Systematic Review and Meta-Analysis

Medicine®

OPEN

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

Shu-Jui Kuo, MD, PhD[a,b], Ping-Wen Hsua, MD, Szu-Yu Chien, PhD[a], Chien-Chung Huang, MD, PhD[a,c], Sung-Lin Hu, MD, PhD[a,d], Chun-Hao Tsai, MD, PhD[a,b], Chen-Ming Su, PhD[e,*], Chih-Hsin Tang, PhD[a,f,g,h,*]

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 rs2977537 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 rs2929970 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, HMGB1 = high-mobility group box protein 1, HWE = 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

Editor: Adel Gouri.

S-JK and P-WH equally contributed as first authors.

This work was supported by grants from Taiwan's Ministry of Science and Technology (MOST 108-2314-B-039-011-; MOST 108-2320-B-039-057-) and China Medical University (CMU106-ASIA-03; CMU 107-ASIA-09; CMU 108-ASIA-06).

The authors have no financial or personal relationships that could inappropriately influence this research.

[a] School of Medicine, [b] Department of Orthopedic Surgery, [c] Division of Immunology and Rheumatology, Department of Internal Medicine, China Medical University Hospital, [d] Department of Family Medicine, China Medical University Hsinchu Hospital, Hsinchu, [e] Department of Biomedical Sciences Laboratory, Affiliated Dongyang Hospital of Wenzhou Medical University, Dongyang, Zhejiang, China, [f] Graduate Institute of Biomedical Science, [g] Chinese Medicine Research Center, China Medical University, [h] Department of Biotechnology, College of Health Science, Asia University, Taichung, Taiwan.

[*] Correspondence: Chen-Ming Su, Department of Biomedical Sciences Laboratory, Affiliated Dongyang Hospital of Wenzhou Medical University, Dongyang, Zhejiang, China (e-mail: proof814@gmail.com) and Chih-Hsin Tang (e-mail: chtang@mail.cmu.edu.tw).

How to cite this article: Kuo S-J, Hsu P-W, Chien S-Y, Huang C-C, Hu S-L, Tsai C-H, Su C-M, Tang C-H. Associations between WNT1-inducible signaling pathway protein-1 (WISP-1) genetic polymorphisms and clinical aspects of rheumatoid arthritis among Chinese Han subjects. Medicine 2019;98:44(e17604).

Received: 3 June 2019 / Received in final form: 4 September 2019 / Accepted: 20 September 2019

http://dx.doi.org/10.1097/MD.0000000000017604

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.[8,9] 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.[1,2,12,13]

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 introns [16,17] 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 $\alpha v\beta 3$ 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.[3,30] 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.[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 identified 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).[1,36]

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, AG, GT, AG, 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 half as likely to develop RA (adjusted OR [AOR] 0.54; 95% CI, 0.34–0.84). Compared with individuals carrying the GT genotype of SNP rs2929973, those carrying the TT genotype were about one-fourth as likely to develop RA (AOR 0.24; 95% CI, 0.13–0.39), while those with the GG genotype were even less likely (0.05; 0.03–0.10). When compared with individuals carrying the AG genotype of SNP rs2977530, those carrying the AA genotype were around one-third as likely to develop RA (AOR 0.34; 95% CI, 0.22–0.54) and those with the GG genotype were around half as likely (0.52; 0.31–0.87).

When the respective SNPs were analyzed for their correlations with demographic and clinical parameters in the RA cohort, compared with patients carrying the AG genotype of SNP rs2977537, being RF-positive was around one-third as 0.38 likely in patients carrying the AA genotype (AOR 0.16; 95% CI, 0.16–0.94) and around half as likely in those with the AG genotype (0.54; 0.21–1.40) (Table 3). When compared with patients carrying the AG genotype of rs2977537, those with the non-AG genotype (A/A + G/G) were nearly twice as likely to have high serum CRP levels (>8 mg/L) (AOR 1.84; 95% CI, 1.05–3.21). When compared with patients carrying the AG genotype of rs2977530, those with the AA genotype were almost 3 times as likely to have high serum CRP levels (>8 mg/L) (AOR 2.62; 95% CI, 1.35–5.09).

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).

Table 1

Clinicodemographic 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%)	
Positive		182 (85.0%)	
ACPA			
Negative		45 (21.0%)	
Positive		169 (79.0%)	
Serum CRP			
≤8 mg/L		128 (59.8%)	
>8 mg/L		86 (40.2%)	
ESR			
≤20 mm/h		103 (48.1%)	
>20 mm/h		111 (51.9%)	
TNF-α inhibitors		86 (40.2%)	
Methotrexate		119 (55.6%)	
Prednisolone		116 (54.2%)	

ACPA = anti-citrullinated protein antibodies, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, RF = rheumatoid factor, TNF = tumor necrosis factor.

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,[1,2,37] 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.[3,38] In RA, excessive migration of circulating leukocytes into the inflamed joint mandate the formation of new vessels for the trafficking of oxygen and nutrients.[39] 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.[40] 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.[41] 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 1 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).[26] The same researchers also found associations between certain *WISP1* polymorphisms (rs2929965, rs2929969, rs2929970, rs2929973 and rs754958) and the

Table 2

Distribution frequencies of WISP1 genotypes and allele frequencies of 6 tag SNPs in rheumatoid arthritis cases and healthy controls.

Variable	Controls N = 293 (%)	Patients N = 214 (%)	OR (95% CI)	AOR (95% CI)
rs2977537				
AG	127 (43.4%)	110 (51.4%)	1 (reference)	1 (reference)
AA	118 (40.3%)	53 (18.1%)	0.52 (0.34–0.78)	0.54 (0.34–0.84)
GG	48 (16.4%)	51 (17.4%)	1.23 (0.77–1.96)	1.15 (0.69–1.92)
AA + GG	166 (56.7%)	104 (35.5%)	0.72 (0.51–1.03)	0.72 (0.49–1.06)
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)
rs2929973				
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)
rs2977530				
AG	68 (23.2%)	96 (44.9%)	1 (reference)	1 (reference)
AA	147 (50.2%)	65 (30.4%)	0.31 (0.20–0.48)	0.34 (0.22–0.54)
GG	78 (26.6%)	53 (24.8%)	0.48 (0.30–0.77)	0.52 (0.31–0.87)
AA + GG	225 (76.8%)	118 (55.1%)	0.37 (0.25–0.54)	0.40 (0.27–0.61)
rs1689334				
CC	5 (1.7%)	2 (0.9%)	1 (reference)	1 (reference)
CT	68 (23.2%)	14 (6.5%)	0.52 (0.09–2.93)	0.79 (0.12–5.22)
TT	220 (75.1%)	198 (92.5%)	2.25 (0.43–11.73)	2.91 (0.50–16.98)
CT + TT	288 (98.3%)	212 (99.1%)	1.84 (0.35–9.58)	2.54 (0.43–15.05)
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.

overall chemotherapy toxicity of lung cancer, while rs16904853, rs2929970, rs2977549, and rs2977551 polymorphisms were significantly associated with hematologic toxicity ($P = .021$, $P = .028$, $P = .024$ and $P = .048$, respectively) and the rs2929946, rs2929970, rs2977519, rs2977536, rs3739262, and rs754958 variants were significantly associated with the gastrointestinal toxicity of lung cancer ($P = .031$, $P = .046$, $P = .029$, $P = .016$, $P = .042$ and $P = .035$, respectively). Chen et al have therefore suggested that *WISP1* SNPs may be useful biomarkers for predicting platinum-based chemotherapy toxicity in lung cancer.[25] Those researchers have also investigated the correlation between *WISP-1* SNP and the risk of developing hepatocellular carcinoma (HCC).[29] 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, 9.78–72.30). 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, Urano et al analyzed the association of rs2929970 SNP with the development of radiographically observable spine osteoarthritis.[28] Those authors found that individuals without the G allele (AA) were significantly over-represented in the cohort that had higher endplate sclerosis scores ($P = .0069$; AOR 2.91; 95% CI, 1.34–6.30). The authors suggest that the rs2929970 SNP genotype correlates with risk for spinal osteoarthritis.[28]

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, rs2929973, 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 rs2929973, and the AA and GG genotypes of SNP

Table 3

Genotype frequencies of *WISP1* rs2977537 and rs2977530 polymorphisms in RA patients, stratified by RA marker status.

Gene Genotype	Controls N = 293 (%)	Patients N = 214 (%)	OR (95% CI)	AOR (95% CI)
rs2977537				
RF positivity				
	Positive	Negative		
AG	99 (46.3%)	11 (5.1%)	1 (reference)	1 (reference)
AA	41 (19.2%)	12 (5.6%)	0.38 (0.16–0.93)	0.38 (0.16–0.94)
GG	42 (19.6%)	9 (4.2%)	0.52 (0.20–1.34)	0.54 (0.21–1.40)
AA + GG	83 (38.8%)	21 (9.8%)	0.44 (0.20–0.96)	0.44 (0.20–0.96)
	Positive	Negative		
AG	86 (40.2%)	24 (11.2%)	1 (reference)	1 (reference)
AA	45 (21.0%)	8 (3.7%)	1.57 (0.65–3.78)	1.83 (0.73–4.56)
GG	38 (17.8%)	13 (6.1%)	0.82 (0.38–1.77)	0.85 (0.39–1.88)
AA + GG	83 (38.8%)	21 (9.8%)	1.10 (0.57–2.13)	1.18 (0.60–2.30)
CRP				
	>8 mg/L	≤8 mg/L		
AG	36 (16.8%)	74 (35.5%)	1 (reference)	1 (reference)
AA	26 (12.2%)	27 (12.6%)	1.98 (1.01–3.87)	1.92 (0.98–3.76)
GG	24 (11.2%)	27 (12.6%)	1.83 (0.93–3.60)	1.75 (0.88–3.48)
AA + GG	50 (23.4%)	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%)	25 (11.7%)	1.25 (0.65–2.41)	1.28 (0.66–2.49)
GG	31 (14.5%)	20 (9.4%)	1.73 (0.88–3.40)	1.76 (0.89–3.49)
AA + GG	59 (27.6%)	45 (21.0%)	1.46 (0.85–2.51)	1.51 (0.87–2.60)
rs2977530				
RF positivity				
	Positive	Negative		
AG	84 (39.3%)	12 (5.6%)	1 (reference)	1 (reference)
AA	52 (24.3%)	13 (6.1%)	0.57 (0.24–1.35)	0.54 (0.23–1.29)
GG	46 (21.5%)	7 (3.3%)	0.94 (0.35–2.55)	0.97 (0.36–2.66)
AA + GG	98 (45.8%)	20 (9.4%)	0.70 (0.32–1.51)	0.69 (0.32–1.51)
	Positive	Negative		
AG	75 (35.1%)	21 (9.8%)	1 (reference)	1 (reference)
AA	55 (25.7%)	10 (4.7%)	1.54 (0.67–3.53)	1.62 (0.69–3.79)
GG	39 (28.2%)	14 (6.5%)	0.78 (0.35–1.70)	0.82 (0.37–1.80)
AA + GG	94 (43.9%)	24 (11.2%)	1.10 (0.57–2.12)	1.14 (0.58–2.23)
CRP				
	>8 mg/L	≤8 mg/L		
AG	30 (14.0%)	66 (30.8%)	1 (reference)	1 (reference)
AA	35 (16.4%)	30 (14.0%)	2.57 (1.34–4.99)	2.62 (1.35–5.09)
GG	21 (9.8%)	32 (15.0%)	1.44 (0.72–2.91)	1.40 (0.68–2.87)
AA + GG	56 (26.2%)	62 (29.0%)	1.99 (1.13–3.49)	2.00 (1.13–3.54)
ESR				
	>20 mm/h			
AG	45 (21.0%)	51 (23.8%)	1 (reference)	1 (reference)
AA	36 (16.8%)	29 (13.6%)	1.41 (0.75–2.65)	1.40 (0.74–2.66)
GG	30 (14.0%)	23 (10.8%)	1.48 (0.75–2.90)	1.53 (0.78–3.03)
AA + GG	66 (30.8%)	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.

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.

Table 4

Genotype frequencies of *WISP1* rs2929970/rs2977530 polymorphisms in RA patients, stratified by RA medications.

Gene, genotype	Patients N = 214 (%)		OR (95% CI)	AOR (95% CI)
rs2929970				
TNF-α inhibitors				
	negative	positive		
AG	60 (28.0%)	45 (21.0%)	1 (reference)	1 (reference)
AA	52 (24.3%)	29 (13.6%)	0.74 (0.41–1.35)	0.76 (0.42 to 1.38)
GG	16 (7.5%)	12 (5.6%)	1.00 (0.43–2.32)	0.99 (0.42 to 2.31)
AA + GG	68 (31.8%)	41 (19.2%)	0.80 (0.47–1.39)	0.81 (0.47 to 1.41)
Methotrexate				
	negative	positive		
AG	43 (20.1%)	62 (29.0%)	1 (reference)	1 (reference)
AA	37 (17.3%)	44 (20.6%)	0.83 (0.46–1.48)	0.89 (0.48–1.65)
GG	15 (7.0%)	13 (6.1%)	0.60 (0.26–1.39)	0.63 (0.26–1.51)
AA + GG	52 (24.3%)	57 (26.6%)	0.76 (0.44–1.31)	0.81 (0.46–1.42)
Prednisolone				
	negative	positive		
AG	40 (18.7%)	65 (30.4%)	1 (reference)	1 (reference)
AA	45 (21.0%)	36 (16.8%)	0.49 (0.27–0.89)	0.49 (0.27–0.88)
GG	13 (6.1%)	15 (7.0%)	0.71 (0.31–1.65)	0.72 (0.31–1.70)
AA + GG	58 (27.1%)	51 (23.8%)	0.54 (0.31–0.99)	0.54 (0.31–0.94)
rs2977530				
TNF-α inhibitors				
	negative	positive		
AG	63 (29.4%)	33 (15.4%)	1 (reference)	1 (reference)
AA	31 (14.5%)	34 (15.9%)	2.09 (1.10–3.99)	2.07 (1.08–3.98)
GG	34 (15.9%)	19 (8.9%)	1.07 (0.53–2.15)	1.06 (0.52–2.15)
AA + GG	65 (30.4%)	53 (24.8%)	1.56 (0.89–2.71)	1.53 (0.87–2.68)
Methotrexate				
	negative	positive		
AG	43 (20.1%)	53 (24.8%)	1 (reference)	1 (reference)
AA	30 (14.0%)	35 (16.4%)	0.95 (0.50–1.78)	0.91 (0.46–1.77)
GG	22 (10.3%)	31 (14.5%)	1.14 (0.58–2.25)	1.25 (0.61–2.55)
AA + GG	52 (24.3%)	66 (30.8%)	1.03 (0.60–1.77)	1.05 (0.59–1.85)
Prednisolone				
	negative	positive		
AG	47 (22.0%)	49 (22.9%)	1 (reference)	1 (reference)
AA	25 (11.7%)	40 (18.7%)	1.54 (0.81–2.91)	1.61 (0.84–3.08)
GG	26 (12.2%)	27 (12.6%)	1.00 (0.51–1.95)	1.04 (0.53–2.05)
AA + GG	51 (23.8%)	67 (31.3%)	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.

Author contributions

Conceptualization: Su Chen-Ming.
Formal analysis: Kuo Shu-Jui, Ping-Wen Hsu.
Methodology: Kuo Shu-Jui, Ping-Wen Hsu.
Resources: Szu-Yu Chien, Huang Chien-Chung, Sung-Lin Hu, Tsai Chun-Hao.
Supervision: Chih-Hsin Tang.
Writing – original draft: Su Chen-Ming, Chih-Hsin Tang.
Writing – review & editing: Chih-Hsin Tang.

References

[1] Kuo SJ, Huang CC, Tsai CH, et al. Chemokine C-C motif ligand 4 gene polymorphisms associated with susceptibility to rheumatoid arthritis. Biomed Res Int 2018;2018:9181647.
[2] Dai C, Kuo SJ, Zhao J, et al. Correlation between genetic polymorphism of angiopoietin-2 gene and clinical aspects of rheumatoid arthritis. Int J Med Sci 2019;16:331–6.
[3] MacDonald IJ, Liu SC, Su CM, et al. Implications of angiogenesis involvement in arthritis. Int J Mol Sci 2018;19: Jul 10.
[4] Kou Y, Koag MC, Cheun Y, et al. Application of hypoiodite-mediated aminyl radical cyclization to synthesis of solasodine acetate. Steroids 2012;77:1069–74.
[5] Cheun Y, Kou Y, Stevenson B, et al. Synthesis of C17-OH-north unit of ritterazine G via "Red-Ox" modifications of hecogenin acetate. Steroids 2013;78:639–43.
[6] Gabriel SE, Crowson CS, O'Fallon WM. Mortality in rheumatoid arthritis: have we made an impact in 4 decades? J Rheumatol 1999;26:2529–33.
[7] Tang CH. Research of pathogenesis and novel therapeutics in arthritis. Int J Mol Sci 2019;20: Apr 2.
[8] Suzuki A, Yamamoto K. From genetics to functional insights into rheumatoid arthritis. Clin Exp Rheumatol 2015;33(4 Suppl 92):S40–3.
[9] Liu IJ MD, Huang SC, Kuo CC, et al. Associations between adipokines in arthritic disease and implications for obesity. Int J Mol Sci 2019;20: Mar 26.
[10] Yang MD, Lin KC, Lu MC, et al. Contribution of matrix metalloproteinases-1 genotypes to gastric cancer susceptibility in Taiwan. BioMedicine 2017;7:10.
[11] Li TC, Li CI, Liao LN, et al. Associations of EDNRA and EDN1 polymorphisms with carotid intima media thickness through interactions with gender, regular exercise, and obesity in subjects in Taiwan: Taichung Community Health Study (TCHS). BioMedicine 2015;5:8.
[12] Wang LH, Wu MH, Chen PC, et al. Prognostic significance of high-mobility group box protein 1 genetic polymorphisms in rheumatoid arthritis disease outcome. Int J Med Sci 2017;14:1382–8.
[13] Wang L, Tang CH, Lu T, et al. Resistin polymorphisms are associated with rheumatoid arthritis susceptibility in Chinese Han subjects. Medicine 2018;97:e0177.

[14] Maiese K. WISP1: clinical insights for a proliferative and restorative member of the CCN family. Curr Neurovasc Res 2014;11:378–89.
[15] Jun JI, Lau LF. Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets. Nat Rev Drug Discov 2011;110: 945–63.
[16] Davies SR, Watkins G, Mansel RE, et al. Differential expression and prognostic implications of the CCN family members WISP-1, WISP-2, and WISP-3 in human breast cancer. Ann Surg Oncol 2007;14:1909–18.
[17] Xu L, Corcoran RB, Welsh JW, et al. WISP-1 is a Wnt-1- and beta-catenin-responsive oncogene. Genes Dev 2000;114:585–95.
[18] Chen PC, Cheng HC, Yang SF, et al. The CCN family proteins: modulators of bone development and novel targets in bone-associated tumors. Biomed Res Int 2014;2014:437096.
[19] Gurbuz I, Chiquet-Ehrismann R. CCN4/WISP1 (WNT1 inducible signaling pathway protein 1): a focus on its role in cancer. Int J Biochem Cell Biol 2015;62:142–6.
[20] Lau LF. Cell surface receptors for CCN proteins. J Cell Commun Signal 2016;10:121–7.
[21] Atkinson SJ, Ellison TS, Steri V, et al. Redefining the role(s) of endothelial alphavbeta3-integrin in angiogenesis. Biochem Soc Trans 2014;42: 1590–5.
[22] Wang CQ, Huang YW, Wang SW, et al. Amphiregulin enhances VEGF-A production in human chondrosarcoma cells and promotes angiogenesis by inhibiting miR-206 via FAK/c-Src/PKCdelta pathway. Cancer Lett 2017;28385:261–70.
[23] Chuang JY, Chen PC, Tsao CW, et al. WISP-1 a novel angiogenic regulator of the CCN family promotes oral squamous cell carcinoma angiogenesis through VEGF-A expression. Oncotarget 2015;286: 4239–52.
[24] Lin CC, Chen PC, Lein MY, et al. WISP-1 promotes VEGF-C-dependent lymphangiogenesis by inhibiting miR-300 in human oral squamous cell carcinoma cells. Oncotarget 2016;7:9993–10005. Mar 1.
[25] Chen J, Yin J, Li X, et al. WISP1 polymorphisms contribute to platinum-based chemotherapy toxicity in lung cancer patients. Int J Mol Sci 2014;15:21011–27. Nov14.
[26] Chen J, Yin JY, Li XP, et al. Association of Wnt-Inducible Signaling Pathway Protein 1 Genetic Polymorphisms With Lung Cancer Susceptibility and Platinum-Based Chemotherapy Response. Clin Lung Cancer 2015;16: 298-304 e291-292.
[27] Lin YH, Hsiao YH, Yang SF, et al. Association between genetic polymorphisms of WNT1 inducible signaling pathway protein 1 and uterine cervical cancer. Reprod Sci 2018;25:1549–56.
[28] Urano T, Narusawa K, Shiraki M, et al. Association of a single nucleotide polymorphism in the WISP1 gene with spinal osteoarthritis in postmenopausal Japanese women. J Bone Miner Metab 2007;25:253–8.
[29] Chen CT, Lee HL, Chiou HL, et al. Impacts of WNT1-inducible signaling pathway protein 1 polymorphism on hepatocellular carcinoma development. PLoS One 2018;13:e0198967.
[30] Chen CY, Su CM, Hsu CJ, et al. CCN1 Promotes VEGF production in osteoblasts and induces endothelial progenitor cell angiogenesis by inhibiting mir-126 expression in rheumatoid arthritis. J Bone Mineral Res 2017;32:34–45.
[31] Paleolog EM. Angiogenesis in rheumatoid arthritis. Arthritis Res 2002;4 (Suppl 3):S81–90.
[32] Su CM, Hsu CJ, Tsai CH, et al. Resistin promotes angiogenesis in endothelial progenitor cells through inhibition of MicroRNA206: potential implications for rheumatoid arthritis. Stem Cells 2015;33:2243–55.
[33] Liu SC, Chuang SM, Hsu CJ, et al. CTGF increases vascular endothelial growth factor-dependent angiogenesis in human synovial fibroblasts by increasing miR-210 expression. Cell Death Dis 2014;235:e1485.
[34] Liu SC, Tsai CH, Wu TY, et al. Soya-cerebroside reduces IL-1 beta-induced MMP-1 production in chondrocytes and inhibits cartilage degradation: implications for the treatment of osteoarthritis. Food Agr Immunol 2019;30:620–32. Jan 1.
[35] Lau HK, Wu ER, Chen MK, et al. Effect of genetic variation in microRNA binding site in WNT1-inducible signaling pathway protein 1 gene on oral squamous cell carcinoma susceptibility. PloS One 2017;12: e0176246.
[36] Lee HP, Chen PC, Wang SW, et al. Plumbagin suppresses endothelial progenitor cell-related angiogenesis in vitro and in vivo. J Funct Foods 2019;52:537–44.
[37] Yuan Y, Shao W, Li Y. Associations between C677T and A1298C polymorphisms of MTHFR and susceptibility to rheumatoid arthritis: a systematic review and meta-analysis. Rheumatol Int 2017;37:557–69.
[38] Chien SY, Huang CY, Tsai CH, et al. Interleukin-1beta induces fibroblast growth factor 2 expression and subsequently promotes endothelial progenitor cell angiogenesis in chondrocytes. Clin Sci 2016;130:667–81.
[39] Elshabrawy HA, Chen Z, Volin MV, et al. The pathogenic role of angiogenesis in rheumatoid arthritis. Angiogenesis 2015;18:433–48.
[40] Tsai HC, Tzeng HE, Huang CY, et al. WISP-1 positively regulates angiogenesis by controlling VEGF-A expression in human osteosarcoma. Cell Death Dis 2017;8:e2750Apr 13.
[41] Wright LH, Herr DJ, Brown SS, et al. Angiokine Wisp-1 is increased in myocardial infarction and regulates cardiac endothelial signaling. JCI Insight 2018;3: Feb 22.