Associations between WNT1-inducible signaling pathway protein-1 (WISP-1) genetic polymorphisms and clinical aspects of rheumatoid arthritis among Chinese Han subjects

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Abstract
This study genotyped blood samples from 214 patients with rheumatoid arthritis (RA) and 293 healthy controls for single nucleotide polymorphisms (SNPs) rs2977537, rs2929970, rs2929973, rs2977530, rs1689334 and rs62514004. We want to investigate whether the SNPs in the WNT1-inducible signaling pathway protein 1 (WISP-1) gene may increase the risk of developing RA. We showed that RA disease was more likely with the AA genotype compared with the AG genotype of SNP rs2977537 (adjusted odds ratio [AOR]: 0.54; 95% confidence interval [CI]: 0.34–0.84), and with the TT genotype (AOR: 0.24; 95% CI: 0.13–0.39) or the GG genotype (AOR: 0.05; 95% CI: 0.03–0.10) compared with the GT genotype of rs2929973, and with the AA genotype (AOR: 0.34; 95% CI: 0.22–0.54) or GG genotype (AOR: 0.52; 95% CI: 0.31 to 0.87) vs the AG genotype of rs2977530. Rheumatoid factor positivity was more likely with the AA genotype than with the AG genotype of the rs2977530 polymorphism (AOR: 0.16; 95% CI: 0.16–0.94). High CRP (>8 mg/L) was more likely with the non-AG genotype (AA + GG) than the AG genotype of rs2977537 (AOR: 1.84; 95% CI: 1.05–3.21), and with the AA genotype vs the AG genotype of rs2977530 (AOR: 2.62; 95% CI: 1.35–5.09). Compared with the AG genotype, the AA genotype of rs2999707 was more likely to require prednisolone (AOR: 0.49; 95% CI: 0.27–0.88), while the AG genotype was more likely than the AA genotype of SNP rs2977530 to require TNF-α inhibitors (AOR: 2.07; 95% CI: 1.08 to 3.98). WISP-1 may be a diagnostic marker and therapeutic target for RA therapy.

Abbreviations: ACPAs = anti-citrullinated protein antibodies, Ang2 = angiopoietin-2, AOR = adjusted odds ratio, CCL4 = chemokine C-C motif ligand 4, CI = confidence interval, Cis = confidence intervals, CRP = C-reactive protein, CYR61 = cysteine-rich 61, ESR = erythrocyte sedimentation rate, FAK = focal adhesion kinase, HCAECs = human coronary artery endothelial cells, HCC = hepatocellular carcinoma, HIF = hypoxia-inducible factor, HMBG1 = high-mobility group box protein 1, HVE = Hardy-Weinberg equilibrium, JNK = Jun amino-terminal kinase, NOV = nephroblastoma overexpressed, OR = odds ratios, PCR = polymerase chain reaction, RA = rheumatoid arthritis, RETN = resistin, RF = rheumatoid factor, SNP = single nucleotide polymorphisms, TNF-α = tumor necrosis factor-α, VEGF-A = vascular-endothelial growth factor-A, WISP-1 = WNT1-inducible signaling pathway protein-1.

Keywords: rheumatoid arthritis, single nucleotide polymorphism, WISP1

1. Introduction
Rheumatoid arthritis (RA) presents with prominent hypertrophy and hypervascularity of the synovial tissues and consequent joint destruction, and affects around 1% of the global population.[1-3] Anti-inflammation drugs are most common for RA treatment.[4,5] In spite of the recent advent of biological agents enabling some RA patients to reach disease remission or at least low disease activity, a substantial proportion remain treatment-refractory and suffer from progressive joint destruction, functional deterioration or even premature mortality.[6,7] The fact that
genetic factors are responsible for more than one-half of the overall susceptibility to RA highlights the importance of research into genetic anomalies of this disease.2,3,9] Investigations into RA genetics might tailor personalized risk prediction and treatment regimens.

Single nucleotide polymorphisms (SNPs) are single nucleotide variations occurring at specific sites in the genome with substantial frequency within the general population.1,2,10,11] Genotyping SNPs and comparing the frequency of SNPs among subgroups (e.g., controls and patients) are frequently used to stratify the risk and prognosis of human diseases, including RA.1,2,12] The SNPs of high-mobility group box protein 1 (HMGB1), resistin (RETN), chemokine C-C motif ligand 4 (CCL4), and angiopoietin-2 (Ang2) genes have been shown to be associated with RA susceptibility.1,2,3,13]

WNT1 inducible signaling pathway protein-1 (WISP-1), also known as CCN4 or Elm1, is a cysteine-rich protein belonging to the CCN superfamily.14] The “CCN” denotes the initialism of its first 3 family members; connective tissue growth factor, cysteine-rich 61 (CYR61), and nephroblastoma overexpressed (NOV).15] WISP-1 is a Wnt-1 and β-catenin responsive gene that maps to chromosome 8q24.1 – 8q24.3, has 5 exons and 4 introns16–17] and is expressed during the processes of embryonic development and tissue repair.18] Anomalous WISP-1 expression is correlated with various pathologies including arthritis, pathologic fibrosis, and malignancy.19] Recent studies have indicated that WISP-1 interacts with integrins.20] Integrin αvβ3 is known to paradoxically harbor both pro- and anti-angiogenic functions,21] while WISP-1 is involved in the angiogenesis of various human diseases, including osteosarcoma and oral squamous cell carcinoma.22–24] The genetic polymorphism of WISP-1 is predictive of platinum-based chemotherapy response as well as platinum-based chemotherapy toxicity in patients with lung cancer.25–26] WISP-1 SNPs are also predictive for susceptibility to uterine cervical cancer, hepatocellular carcinoma, and osteoarthritis.27–29]

The process of angiogenesis is crucial in the pathogenesis of RA.1,19] The proliferation of the articular synovial lining and the consequent invasion by the pannus of underlying cartilage and bone necessitate an increase in the vascular supply to the synovium in RA.31–33] Angiogenesis is also pivotal in facilitating the invasion of inflammatory cells and increase in local pain receptors that lead to structural damage and pain.1,2,3,34] Despite the known impact of WISP-1 on angiogenesis, the key component of RA pathogenesis, and the recognized prognostic value of WISP-1 SNPs for human disease, little is known about the association between WISP-1 SNPs and the risk of RA. In this study, we sought to determine the association between the distribution of WISP-1 SNPs and the susceptibility and clinical aspects of RA.

2. Materials and methods

2.1. Patients and blood samples

We collected 214 blood specimens from patients diagnosed with RA at Dongyang People’s Hospital from 2007 and 2015. A total of 293 healthy participants without history of RA or cancer were recruited and served as controls. All of the enrolled participants indicated as Chinese Han ethnicity. All study participants provided written informed consent, and this study was approved by the Ethics Committee of Dongyang People’s Hospital Ethics Committee and Institutional Review Board (2015-YB002). Clinical and pathological characteristics of all patients were determined based on medical records. A standardized questionnaire and data from the electronic medical record system were used to obtain demographic and clinical information on age, gender, and disease duration, as well as concurrent treatment with methotrexate, prednisolone, and tumor necrosis factor-α (TNF-α) inhibitors. At baseline, serum samples were collected from all RA patients and analyzed for levels of anti-citrullinated protein antibodies (ACPAs), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Samples were ACPA-positive if anti-CCP2 titers were ≥17 IU/ml and RF-positive if IgM RF titers were ≥30 IU/ml. Whole blood samples (3 ml) were collected from all study participants and stored at −80°C for subsequent DNA extraction.

2.2. Selection of WISP-1 polymorphisms

Six WISP-1 SNPs were selected from the intron of WISP-1; all SNPs had minor allele frequencies of greater than 5% to prevent false negative results. Also, these SNPs were examined with large sample size of oral squamous cell carcinoma as previous described.35]

2.3. Genomic DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using a QiAamp DNA blood kit (Qiagen, CA, USA), following the manufacturer’s instructions. Extracted DNA was stored at −20°C and prepared for genotyping by polymerase chain reaction (PCR).

2.4. Genotyping by real-time PCR using allele-specific RT-PCR

Total genomic DNA was isolated from whole blood specimens using QiAamp DNA blood mini kits (Qiagen, Valencia, CA), following the manufacturer’s instructions. DNA was dissolved in TE buffer (10 mM Tris, pH 7.8, 1 mM EDTA) and stored at −20°C until quantitative PCR analysis. Six WISP-1 SNP probes were purchased from Thermo Fisher Scientific Inc. (CA, USA), and assessment of allelic discrimination for WISP-1 SNPs was conducted using a QuantStudio™ 5 Real-Time PCR system (Applied Biosystems, CA, USA), according to the manufacturer’s instructions. Data were further analyzed with QuantStudio Design & Analysis Software (Applied Biosystems).31,36]

2.5. Statistical analysis

Between-group differences were considered significant if P values were less than 0.05. Hardy-Weinberg equilibrium (HWE) was assessed using Chi-Squared goodness-of-fit tests for biallelic markers. As the data sets were independent and normally distributed, the Fisher exact test was used to compare differences in demographic characteristics between healthy controls and patients with RA. The odds ratios (ORs) and 95% confidence intervals (CIs) for associations between genotype frequencies and the risk of RA or clinical and pathological characteristics were estimated by multiple logistic regression models, after controlling for age and gender in each comparison. All data were analyzed using Statistical Analytic System software (v. 9.1, 2005; SAS Institute, Cary, NC, USA).
3. Results

All of the enrolled participants identified as Chinese Han ethnicity. As shown in Table 1, a significantly greater proportion of individuals in the RA cohort compared with controls were aged >60 years (33.2% vs 24.2%; P < .001) and were female (83.6% vs 50.9%; P < .001). At the time of blood sampling, 40.2% of the RA cohort were receiving TNF-α inhibitors, 55.6% were receiving methotrexate, and 54.2% were receiving prednisolone. The majority of RA patients were rheumatoid factor (RF)-positive (85.0%) and anti-citrullinated protein antibody (ACPA)-positive (79.0%) (Table 1).

All genotypes were in Hardy–Weinberg equilibrium (P > .05). The most frequent genotypes for SNPs rs2977537, rs2929970, rs2929973, rs2977530, rs1689334, and rs62514004 were AG, AA, GG, AA, TT, and AA, respectively, for the control cohort and AG, GG, AA, TT, and AA, respectively, for the RA cohort (Table 2). Compared with individuals carrying the AG genotype of SNP rs2977537, those carrying the AA genotype were around one-third as likely to develop RA (AOR 0.34; 95% CI, 0.22–0.54) compared with those carrying the AG genotype of SNP rs2977537, those carrying the AA genotype were almost 3 times as likely to develop RA (AOR 3.21). When compared with patients carrying the AG genotype of SNP rs2977530, those with the AA genotype were around half as likely to require prednisolone (AOR 0.49; 95% CI, 0.27–0.88). When compared with patients carrying the AG genotype of SNP rs2977530, those with the AA genotype were twice as likely to require TNF-α inhibitors (AOR 2.07; 95% CI, 1.08–3.98).

Finally, we analyzed the impact of genotypes on RA medications (Table 4). When compared with patients carrying the AG genotype of rs2929970, those with the AA genotype were around half as likely to require prednisolone (AOR 0.49; 95% CI, 0.27–0.88). When compared with patients carrying the AG genotype of SNP rs2977530, those with the AA genotype were twice as likely to require TNF-α inhibitors (AOR 2.07; 95% CI, 1.08–3.98).

4. Discussion

The risk of developing RA is influenced by genetic factors. Although the advent of biologics has enabled some patients to achieve minimal disease activity, a substantial proportion remain treatment-refractory, which underlines the importance of continuing to investigate the pathogenesis of RA. Determining RA-related SNPs could help to elucidate the pathogenesis of RA and enable clinicians to stratify individuals at risk of developing the disease.

Angiogenesis, the formation of new capillaries from existing vasculature, plays a key role in RA pathogenesis. In RA, excessive migration of circulating leukocytes into the inflamed joint mandate the formation of new vessels for the trafficking of oxygen and nutrients. WISP-1 is the pivotal mediator for angiogenesis in cancerous and non-cancerous diseases. Tsai et al have demonstrated that WISP-1 promotes vascular-endothelial growth factor-A (VEGF-A) expression in human osteosarcoma cells through the focal adhesion kinase (FAK)/jun amino-terminal kinase (JNK)/hypoxia-inducible factor (HIF)-1α signaling pathways, as well as via downregulation of microRNA-381 expression. This study nicely demonstrated the correlation between WISP-1, angiogenesis, and osteosarcoma cells. In noncancerous disease, Wright et al have shown that human coronary artery endothelial cells (HCAECs) produce WISP-1 and are responsive to autocrine WISP-1-mediated signaling, which functionally promotes their proangiogenic behavior. That investigation showed that altering endogenous expression of WISP-1 in HCAECs directly impacts their network density in vitro.

WISP-1 SNPs have been shown to predict the susceptibility to lung cancer as well as platinum-related response and toxicity. In a comparison of 556 lung cancer patients and 254 healthy controls, WISP1 SNPs rs16893344, rs2977530, rs2977537, and rs62514004 were significantly associated with susceptibility for lung cancer (P = .009, P = .033, P = .049 and P = .036, respectively), while marked correlations were found between the following WISP1 SNPs and response to platinum-based chemotherapy in the lung cancer cohort: rs11778573 (P = .023, nonsmokers), rs16893344 (P = .013, ≥ 50 years, rs2977536 (P = .039, ≥ 50 years; P = .044, nonsmokers; P = .047, non-small-cell lung cancer), rs2977549 (P = .013, smokers) and rs62514004 (P = .033, ≥ 50 years). The same researchers also found associations between certain WISP1 polymorphisms (rs2929965, rs2929969, rs2929970, rs2929973 and rs754958) and the

### Table 1: Clinodemographic characteristics of the study subjects at baseline.

| Variable          | Controls n=293 (%) | RA patients n=214 (%) | P value |
|-------------------|--------------------|-----------------------|---------|
| Age (years)       |                    |                       |         |
| <60               | 222 (75.8%)        | 143 (66.8%)           | <.001   |
| >60               | 71 (24.2%)         | 71 (33.2%)            |         |
| Gender            |                    |                       |         |
| Female            | 149 (50.9%)        | 179 (83.6%)           | <.001   |
| Male              | 144 (49.1%)        | 35 (16.4%)            |         |
| RF                |                    |                       |         |
| Negative          | 32 (15.0%)         | 32 (15.0%)            |         |
| Positive          | 182 (85.0%)        | 182 (85.0%)           |         |
| ACPA              |                    |                       |         |
| Negative          | 45 (21.0%)         | 45 (21.0%)            |         |
| Positive          | 169 (79.0%)        | 169 (79.0%)           |         |
| Serum CRP         |                    |                       |         |
| <8 mg/L           | 128 (59.8%)        | 128 (59.8%)           |         |
| >8 mg/L           | 86 (40.2%)         | 86 (40.2%)            |         |
| ESR               |                    |                       |         |
| <20 mm/h          | 103 (48.1%)        | 103 (48.1%)           |         |
| >20 mm/h          | 111 (51.9%)        | 111 (51.9%)           |         |
| TNF-α inhibitors  | 86 (40.2%)         | 86 (40.2%)            |         |
| Methotrexate      | 119 (56.6%)        | 119 (56.6%)           |         |
| Prednisolone      | 116 (54.2%)        | 116 (54.2%)           |         |

ACPA = anti-citrullinated protein antibodies, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, RF = rheumatoid factor, TNF = tumor necrosis factor.
rs2977530 reduced the risk of invasive cervical cancer, when capacity of patients with preinvasive lesions (n = 293) patients with invasive cervical cancer (n = 214), of our knowledge, our study is the first to identify that SNPs may be useful biomarkers for predicting platinum-based chemotherapy toxicity in lung cancer.[21] Those researchers have also investigated the correlation between WISP-1 SNP and the risk of developing hepatocellular carcinoma (HCC). Among 332 HCC patients and 664 cancer-free controls, individuals with higher frequencies of WISP1 rs62514004 (AG+GG) and rs16893344 (CT+TT) haplotypes were at lower risk than wild-type carriers of being diagnosed with advanced cancer, while alcohol drinkers carrying the WISP1 rs62514004 and rs16893344 GT haplotypes were at significantly increased risk of developing HCC compared with the reference group (AOR 26.59; 95% CI, 9.77–71.96). The study authors therefore suggested that WISP1 SNPs may serve as markers or therapeutic targets for HCC.[29] In addition to the study by Chen et al.,[25,29] Lin et al have suggested the predictive capacity of WISP1 SNPs for cervical cancer.[27] Lin et al recruited Taiwanese patients with invasive cervical cancer (n = 115), patients with preinvasive lesions (n = 95) and normal controls (n = 316), and showed that genotypes AG/GG in WISP1 SNP rs2977530 reduced the risk of invasive cervical cancer, when using AA as a reference. In contrast, genotype AA in WISP1 SNP rs2977537 elevated the risk of invasive cervical cancer, using GG/GA as a reference. Thus, the study authors concluded that genotypes AG/GG in WISP1 SNP rs2977530 reduce the susceptibility of Taiwanese women to invasive cervical cancer, whereas genotype AA in rs2977537 increases this risk.[27] In addition to the correlation with the development of RA mentioned in our study, WISP-1 SNPs are prognostic for other arthritic diseases, such as osteoarthritis. For instance, Uranio et al analyzed the association of rs2929970 SNP with the development of radiographically observable spine osteoarthritis.[23] Those authors found that individuals without the G allele (AA) were significantly over-represented in the cohort that had higher endplate sclerosis scores (P = 0.0069; AOR 2.91; 95% CI, 1.34–6.30). The authors suggest that the rs2929970 SNP genotype correlates with risk for spinal osteoarthritis.[23] Despite the evidence inferring a role for WISP-1 in pathologic angiogenesis, a critical component of RA, and the prognostic value of WISP-1 SNPs in various human diseases, including arthritic disease, few studies have investigated the relationship between WISP-1 SNPs and the risk of developing RA. To the best of our knowledge, our study is the first to identify that SNPs rs2977537, rs2929970, rs2977530, rs1689334, and rs62514004 are associated with RA development in our cohorts of 293 healthy controls and 214 RA patients. We found that the AA genotype of SNP rs2977537, the TT and GG genotypes of SNP rs2929970, and the AA and GG genotypes of SNP.

### Table 2

| Variable | Controls N = 293 (%) | Patients N = 214 (%) | OR (95% CI) | AOR (95% CI) |
|----------|----------------------|----------------------|------------|------------|
| rs2977537 |                      |                      |            |            |
| AG       | 127 (43.4%)          | 110 (49.1%)          | 1 (reference) | 1 (reference) |
| AA       | 118 (40.3%)          | 81 (37.9%)           | 0.67 (0.45–0.98) | 0.71 (0.47–1.08) |
| GG       | 48 (16.4%)           | 28 (13.1%)           | 0.65 (0.38–1.11) | 0.72 (0.40–1.28) |
| AA + GG  | 179 (61.1%)          | 109 (50.9%)          | 0.66 (0.46–0.94) | 0.71 (0.48–1.04) |
| rs2929970 |                      |                      |            |            |
| AG       | 114 (38.9%)          | 105 (49.1%)          | 1 (reference) | 1 (reference) |
| AA       | 132 (45.1%)          | 81 (37.9%)           | 0.67 (0.45–0.98) | 0.71 (0.47–1.08) |
| GG       | 47 (16.0%)           | 28 (13.1%)           | 0.65 (0.38–1.11) | 0.72 (0.40–1.28) |
| AA + GG  | 179 (61.1%)          | 109 (50.9%)          | 0.66 (0.46–0.94) | 0.71 (0.48–1.04) |
| rs2977530 |                      |                      |            |            |
| GT       | 33 (11.3%)           | 100 (46.7%)          | 1 (reference) | 1 (reference) |
| TT       | 127 (43.3%)          | 92 (43.0%)           | 0.24 (0.15–0.39) | 0.24 (0.13–0.39) |
| GG       | 133 (45.4%)          | 22 (10.3%)           | 0.06 (0.03–0.10) | 0.05 (0.03–0.10) |
| TT + GG  | 260 (88.7%)          | 114 (53.3%)          | 0.16 (0.09–0.23) | 0.14 (0.08–0.23) |
| rs1689334 |                      |                      |            |            |
| CC       | 5 (1.7%)             | 2 (0.9%)             | 1 (reference) | 1 (reference) |
| CT       | 68 (23.2%)           | 65 (30.4%)           | 0.31 (0.20–0.48) | 0.34 (0.22–0.54) |
| TT       | 220 (75.1%)          | 192 (90.5%)          | 2.25 (0.43–11.73) | 2.91 (0.50–16.98) |
| CT + TT  | 288 (98.3%)          | 212 (99.1%)          | 1.84 (0.35–9.56) | 2.54 (0.43–15.03) |
| rs62514004|                     |                      |            |            |
| AA       | 230 (78.5%)          | 169 (79.0%)          | 1 (reference) | 1 (reference) |
| AG       | 61 (20.8%)           | 44 (20.6%)           | 0.98 (0.64–1.52) | 1.15 (0.71–1.86) |
| GG       | 2 (0.7%)             | 1 (0.5%)             | 0.68 (0.06–7.57) | 0.38 (0.03–4.47) |
| AG + GG  | 63 (21.5%)           | 45 (21.3%)           | 0.97 (0.63–1.50) | 1.11 (0.69–1.78) |

AOR adjusted odds ratio, OR odds ratio, SNP single nucleotide polymorphism, WISP-1 = WNT1-inducible signaling pathway protein 1.
rs2977530 may help to protect against RA development, while the AA genotype of SNP rs2977537 was associated with RF negativity. The AA+GG genotype of rs2977537 and AA genotype of rs2977530 were associated with high serum CRP levels, while the presence of the AG genotype of rs2929970 and AA genotype of rs2977530 necessitated prednisolone and TNFα inhibitors, respectively. These correlations between clinical data and genetic function require further exploration.

A major limitation to our study is that the findings of our study might merely represent interconnections between SNPs and RA disease rather than causality. This is also an ubiquitous problem for similar studies, which might partly be overcome by in-depth evaluations that select and analyze relationships between all the known SNP elements. In conclusion, our study offers evidence for correlations between WISP-1 SNPs and susceptibility to RA. The AA genotype of SNP rs2977537, TT and GG genotypes of SNP rs2929973, and the AA and GG genotypes of SNP rs2977530 are associated with lower susceptibility to RA. This is the first study to demonstrate that a correlation exists between WISP-1 polymorphisms and RA susceptibility. The potential of WISP-1 serving as the diagnostic marker and therapeutic target for RA might warrant future studies.

| Gene Genotype | Controls N=293 (%) | Patients N=214 (%) | OR (95% CI) | AOR (95% CI) |
|---------------|-------------------|-------------------|-------------|-------------|
|               | Positive          | Negative          |             |             |
| rs2977537     |                   |                   |             |             |
| RF positivity | Positive          | Negative          |             |             |
| AG            | 11 (5.1%)         | 1 (reference)     | 1 (reference) |             |
| AA            | 12 (5.6%)         | 0.38 (0.16–0.93)  | 0.38 (0.16–0.94) |             |
| AG + AA       | 21 (9.8%)         | 0.44 (0.20–0.96)  | 0.44 (0.20–0.96) |             |
| CRP           | >8 mg/L           | ≤8 mg/L           |             |             |
| AG            | 36 (18.6%)        | 74 (35.5%)        | 1 (reference) | 1 (reference) |
| AA            | 27 (12.6%)        | 1.98 (1.01–3.87)  | 1.92 (0.98–3.76) |             |
| AA + GG       | 54 (25.2%)        | 1.90 (1.09–3.31)  | 1.84 (1.05–3.21) |             |
| ESR           | >20 mm/h          | ≤20 mm/h          |             |             |
| AG            | 52 (24.3%)        | 58 (27.1%)        | 1 (reference) | 1 (reference) |
| AA            | 28 (13.1%)        | 1.25 (0.65–2.41)  | 1.28 (0.66–2.49) |             |
| AA + GG       | 50 (24.3%)        | 1.10 (0.57–2.13)  | 1.18 (0.60–2.30) |             |
| rs2977530     |                   |                   |             |             |
| RF positivity | Positive          | Negative          |             |             |
| AG            | 12 (5.6%)         | 1 (reference)     | 1 (reference) |             |
| AA            | 13 (6.1%)         | 1.25 (0.65–2.41)  | 1.28 (0.66–2.49) |             |
| AA + GG       | 20 (9.8%)         | 1.10 (0.57–2.13)  | 1.18 (0.60–2.30) |             |
| CRP           | >8 mg/L           | ≤8 mg/L           |             |             |
| AG            | 30 (14.0%)        | 66 (30.8%)        | 1 (reference) | 1 (reference) |
| AA            | 30 (14.0%)        | 2.57 (1.34–4.99)  | 2.62 (1.35–5.00) |             |
| AA + GG       | 62 (29.0%)        | 1.90 (1.13–3.49)  | 2.00 (1.13–3.54) |             |
| ESR           | >20 mm/h          | ≤20 mm/h          |             |             |
| AG            | 51 (23.8%)        | 1 (reference)     | 1 (reference) |             |
| AA            | 29 (13.6%)        | 1.41 (0.75–2.65)  | 1.40 (0.74–2.66) |             |
| AA + GG       | 52 (24.3%)        | 1.44 (0.84–2.47)  | 1.49 (0.86–2.57) |             |

ACPA = anti-citrullinated protein antibodies, AOR = adjusted odds ratio, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, OR = odds ratio, RA = rheumatoid arthritis, RF = rheumatoid factor, WISP-1 = WNT1-inducible signaling pathway protein 1.
| Gene, genotype | Patients N=214 (%) | OR (95% CI) | AOR (95% CI) |
|---------------|------------------|-------------|--------------|
| rs2929970     |                  |             |              |
| **TNF-α inhibitors** |                  |             |              |
| AG            | 60 (28.0%)       | 1 (reference) | 1 (reference) |
| AA            | 52 (24.3%)       | 0.74 (0.41–1.35) | 0.76 (0.42 to 1.38) |
| GG            | 16 (7.5%)        | 1.00 (0.43–2.32) | 0.99 (0.42 to 2.31) |
| AA + GG       | 52 (24.3%)       | 0.80 (0.47–1.39) | 0.81 (0.47 to 1.41) |
| Methotrexate  |                  |             |              |
| AG            | 43 (20.1%)       | 1 (reference) | 1 (reference) |
| AA            | 37 (17.3%)       | 0.83 (0.46–1.48) | 0.89 (0.48–1.65) |
| GG            | 15 (7.0%)        | 0.60 (0.26–1.39) | 0.63 (0.26–1.51) |
| AA + GG       | 52 (24.3%)       | 0.76 (0.44–1.31) | 0.81 (0.46–1.42) |
| Prednisolone  |                  |             |              |
| AG            | 40 (18.7%)       | 1 (reference) | 1 (reference) |
| AA            | 45 (21.0%)       | 0.49 (0.27–0.89) | 0.49 (0.27–0.89) |
| GG            | 22 (10.3%)       | 0.71 (0.31–1.65) | 0.72 (0.31–1.70) |
| AA + GG       | 58 (27.1%)       | 0.54 (0.31–0.99) | 0.54 (0.31–0.94) |
| rs2977530     |                  |             |              |
| **TNF-α inhibitors** |                  |             |              |
| AG            | 63 (29.4%)       | 1 (reference) | 1 (reference) |
| AA            | 51 (14.5%)       | 2.09 (1.10–3.99) | 2.07 (1.08–3.99) |
| GG            | 34 (15.9%)       | 1.07 (0.53–2.15) | 1.06 (0.52–2.15) |
| AA + GG       | 65 (30.4%)       | 1.56 (0.89–2.71) | 1.53 (0.87–2.68) |
| Methotrexate  |                  |             |              |
| AG            | 43 (20.1%)       | 1 (reference) | 1 (reference) |
| AA            | 30 (14.0%)       | 0.95 (0.50–1.78) | 0.91 (0.46–1.77) |
| GG            | 22 (10.3%)       | 1.14 (0.58–2.25) | 1.25 (0.61–2.50) |
| AA + GG       | 52 (24.9%)       | 1.03 (0.60–1.77) | 1.05 (0.59–1.85) |
| Prednisolone  |                  |             |              |
| AG            | 47 (22.0%)       | 1 (reference) | 1 (reference) |
| AA            | 25 (11.7%)       | 1.54 (0.81–2.91) | 1.61 (0.84–3.08) |
| GG            | 26 (12.2%)       | 1.00 (0.51–1.95) | 1.04 (0.53–2.05) |
| AA + GG       | 51 (23.8%)       | 1.26 (0.73–2.17) | 1.31 (0.76–2.26) |

AOR = adjusted odds ratio, OR = odds ratio, RA = rheumatoid arthritis, TNF-α = tumor necrosis factor alpha, WISP-1 = WNT1-inducible signaling pathway protein 1.

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