese Han population. The allele and the genotype were compared between patients with RA and healthy controls. Association analyses were performed on the entire data set and on different RA subsets based on the status of the anti-cyclic citrullinated peptide antibody and the rheumatoid factor by logistic regression, adjusting for age and gender.

**Results:** Trend associations were detected between rs2723186, rs4241122, rs4392270, and RA in Stage I (160 patients with RA; 252 healthy controls). Further validation in Stage II comprised 730 unrelated patients with RA (mean age: 54.9 ± 12.6 years; 81.6% females) and 778 unrelated healthy individuals (mean age: 53.5 ± 15.7 years; 79.5% females). No significant differences in the distributions of alleles and genotypes were observed between the case and control groups in both the entire set and the different RA subsets. Disease activity and age of RA onset were also not associated with genotype distributions.

**Conclusion:** *IL1F7* gene polymorphism does not significantly influence RA susceptibility in the Northern Chinese Han population.

**Key words:** *IL1F7* Gene; Interleukin-37; Rheumatoid Arthritis; Single-nucleotide Polymorphisms

**Introduction**

Rheumatoid arthritis (RA) is an autoimmune disease associated with progressive disability, systemic complications, early death, and socioeconomic costs. Although the etiology of the disease is still obscure, the genetic factor is of great importance for the development of RA. The heritability of RA is estimated to be about 60%. At present, 101 loci have been identified with susceptibility to RA across multiple populations; these loci are at least partially shared between ethnicities. The most important genetic factors were HLA-DRB1-shared epitope alleles.

Genetic susceptibility also contributes to different clinical manifestations, laboratory phenotypes, and radiographic bone erosions of patients with RA. Genetic susceptibility also contributes to different clinical manifestations, laboratory phenotypes, and radiographic bone erosions of patients with RA.

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Interleukin (IL)-37, originally defined as IL-1 family member 7 (IL1F7), is the most recently identified cytokine and acts as a fundamental inhibitor of innate immunity.[9] The human IL1F7 gene is located on human chromosome 2q13 and undergoes alternative splicing, resulting in the expression of five different isoforms, namely, IL-37a to IL-37e.[7] IL-37a, b, and d share the β-trefoil structural pattern of the IL-1 family and might play a role as functional cytokines. Pro-inflammatory cytokines could induce IL-37 production in peripheral blood mononuclear cells. Conversely, the expression of IL-37 in macrophages or epithelial cells inhibited the secretion of pro-inflammatory cytokines, such as tumor necrosis factor (TNF), IL-6, and IL-1β.[6,8,10] IL-37 was not constitutively expressed in tissues from healthy controls, but could be upregulated by inflammatory stimuli and correlated with disease activity in various autoimmune diseases, such as RA, Graves’ disease, and Guillain–Barré syndrome.[10–15] Apart from this, the single-nucleotide polymorphism (SNP) rs3811047 of the IL-37 might contribute to the susceptibility of ankylosing spondylitis.[16]

Previous studies revealed that IL-37 was elevated in the synovial tissue and plasma of patients with RA.[16,12] The plasma level of IL-37 was positively related to pro-inflammatory cytokines, such as TNF-α and IL-6 in RA, and decreased in drug responders after treatment with disease-modifying antirheumatic drugs.[12] IL-37 could downregulate IL-17 and IL-17-triggering cytokine production and curb Th17 cell proliferation in patients with RA and mice with collagen-induced arthritis.[13] These studies suggested that IL-37 was associated with the pathogenesis of RA and implied that variations in the IL1F7 gene might be a genetic risk factor of RA. To date, two studies, involving 184 patients and 184 controls, and 276 patients and 276 controls, respectively, showed that SNP rs3811047 in the IL1F7 gene had no significant relationship with RA susceptibility among Chinese Han population, but was associated with disease activity in one study.[17,18] However, the sample sizes of these two studies were probably too small to reach a statistical power. Besides, one SNP cannot cover the entire region to identify the relationship between the IL1F7 gene and susceptibility to RA. It is also still unclear whether the IL1F7 gene is related to different RA subsets based on the status of anti-cyclic citrullinated peptide (CCP) antibody, rheumatoid factor (RF), or gender. This study aimed to test five SNPs, covering the entire region of IL1F7 gene with a large number of study participants to identify whether the polymorphisms of IL1F7 gene contribute to RA susceptibility and to determine whether the genetic impact of IL1F7 was specifically related to subsets of RA.

**Methods**

**Ethical approval**

This study was approved by the ethical committee of Peking University People’s Hospital (FWA00001384). Oral and written informed consent was obtained from all participants enrolled in this study.

**Study subjects**

In Stage I, the study initially screened 160 patients with RA and 252 controls for identifying the relationship between RA and the IL1F7 gene. Subsequently, in Stage II, the sample size was enlarged for further validation. A total of 730 unrelated patients with RA were recruited from the Department of Rheumatology at Peking University People’s Hospital. All patients satisfied the American College of Rheumatology 1987 revised criteria for the diagnosis of RA.[19] The following clinical characteristics of patients with RA were recorded: age of RA onset, disease duration, Disease Activity Score using 28 joints (DAS28), RF, and anti-CCP status. In this sample, 81.9% of the patients (411/502) were anti-CCP positive, defined, and quantified with results >5 RU/ml using a second-generation anti-CCP antibody enzyme-linked immunosorbent assay kit (Euroimmun, Luebeck, Germany). The control group comprised 778 unrelated healthy individuals who were recruited from the Health Care Center of Peking University People’s Hospital. All participants were Chinese Han, originating from North China. The clinical characteristics of the patients and healthy controls are shown in Table 1. Age and gender were comparable for both the patients with RA and the healthy controls (P > 0.05).

**Deoxyribonucleic acid extraction**

Genomic deoxyribonucleic acid (DNA) samples were extracted from 2 ml of peripheral white blood cells gathered from patients with RA and healthy controls using a DNA extraction kit (Tiangen, Beijing, China). The concentration (>0.5 μg/μl) and purity (A$_{260/280}$ = 1.6–1.8) of

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**Table 1:** Clinical characteristics of patients with RA and controls in the study

| Characteristics                  | Stage I                  | Stage II                  | Patients in two stages |
|----------------------------------|--------------------------|---------------------------|------------------------|
|                                  | Patients | Controls | Statistics | P   | Patients | Controls | Statistics | P   | Statistics | P   |
| Number of cases                  | 160      | 252      |            |     | 730      | 778      |            |     |            |     |
| Gender, female, %                | 81.8     | 83.3     | 0.173*     | 0.678 | 81.6     | 79.5     | 0.916*     | 0.338 | 0.000*     | 0.996 |
| Age, years (mean ± SD)           | 53.1 ± 12.4          | 53.8 ± 16.9   | 0.484†     | 0.629 | 54.9 ± 12.6 | 53.5 ± 15.7   | 1.745†     | 0.081 | 1.559†     | 0.119 |
| Age at RA onset, years (mean ± SD)| 43.4 ± 13.0          | 58.3 ± 16.9   | 0.484†     | 0.629 | 45.8 ± 14.5 | 53.5 ± 15.7   | 1.745†     | 0.081 | 1.559†     | 0.119 |
| Disease duration, median (IQR), months | 96 (36, 180)   | 84 (21, 168) | 1.395‡     | 0.163 | -1.395‡     | 0.163 |
| Anti-CCP status, percentage positive | 77.1     | 81.9     | 1.749*     | 0.186 | 80.3     | 2.232*     | 0.135 |
| RF status, percentage positive   | 74.3     | 80.3     |            |     |            |           |            |

*: χ² value. †: t value. ‡: U value. CCP: Cyclic citrullinated peptide; IQR: Interquartile range; RA: Rheumatoid arthritis; RF: Rheumatoid factor; SD: Standard deviation.
DNA were measured by an ultraviolet spectrophotometer. The DNA showed no degradation through agarose gel electrophoresis and was stored at −80°C.

**Single-nucleotide polymorphism selection and genotyping**

In the entire region around the IL1F7 gene, SNPs with minor allele frequencies (MAFs) >0.05 were identified in HapMap Chinese Han in Beijing (CHB) data sets (http://hapmap.ncbi.nlm.nih.gov). Tag SNPs were selected based on linkage disequilibrium (LD) between SNPs, according to HaploView 4.2 (Broad Institute of MIT and Harvard, Cambridge, USA) based on HapMap CHB with thresholds of $r^2 > 0.8$ to reduce redundancy.

Five tagged SNPs, rs2723186, rs3811046, rs4241122, rs4364030, and rs4392270, were selected for the study (Table 2 and Figure 1). Predesigned TaqMan Genotyping Assays were applied for the genotyping of SNPs rs2723186, rs3811046, rs4241122, rs4364030, and rs4392270 (ID: C_16061041_10, ID: C_27487174_10, ID: C_11725774_10, ID: C_27161165_10, and ID: C_38367620, respectively, Applied Biosystems, CA, USA). Allelic discrimination was performed using the ABI 7300 Real-Time (Applied Biosystems, CA, USA) polymerase chain reaction system. The genotyping success rate was >95%. A total of 100 individuals of cases and controls for each SNP were sequenced to validate the genotyping accuracy, and the confirmation rate was found to be 100%.

**Statistical analysis**

Genotype frequencies for SNPs of IL1F7 gene were tested against the Hardy–Weinberg equilibrium (HWE) using the Pearson’s Chi-square goodness-of-fit test. The differences in allelic distributions between cases and controls were analyzed using the Chi-square or Fisher’s exact test with two-tailed P values. The P values for odds ratios (ORs) and 95% confidence intervals were calculated for an alternative genetic model analysis using logistic regression, adjusting for age and gender. First, an explorative analysis was performed and no correction for multiple testing was applied in Stage I. Significance was considered at $P < 0.05$. Subsequently, tagged SNPs were tested in the validation cohort. For multiple testing corrections, statistical significance was specified by Bonferroni correction. Sample size calculation was done by PS software (Version 3.0.43; available at http://www.mc.vanderbilt.edu/prevmed/ps). A total of 707 cases and controls were needed to detect 80% power with a fixed MAF of 0.1, assuming an OR of 1.4 and a Type I error $P$ of 0.05. Comparisons of DAS28 and the age of RA onset for three genotypes were made by a one-way analysis of variance. The Mann–Whitney U-test, t-test, and Chi-square test were used to compare demographic characteristics between patients with RA and healthy controls. All analyses were performed on IBM SPSS Statistical Software version 23.0 (Chicago, IL, USA).

**Results**

The genotype distributions of rs2723186, rs3811046, rs4241122, rs4364030, and rs4392270 polymorphisms were in accordance with the HWE in both cases and controls ($P > 0.05$). In HapMap CHB (data resulted from the sequencing of 137 individuals), the allelic distributions for the five SNPs are shown in Table 2. In 160 healthy controls, the allele frequency for rs4364030 C and rs3464030 G was found to be 0.519 and 0.481, respectively, different from the data in HapMap CHB. The allelic distributions of other four SNPs between the data from this study and HapMap CHB were comparable.

**Distributions of alleles and genotypes for the five single-nucleotide polymorphisms in Stage I**

In Stage I, the genotype frequencies for rs2723186, rs4241122, and rs4392270 were significantly different between patients with RA and healthy controls ($\chi^2 = 4.817, P = 0.028; \chi^2 = 4.573, P = 0.032; \text{and} \chi^2 = 7.121, P = 0.008$, respectively). Meanwhile, a marginal difference was observed in the dominant model of rs4392270 ($B = −0.520, P = 0.028$); otherwise, the genotype and allele frequencies of rs4364030 and rs3811046 were comparable between patients with RA and healthy controls ($P > 0.05$, Table 3).

**Distributions of alleles and genotypes for IL1F7 single-nucleotide polymorphisms in Stage II**

Based on the results of Stage I, the sample size was enlarged for rs2723186, rs4241122, and rs4392270 in Stage II. For Bonferroni correction, the current data were found to be equivalent to three independent markers with no LD, and, consequently, a corrected $P = 0.017$ was equivalent to uncorrected $P = 0.05$. For rs2723186, the genotype distributions were GG (73.1%), AG (25.4%), and AA (1.5%) in patients with RA and GG (75.8%), AG (21.8%), and AA (2.4%) in controls. No significant differences were found between cases and controls ($P > 0.017$) in either the recessive model or the dominant model. In addition, no difference was observed in the allelic frequency between the two groups (A: 14.2% in cases versus 13.3% in controls, $\chi^2 = 0.542, P = 0.461$). Similarly, the distributions of alleles and genotypes for rs4241122 and rs4392270 were comparable between patients with RA and healthy controls (Table 4).

**Relationship between clinical characteristics and genotype distribution**

The clinical characteristics of RA, according to the IL1F7 gene SNP genotypes, including age of RA onset and DAS28 score, did not show significant differences ($P > 0.05$, Table 5). The contribution of the IL1F7 region to different anti-CCP and RF subsets was also analyzed. No associations were found between rs2723186, rs4241122, rs4392270, and different anti-CCP or RF subsets of patients ($P > 0.017$, Table 6). In addition, the distributions of alleles and genotypes of the three SNPs were comparable between RA and healthy controls in both female and male subsets (Table 7).

**Discussion**

This study was aimed to demonstrate the relationship of SNPs, covering the entire region of IL1F7 gene with RA in a Northern Chinese Han population. The results of the
**Table 2: Information of IL1F7 SNPs from the HapMap database**

| rs number     | Chr position | Contig position | Allele | MAF  | Genotype | Frequency* | Function class | mRNA | Protein |
|---------------|--------------|-----------------|--------|------|----------|------------|----------------|-------|---------|
| rs2723186     | 113675080    | 3423743         | A      | 0.102| AA/AG/GG | 0.0/0.204/0.796 | Intron | A→G    |
| rs3811046     | 113671378    | 3420041         | G      | 0.146| GG/GT/TT | 0.0/0.292/0.708 | GGA→GTA | Gly⇒Val |
| rs3464030     | 113664409    | 3420041         | C      | 0.493| CC/CG/GG | 0.234/0.518/0.248 |       |         |
| rs3492270     | 113678629    | 3427292         | A      | 0.151| AA/AG/GG | 0.037/0.229/0.734 |       |         |
| rs4241122     | 113678856    | 3427519         | G      | 0.146| GG/AG/AA | 0.0/0.292/0.708 |       |         |

*The total number of samples is 137. MAF: Minor allele frequency; SNPs: Single-nucleotide polymorphisms.

**Table 3: Association analysis of SNPs in the IL1F7 gene with RA in the discovery population*%

| Genotype | RA, n (%) | CON, n (%) | Alleles | RA, n (%) | CON, n (%) | Genotypic | χ²   | P†  |
|----------|-----------|------------|---------|-----------|------------|-----------|-------|-----|
| GG       | 123 (76.9)| 167 (66.3) | G       | 280 (87.5)| 412 (81.7) | GG + AG   | 4.817 | 0.028|
| AG       | 34 (21.2) | 78 (31.0)  | A       | 40 (12.5) | 92 (18.3)  | AG + AA   |       |     |
| AA       | 3 (1.9)   | 7 (2.8)    |         |           |            | AA       |       |     |
| TT       | 108 (67.5)| 167 (66.2) | T       | 264 (82.5)| 413 (81.9) | TT + TG   | 0.041 | 0.839|
| TG       | 48 (30.0)| 79 (31.3)  | G       | 56 (17.5) | 91 (18.1)  | TG + GG   |       |     |
| GG       | 4 (2.5)   | 6 (2.4)    |         |           |            | GG       |       |     |
| AA       | 108 (67.5)| 147 (58.3) | A       | 265 (82.8)| 386 (76.6) | AA + AG   | 4.573 | 0.032|
| AG       | 49 (30.6)| 92 (36.5)  | G       | 55 (17.2) | 118 (23.4) | AG + GG   |       |     |
| GG       | 3 (1.9)   | 13 (5.2)   |         |           |            | GG       |       |     |
| CC       | 44 (27.5)| 62 (24.6)  | C       | 166 (51.9)| 264 (52.4) | CC + CG   | 0.020 | 0.887|
| CG       | 78 (48.7)| 140 (55.6)| G       | 154 (48.1)| 240 (47.6) | CG + GG   |       |     |
| GG       | 38 (23.8)| 50 (19.8)  |         |           |            | GG       |       |     |
| GG       | 123 (76.9)| 163 (64.7) | G       | 280 (87.5)| 405 (80.4) | GG + AG   | 7.121 | 0.008|
| AG       | 34 (21.2)| 79 (31.3)  | A       | 40 (12.5) | 99 (19.6)  | AG + AA   |       |     |
| AA       | 3 (1.9)   | 10 (4.0)   |         |           |            | AA       |       |     |

*Association analysis is adjusted for age and gender; †Numbers in bold represent P<0.05. CI: Confidence interval; CON: Healthy controls; OR: Odds ratio; RA: Patients with rheumatoid arthritis; SNPs: Single-nucleotide polymorphisms.
The present study showed that SNPs rs2723186, rs3811046, rs4241122, rs4364030, and rs4392270 were not associated with RA, suggesting that the IL1F7 polymorphisms were not involved in the susceptibility of both the whole set and different RA subsets in the Northern Chinese Han population.

Table 4: Association analysis of SNPs in the IL1F7 gene with RA in the validation population*

| Genotype | RA, n (%)† | CON, n (%)† | Alleles | RA, n (%)† | CON, n (%)† | χ² | P‡ |
|----------|------------|-------------|---------|------------|-------------|-----|-----|
| rs2723186 |            |             |         |            |             |     |     |
| GG       | 523 (73.1) | 564 (75.8)  | G       | 1228 (85.8)| 1290 (86.7) | 0.542| 0.461|
| AG       | 182 (25.4) | 162 (21.8)  | A       | 204 (14.2) | 198 (13.3)  |     |     |
| AA       | 11 (1.5)   | 18 (2.4)    |         |            |             |     |     |
| rs4241122 |            |             |         |            |             |     |     |
| AA       | 458 (64.0) | 474 (64.1)  | A       | 1129 (79.0)| 1175 (79.5) | 0.133| 0.716|
| AG       | 213 (29.8) | 227 (30.7)  | G       | 301 (21.0) | 303 (20.5)  |     |     |
| GG       | 44 (6.2)   | 38 (5.1)    |         |            |             |     |     |
| rs4392270 |            |             |         |            |             |     |     |
| GG       | 517 (71.9) | 545 (72.6)  | G       | 1225 (85.2)| 1282 (85.4) | 0.16 | 0.900|
| AG       | 191 (26.6) | 192 (25.6)  | A       | 213 (14.8) | 220 (14.6)  |     |     |
| AA       | 11 (1.5)   | 14 (1.9)    |         |            |             |     |     |

*Association analysis is adjusted for age and gender; †Numbers may not add up to the expected total because of genotyping failure; ‡For Bonferroni correction, a corrected P<0.017 is considered significant. CI: Confidence interval; CON: Healthy controls; OR: Odds ratio; RA: Patients with rheumatoid arthritis; SNPs: Single-nucleotide polymorphisms.

Figure 1: Information about IL1F7 SNPs from the HapMap CHB database. Five SNPs (rs2723186, rs3811046, rs4241122, rs4364030, and rs4392270) were selected in the study. CHB: Chinese Han in Beijing; SNP: Single-nucleotide polymorphism.
Table 5: Analysis of DAS28 score and age of RA onset according to the genotype distribution of SNPs in the IL1F7 gene

| Genotypes | n  | DAS28 score | n  | Age of RA onset |
|-----------|----|-------------|----|-----------------|
|           |    | Mean ± SD   |    | Mean ± SD       |
|           |    | F           | P  | F               |
| rs2723186 |    |             |    |                |
| GG        | 275| 5.39 ± 1.61 | 2.036| 0.132| 380 | 46.1 ± 14.3 | 0.369 | 0.691 |
| AG        | 102| 5.64 ± 1.53 | 1.43 | 0.043 | 143 | 46.2 ± 12.9 | 0.067 | 0.691 |
| AA        | 6  | 4.50 ± 1.06 |     |        | 9   | 50.1 ± 13.5 |       |       |
| rs4241122 |    |             |    |                |
| AA        | 250| 5.43 ± 1.63 | 0.143| 0.043 | 334 | 46.5 ± 14.5 | 1.924 | 0.147 |
| AG        | 118| 5.52 ± 1.45 | 1.63 | 0.067 | 163 | 44.7 ± 12.7 | 4.47 | 0.127 |
| GG        | 24 | 4.52 ± 1.06 |     |        | 9   | 50.1 ± 13.5 |       |       |
| rs4392270 |    |             |    |                |
| GG        | 273| 5.42 ± 1.64 | 2.374| 0.095 | 377 | 46.4 ± 14.3 | 0.450 | 0.638 |
| AG        | 109| 5.65 ± 1.51 |     |        | 145 | 45.8 ± 13.0 |       |       |
| AA        | 7  | 4.40 ± 1.00 |     |        | 9   | 50.1 ± 14.5 |       |       |

DAS28: Disease Activity Score using 28 joints; RA: Rheumatoid arthritis; SD: Standard deviation; SNPs: Single-nucleotide polymorphisms.

Table 6: Association analysis of SNPs in the IL1F7 gene with subsets of patients with RA based on antibody status

| Genotypes | n   | 1, n (%) | 2, n (%) | 11, n (%) | 12, n (%) | 22, n (%) |
|-----------|-----|----------|----------|-----------|-----------|----------|
|           |     |          |          |           |           |          |
| rs2723186 |     |          |          |           |           |          |
| CON       | 744 | 1290 (86.7) | 198 (13.3) | 564 (75.8) | 162 (21.8) | 18 (2.4) |
| RA CCP+   | 408 | 695 (85.2) | 121 (14.8) | 294 (72.1) | 107 (26.2) | 7 (1.7)  |
| RA CCP−   | 90  | 157 (87.2) | 23 (12.8)  | 68 (75.6)  | 21 (23.3)  | 1 (1.1)  |
| RA RF+    | 383 | 653 (89.9) | 113 (10.1) | 276 (72.1) | 101 (26.4) | 6 (1.6)  |
| RA RF−    | 93  | 157 (84.4) | 29 (15.6)  | 65 (69.9)  | 27 (29.0)  | 1 (1.1)  |
| rs4241122 |     |          |          |           |           |          |
| CON       | 739 | 1175 (79.5) | 303 (20.5) | 474 (64.1) | 227 (30.7) | 38 (5.1) |
| RA CCP+   | 407 | 637 (78.3) | 177 (21.7) | 259 (63.6) | 119 (29.2) | 29 (7.2) |
| RA CCP−   | 91  | 151 (83.0) | 31 (17.0)  | 61 (67.0)  | 29 (31.9)  | 1 (1.1)  |
| RA RF+    | 384 | 603 (78.5) | 165 (21.5) | 245 (63.8) | 113 (29.4) | 26 (6.8) |
| RA RF−    | 95  | 149 (78.4) | 41 (21.6)  | 60 (63.2)  | 29 (30.5)  | 6 (6.3)  |
| rs4392270 |     |          |          |           |           |          |
| CON       | 751 | 1282 (85.4) | 220 (14.6) | 545 (72.6) | 192 (25.6) | 14 (1.9) |
| RA CCP+   | 409 | 693 (84.7) | 125 (15.3) | 291 (71.1) | 111 (27.1) | 7 (1.7)  |
| RA CCP−   | 87  | 151 (86.8) | 23 (13.2)  | 65 (74.7)  | 21 (24.1)  | 1 (1.1)  |
| RA RF+    | 382 | 649 (84.9) | 115 (15.1) | 273 (71.4) | 103 (27.0) | 6 (1.6)  |
| RA RF−    | 95  | 159 (83.7) | 31 (16.3)  | 65 (68.4)  | 29 (15.3)  | 1 (1.3)  |

Contd...
Table 7: Association analysis of SNPs in the *IL1F7* gene with different genders of patients with RA*

| Genotypes | rs2723186 | rs4241122 | rs4392270 |
|-----------|------------|------------|------------|
|          | Female     | Male       | Female     | Male       | Female     | Male       |
| RA CCP+  | CON        | RA         | CON        | RA         | CON        | RA         |
|          |            |            |            |            |            |            |
|          | 554        | 469        | 523        | 468        | 140        | 106        |
|          | 961 (86.7) | 795 (84.7) | 835 (79.8) | 731 (78.1) | 226 (80.7) | 168 (79.2) |
|          | 147 (13.3) | 143 (15.2) | 211 (20.2) | 205 (21.9) | 54 (19.3)  | 44 (20.8)  |
|          | 419 (75.6) | 332 (70.8) | 337 (64.4) | 290 (62.0) | 93 (66.4)  | 72 (67.9)  |
|          | 123 (22.2) | 131 (27.9) | 161 (30.8) | 151 (32.3) | 40 (28.6)  | 24 (22.6)  |
|          | 12 (2.2)   | 6 (1.3)    | 25 (4.8)   | 27 (5.8)   | 7 (5.0)    | 10 (9.5)   |
| RA CCP−  | CON        | RA         | CON        | RA         | CON        | RA         |
|          |            |            |            |            |            |            |
|          | 0.168      | 0.058      | 0.163      | 1.026      | 0.257      | 0.891      |
|          | 0.682      | 0.809      | 0.345      | 0.311      | 0.612      | 0.354      |
|          | −0.240     | −0.007     | 0.221      | −0.670     | −0.668     | 0.510      |
|          | 0.610      | 0.991      | 0.439      | 0.180      | 0.523      | 0.330      |
|          | 0.79 (0.31–1.98) | 1.01 (0.26–3.87) | 1.25 (0.71–2.18) | −0.255 | 0.058 | 0.809 |
|          | 0.15 (0.87–1.52) | 0.97 (0.57–1.65) | 1.12 (0.87–1.46) | 1.29 (0.98–1.71) | 1.05 (0.57–1.91) | 0.87 (0.51–1.51) |

*Association analysis is adjusted for age and gender; Numbers may not add up to the expected total because of genotyping failure; \(^1\): Major (common) allele; \(^2\): Minor (rare) allele. 2/1: rs2723186, A/G; rs4241122, G/A; rs4392270, A/G; \(^3\)For Bonferroni correction, a corrected \(P<0.017\) is considered significant. CCP: Anti-cyclic citrullinated peptide; CI: Confidence interval; CON: Healthy controls; OR: Odds ratio; RA: Patients with rheumatoid arthritis; RF: Rheumatoid factor; SNPs: Single-nucleotide polymorphisms.
Previous studies suggested that IL-37, as a natural suppressor of innate inflammation, might participate in the development of autoimmune disease, including RA. The studies by Shi et al. and Pei et al. showed no relationship between the SNP of IL1F7 gene with RA. However, the small sample size limited the reliability of the studies. Therefore, a large-sample study was conducted to identify the relationship of IL1F7 gene with RA susceptibility. Meanwhile, five SNPs spanning the entire region were involved in comprehensively investigate the contribution of IL1F7 genetic variant to the development of RA. Even though the trend associations of three SNPs were detected in Stage I, the result of further validation with the statistical power >80% could be sufficient to confirm that IL1F7 genes were not significantly associated with susceptibility.

Genetic risk factors might be responsible for the distinct clinical characteristics of the disease, which was important for understanding the pathogenesis of the disease. For example, the PTPN22 R620W risk allele was restricted to anti-CCP-positive RA. The present study also analyzed the association of different RA subsets according to antibody status and gender. The results demonstrated that IL-37-tagging SNPs had no contribution to the susceptibility of RA, neither in the entire study cohort nor in the subsets. Furthermore, no statistical difference of disease activity was detected between SNP genotype subgroups, indicating that IL-37 might not participate in the inflammation process of RA through direct gene variants. The findings of this study did not rule out the pathogenic role of IL-37 in RA. A number of examples pertaining to inconsistency were available. For example, the IL17A and IL17F gene polymorphisms were not associated with the susceptibility of RA, while they were among the most important cytokines in the pathogenesis of RA.

One of the limitations of the current study was that only Chinese Han population originating from North China was enrolled. The significant role of IL1F7 gene in other ethnic populations could not be excluded in the pathogenesis of RA. In addition, paired serum specimens of the individuals were not collected to evaluate serum IL-37 expression and clarify the possible relationship between IL1F7 gene polymorphism and IL-37 protein level. Further studies are warranted to explore the role of IL-37 in the development of RA.

In conclusion, the present study with a large sample size showed that the five SNPs covering the entire region of IL1F7 gene were not risk factors for RA susceptibility. Considering the power calculation and sample size of the present study, it was concluded that no association existed between the IL1F7 gene and RA in the Northern Chinese Han population. However, larger studies are needed to definitively exclude the overall significance of IL1F7 gene in RA.

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### Conflicts of interest
There are no conflicts of interest.

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### Table 7: Cont'd...

| Genotypes  | 2 versus 1 | Recessive model (11 + 21 versus 22) | Dominant model (11 versus 21 + 22) |
|------------|------------|-----------------------------------|-----------------------------------|
|            | χ²         | B       | P² |
| Male CON   | 1.263      | 0.261  | 1.609        | 0.154      | 5.0 (0.55−45.67) | 0.233 | 0.451 | 1.26 (0.69−2.31) |

*Association analysis is adjusted for age; †Numbers may not add up to the expected total because of genotyping failure; 2: Minor (rare) allele; 2/1: rs2723186, A/G; rs4241122, G/A; rs4392270, A/G; §For Bonferroni correction, a corrected P<0.017 is considered significant. CI: Confidence interval; CON: Healthy controls; OR: Odds ratio; RA: Patients with rheumatoid arthritis; SNPs: Single-nucleotide polymorphisms.
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