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Association of Gene Polymorphisms in CXC Chemokine Receptor 5 with Rheumatoid Arthritis Susceptibility

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ABSTRACT

Rheumatoid arthritis (RA) is caused by complicated interactions between genes and the environment. CXC chemokine receptor 5 (CXCR5) is required for B and T follicular helper cell migration and humoral immunity generation. Therefore, this study aimed to assess whether polymorphisms of the CXCR5 gene are implicated in RA development and progression.

This case-control study enrolled 285 RA patients and 291 healthy controls. The polymerase chain reaction-ligase detection reaction method was used to genotype rs630923, rs497916, rs3922, and rs676925 in the CXCR5 gene. Epidemiological, clinical, and laboratory data were collected retrospectively.

The rs630923 A allele was associated with a higher risk of RA (AOR [adjusted odds ratio]=2.00, 95% confidence interval [CI] =1.14–3.53). However, in the RA group, the frequency of the rs497916 T allele was lower (AOR=0.69, 95% CI=0.51–0.93). Regarding rs3922, AG+GG genotype carriers were at a significantly lower risk for RA than AA genotype carriers (AOR=0.70, 95% CI=0.49–0.99). In the RA group, we found that the different genotypes were significantly associated with specific laboratory values, including rheumatoid factor, total bilirubin, total cholesterol, low-density lipoprotein cholesterol, and alkaline phosphatase.

This is the first report indicating that CXCR5 polymorphisms were associated with RA susceptibility. These findings lead to a rising possibility of identifying RA-susceptible individuals based on genetic markers.

Keywords: CXC chemokine receptor 5; Disease susceptibility; Rheumatoid arthritis; Single nucleotide polymorphism

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder that affects approximately 5 in 1,000 adults worldwide.[1] Synovial inflammation and joint damage are cardinal features of RA, and extra-articular manifestations can involve the lungs, cardiovascular system, skin, and nervous system.[2] Patients with RA have higher mortality and disability rates than the general population.[3,4] Early diagnosis and highly effective therapy could markedly improve the

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long-term prognosis; however, these are largely dependent on a better understanding of the RA pathogenesis.[5]

The exact cause of RA is unknown. However, the pathogenesis likely involves complex interactions between genes and the environment.[6] Genetic polymorphisms contribute 30–60% to the overall risk.[7] Recently, more studies have linked RA to immune marker polymorphisms such as human leukocyte antigen (HLA), cytotoxic T-lymphocyte associated protein 4 (CTLA-4), tumor necrosis factor (TNF), GATA binding protein 3 (GATA-3), and forkhead box protein 3 (Foxp3).[6–9] Owing to the disease heterogeneity and interindividual variability, distinct genetic variations may increase the RA risk in different populations.[10] Further studies on the association between RA susceptibility and the genes involved in autoimmunity are warranted.

CXC chemokine receptor 5 (CXCR5) is a seven-transmembrane G-protein–coupled receptor that is predominantly expressed on the surface of B cells and a subset of T cells called T follicular helper (Tfh) cells. It plays an important role in secondary lymphoid tissue orchestration and lymphoid neogenesis.[11] Within the lymph node microenvironments, Tfh cells aid B cell maturation and antibody production.[12] A recent study found that CXCR5 polymorphisms are risk factors for diffuse large B-cell lymphoma.[13] This suggests that CXCR5 may affect the homeostasis of humoral immunity. As such, Tfh cells and CXCR5 impact infection, immunodeficiency, and autoimmunity, including RA. Previous studies have indicated that peripheral CD4+CXCR5+PD-1+ Tfh cells are significantly increased in RA patients, and that the proportion of Tfh cells was positively correlated with anti-cyclic citrullinated peptide antibody (anti-CCP).[14] CXCR5 expression increased in RA synovium at the mRNA and protein level.[15] Our recent study revealed that the ratio skewing between CD4+CXCR5+Foxp3- Tfh cells and CD4+CXCR5+Foxp3+ T follicular regulatory (Tfr) cells caused by enhanced interleukin-6/phosphorylated signal transducer and activator of transcription 3 signaling may be critical for disease progression in RA.[16] CXCR5-deficient mice and mice with CXCR5-deficient T or B cells were completely resistant to collagen-induced arthritis–a mouse model of RA.[17] Considering the genetic predisposition in RA pathogenesis, it seems reasonable to hypothesize that CXCR5 polymorphisms may be associated with the risk of RA; however, no such studies have ever been conducted.

Previous candidate gene association studies have identified several single nucleotide polymorphisms (SNPs) in the CXCR5 gene related to multiple sclerosis (MS) and hepatitis B virus or *Chlamydia trachomatis* infection.[18–23] Therefore, we aimed to investigate the relationship of these SNPs with RA development and progression in this study. RA-related clinical and laboratory characteristic distributions in patients carrying different genotypes were evaluated concurrently.

MATERIALS AND METHODS

Study Subjects

This retrospective case-control study was performed at the West China Hospital of Sichuan University. A total of 285 RA patients were included. The revised American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) classification criteria 2010 were used to make a clinical diagnosis.[24] Clinicians experienced in the diagnosis and treatment of RA identified all the patients. The main exclusion criteria were age <18 years, malignancies, serious liver or kidney diseases, and chronic or severe acute infections. The control group included 291 apparently healthy volunteers without infections, tumors, autoimmune diseases, or other inflammatory diseases.

The study was approved by the ethics committee of the West China Hospital of Sichuan University (ethical approval document number: 2019-1191) in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all participants.

Genomic DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral blood samples using a DNA isolation kit (Bioteke Corporation, Beijing, China) following the manufacturer's protocol. During this study, a total of four SNPs in CXCR5 (rs630923, rs497916, rs3922, and rs676925) (Supplementary Table 1) were genotyped. The polymerase chain reaction-ligase detection reaction (PCR-LDR) method was used (Supplementary Table 2 illustrates the primer and probe sequences).

The 20-μL PCR reaction mixture contained 1×GC buffer I (Takara Shuzo Co., Ltd., Kyoto, Japan), 3.0 mM Mg^{2+}, 2.0 mM dNTPs, 1 U Taq DNA polymerase (Takara), 2 μL primer mixture, and 1 μL genomic DNA.

The following conditions were used for PCR cycling: 2 min at 95°C; 40 cycles of 30 s at 94°C, 90 s at 56°C, 30 s at 65°C; and a final extension step of 10 min at 65°C. Subsequently, the LDR was performed with a final volume of 10 μL containing 1× ligation buffer (New England Biolabs, Beverly, MA, USA), 2 U Taq DNA ligase (New England Biolabs), 1 μL of each probe mixture, and 4 μL PCR product. The LDR parameters were as follows: 2 min at 95°C; 40 cycles of 15 s at 94°C, and 25 s at 50°C. After the reaction, the LDR product was analyzed using the ABI 3730 PRISM DNA Sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc. Foster City, CA, USA). Repeated genotyping of 10% of randomly selected samples revealed 100% concordance.

Clinical and Laboratory Data Collection

All participants' demographic and clinical data were obtained from the hospital records or via a questionnaire. The information obtained included age, sex, age at RA onset, presence or absence of osteoporosis, number of painful and swollen joints, and several laboratory test results. Total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were quantified using Cobas c702 (Roche Diagnostics, Mannheim, Germany). Anti-CCP was determined using the Roche E170 Modular immunoassay analyzer (Roche Diagnostics). C-reactive protein (CRP) and rheumatoid factor (RF) were determined by Immage 800 nephelometry (Beckman Coulter, CA, USA). The erythrocyte sedimentation rate (ESR) was determined using TEST 1 (Alifax, Padova, Italy).

Statistical Analysis

SPSS 25.0 (IBM Corp, Armonk, NY, USA), PLINK v1.07,[25] and GraphPad Prism 8 (San Diego, CA, USA) were used for statistical analyses. The Haploview software package (version 4.2) was used to evaluate linkage disequilibrium (LD) and to construct haplotypes. Statistical significance was defined as a two-tailed $p<0.05$.

The goodness-of-fit χ^2 test was used to assess Hardy-Weinberg equilibrium (HWE). Continuous data were expressed as mean±standard deviation (SD) or median (25^{th} and 75^{th} percentiles) and compared between groups using either the independent sample *t*-test or the Mann-Whitney *U* test. Categorical data were expressed as frequency (percentage), and the χ^2 test with Fisher's exact test was used for comparison. Binary logistic regression adjusting for possible confounders was used to examine the relationship between genetic polymorphisms and RA susceptibility.

RESULTS

Baseline Characteristics of the Participants

The participants' basic characteristics are shown in Table 1. A striking female preponderance (76.49%) was found among RA patients. However, the female-to-male ratio was nearly 1:1 in the healthy controls. The RA patients were older, more likely to have lower blood lipids and total bilirubin, but higher alkaline phosphatase compared with healthy controls.

The average age at RA onset was 43.6 years for these recruited patients. RA coexisted with osteoporosis in 25.56% of the patients. Anti-CCP and multiple inflammation markers were substantially elevated in RA patients. Furthermore, the means of DAS28-ESR (28-joint disease activity score based on the erythrocyte sedimentation rate) and DAS28-CRP (28-joint disease activity score based on the C-reactive protein level) were 5.87 (SD, 1.57) and 5.06 (SD, 1.54), respectively, suggesting that there was ongoing disease activity in most patients.[26]

Associations between Genetic Polymorphisms of CXCR5 and RA Susceptibility

The genotype distribution of these four polymorphisms agreed with the HWE in all 576 participants (Supplementary Table 1). We calculated the age- and sex-adjusted odds ratios (AORs) for developing RA among different alleles or genotypes (Table 2). The minor allele A of rs630923 was significantly associated with an increased risk of RA (p=0.016, AOR=2.00, 95% confidence interval [CI]=1.14–3.53). The CA+AA genotype frequency of rs630923 was significantly higher in RA patients (13.68%) than in controls (7.56%) (p=0.010, AOR=2.17, 95% CI=1.20–3.90). The frequencies of T allele and CT+TT genotypes of rs497916 were decreased in the RA group (T allele: p=0.016, AOR=0.69, 95% CI=0.51–0.93; CT+TT genotypes: p=0.004, AOR=0.60, 95% CI=0.42–0.85). Regarding rs3922, allele G was associated with a reduced risk of RA with borderline statistical

significance (p=0.057, AOR=0.76, 95% CI=0.58–1.01); however, the AG+GG genotype carriers were at significantly lower risk for RA than the AA genotype carriers (p=0.043, AOR=0.70, 95% CI=0.49–0.99).

Table 1. Baseline characteristics of the study participants

Characteristics		Healthy controls (n =291)	RA patients (n =285)	*p*
Demographics				
Sex	**Male**	144 (49.48%)	67 (23.51%)	**<0.001**
	Female	147 (50.52%)	218 (76.49%)	
Age (years)		48.0 ± 9.5	51.9 ± 12.9	**<0.001**
Age at RA onset (years)		NA	43.6 ± 14.8	NA
Clinical manifestations				
Osteoporosis [a]		NA	68 (25.56%)	NA
DAS28-ESR		NA	5.87 ± 1.57	NA
DAS28-CRP		NA	5.06 ± 1.54	NA
Anti-CCP (U/mL)		NR	360.25 (154.28, 501.00)	NA
RF (IU/mL)		NR	180.0 (41.0, 650.5)	NA
ESR (mm/h)		NR	55.9 ± 33.7	NA
CRP (mg/L)		NR	11.85 (5.09, 43.60)	NA
TBIL (μmol/L)		12.7 (9.7, 15.7)	9.4 (6.8, 11.7)	**<0.001**
ALT (IU/L)		18 (15, 26)	19 (12, 27)	0.529
AST (IU/L)		21 (18, 25)	21 (17, 29)	0.743
GGT (IU/L)		19 (13, 33)	20 (13, 34)	0.662
ALP (IU/L)		73 (59, 84)	82 (67, 102)	**<0.001**
TG (mmol/L)		1.14 (0.87, 1.59)	1.06 (0.83, 1.49)	0.183
TC (mmol/L)		4.77 ± 0.77	4.22 ± 1.02	**<0.001**
HDL-c (mmol/L)		1.44 ± 0.38	1.35 ± 0.45	**0.022**
LDL-c (mmol/L)		2.87 ± 0.66	2.37 ± 0.72	**<0.001**

ALP, alkaline phosphatase; ALT, alanine aminotransferase; Anti-CCP, anti-cyclic citrullinated peptide antibody; AST, aspartate aminotransferase; CRP, C-reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; GGT, gamma-glutamyl transferase; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; NA, not available; NR, not reported; RA, rheumatoid arthritis; RF, rheumatoid factor; TBIL, total bilirubin; TC, total cholesterol; TG, triglycerides.

Data are expressed as mean ± standard deviation (SD), frequency (percentage), or median (25^{th} and 75^{th} percentiles), as appropriate. The Mann-Whitney *U* test and Student's *t*-test were applied for quantitative data analysis, whereas the χ^2 test was applied for categorical data.

[a] Osteoporosis-related data were only collected in 266 RA patients.

As illustrated in Figure 1, other than rs497916 and rs3922, which showed moderate LD (r^2=0.66), the other pairwise SNPs exhibited low LD (r^2 ranging from 0.08 to 0.31). Six common haplotypes with frequencies ≥0.01 were inferred (Table 3). We compared each haplotype distribution with all the others and adjusted for age and sex and found that the rs630923-rs497916-rs3922-rs673925 haplotype ATGG was more frequent in RA patients than in controls (p=0.042, AOR=1.93). However, the distributions of haplotypes CTGC and CTGG, in contrast, were observed to be significantly increased among controls (CTGC: p=0.002, AOR=0.54; CTGG: p=0.012, AOR=0.52).

Table 2. Associations between alleles and genotypes of CXCR5 polymorphisms and RA susceptibility

SNPs	Model	Allele or genotype	Controls (n=291)	RA patients (n=285)	Adjusted OR (95% CI)	Adjusted *p*-value
rs630923	Allele	C	559 (96.05)	530 (92.98)	1	**0.016**
		A	23 (3.95)	40 (7.02)	**2.00 (1.14–3.53)**	
	Codominant	CC	269 (92.44)	246 (86.32)	1	
		CA	21 (7.22)	38 (13.33)	**2.25 (1.23–4.09)**	**0.029**
		AA	1 (0.34)	1 (0.35)	0.85 (0.05–13.73)	
	Dominant	CC	269 (92.44)	246 (86.32)	1	**0.010**
		CA+AA	22 (7.56)	39 (13.68)	**2.17 (1.20–3.90)**	
	Recessive	CC+CA	290 (99.66)	284 (99.65)	1	0.868
		AA	1 (0.34)	1 (0.35)	0.79 (0.05–12.74)	
rs497916	Allele	C	434 (74.57)	458 (80.35)	1	**0.016**
		T	148 (25.43)	112 (19.65)	**0.69 (0.51–0.93)**	
	Codominant	CC	156 (53.61)	186 (65.26)	1	
		CT	122 (41.92)	86 (30.18)	**0.58 (0.40–0.83)**	**0.013**
		TT	13 (4.47)	13 (4.56)	0.80 (0.35–1.84)	
	Dominant	CC	156 (53.61)	186 (65.26)	1	**0.004**
		CT+TT	135 (46.39)	99 (34.74)	**0.60 (0.42–0.85)**	
	Recessive	CC+CT	278 (95.53)	272 (95.44)	1	0.964
		TT	13 (4.47)	13 (4.56)	0.98 (0.43–2.23)	
rs3922	Allele	A	395 (67.87)	421 (73.86)	1	0.057
		G	187 (32.13)	149 (26.14)	0.76 (0.58–1.01)	
	Codominant	AA	126 (43.30)	154 (54.04)	1	
		AG	143 (49.14)	113 (39.65)	0.70 (0.49–1.01)	0.128
		GG	22 (7.56)	18 (6.32)	0.67 (0.33–1.35)	
	Dominant	AA	126 (43.30)	154 (54.04)	1	**0.043**
		AG+GG	165 (56.70)	131 (45.96)	**0.70 (0.49–0.99)**	
	Recessive	AA+AG	269 (92.44)	267 (93.68)	1	0.503
		GG	22 (7.56)	18 (6.32)	0.79 (0.40–1.56)	
rs676925	Allele	C	509 (87.46)	503 (88.25)	1	0.969
		G	73 (12.54)	67 (11.75)	0.99 (0.68–1.45)	
	Codominant	CC	220 (75.60)	222 (77.89)	1	
		CG	69 (23.71)	59 (20.70)	0.88 (0.58–1.33)	0.373
		GG	2 (0.69)	4 (1.40)	2.99 (0.52–17.25)	
	Dominant	CC	220 (75.60)	222 (77.89)	1	0.716
		CG+GG	71 (24.40)	63 (22.11)	0.93 (0.62–1.40)	
	Recessive	CC+CG	289 (99.31)	281 (98.60)	1	0.208
		GG	2 (0.69)	4 (1.40)	3.08 (0.54–17.74)	

CI, confidence interval; CXCR5, CXC chemokine receptor 5; OR, odds ratio; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism. Data are expressed as frequency (percentage). A logistic regression analysis with adjustment for age and sex was used to evaluate the association between CXCR5 polymorphisms and RA susceptibility.

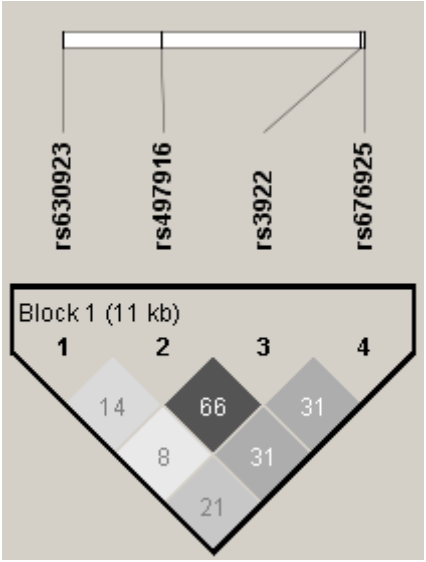

Figure 1. Linkage disequilibrium of single nucleotide polymorphisms (SNPs) within CXC chemokine receptor 5 (CXCR5). The linkage disequilibrium plot shows r^2 values between each pair of SNPs.

Table 3. Associations between CXCR5 haplotypes and RA susceptibility

Haplotype	Controls	RA patients	Adjusted OR (95% CI)	Adjusted *p*-value
CCAC	392.7 (67.47%)	406.7 (71.35%)	1.18 (0.90–1.56)	0.231
CTGC	78.9 (13.56%)	48.0 (8.42%)	**0.54 (0.36–0.81)**	**0.002**
CTGG	47.6 (8.18%)	25.8 (4.53%)	**0.52 (0.30–0.88)**	**0.012**
CCGC	32.6 (5.60%)	32.6 (5.72%)	1.24 (0.72–2.13)	0.442
ATGG	18.5 (3.18%)	29.4 (5.16%)	**1.93 (1.01–3.68)**	**0.042**
CCGG	6.6 (1.13%)	9.5 (1.67%)	1.80 (0.61–5.31)	0.285

CI, confidence interval; CXCR5, CXC chemokine receptor 5; OR, odds ratio; RA, rheumatoid arthritis.

The loci chosen for the hap-analysis were in the following order: rs630923, rs497916, rs3922, and rs673925. Rare haplotypes (frequencies <0.01) were omitted.

Data are expressed as frequency (percentage). The distribution of each haplotype, compared with all the others, was analyzed using logistic regression adjusting for age and sex.

Associations between CXCR5 Polymorphisms and RAA Disease Activity

DAS28 incorporating CRP or ESR has been extensively used for assessing RA disease activity.[26] Additionally, multiple laboratory inflammation markers can directly predict disease activity.[27–29] The long-term activity of RA can be assessed by the presence or absence of osteoporosis.[30] Therefore, we applied various indicators in the current study for RA activity evaluation.

A significant upward trend in disease activity was observed in the AG+GG genotype carriers of rs3922; the mean DAS28-ESR and DAS28-CRP were 6.23 and 5.43, respectively, which were 5.59 and 4.78 in the AA genotype carriers (p=0.003 and p=0.002 for DAS28-ESR and DAS28-CRP, respectively). We also found that patients with CT+TT genotypes of rs497916 had lower RF levels (p=0.017). However, no associations of anti-CCP and other inflammation markers (including CRP and ESR) with CXCR5 polymorphisms were observed in RA patients. The osteoporosis incidence did not differ between patients with distinct genotypes (Table 4).

Table 4. Associations of CXCR5 polymorphisms with RA-related clinical features

Clinical features	rs630923			rs479716			rs3922			rs676925		
	CC	CA+AA	*p*	CC	CT+TT	*p*	AA	AG+GG	*p*	CC	CG+GG	*p*
DAS28-ESR	5.87 ± 1.60	5.82 ± 1.36	0.869	5.72 ± 1.54	6.17 ± 1.58	0.051	5.59 ± 1.51	6.23 ± 1.57	**0.003**	5.81 ± 1.56	6.10 ± 1.58	0.283
DAS28-CRP	5.05 ± 1.58	5.16 ± 1.32	0.689	4.93 ± 1.56	5.34 ± 1.48	0.060	4.78 ± 1.52	5.43 ± 1.51	**0.002**	5.01 ± 1.56	5.28 ± 1.46	0.305
Age of onset (year)	43.7 ± 14.6	43.4 ± 16.5	0.928	44.3 ± 14.1	42.3 ± 16.2	0.367	44.2 ± 14.2	43.0 ± 15.6	0.538	44.1 ± 14.5	41.8 ± 16.1	0.361
Osteoporosis [a]	58 (25.33%)	10 (27.03%)	0.839	48 (27.59%)	20 (21.74%)	0.353	41 (28.67%)	27 (21.95%)	0.456	53 (25.36%)	15 (26.32%)	0.567
Anti-CCP (U/mL)	352.80 (128.95, 501.00)	387.40 (280.60, 501.00)	0.276	362.50 (137.18, 501.00)	355.00 (159.40, 501.00)	0.839	379.10 (150.05, 501.00)	344.40 (149.85, 501.00)	0.437	350.10 (141.00, 501.00)	406.60 (176.30, 501.00)	0.301
RF (IU/mL)	178.0 (41.0, 636.5)	197.5 (35.2, 670.5)	0.978	220.0 (60.3, 726.0)	106.5 (25.1, 513.8)	**0.017**	181.0 (57.7, 740.3)	178.0 (29.1, 581.0)	0.204	209.0 (51.4, 679.0)	105.5 (28.7, 624.0)	0.163
ESR (mm/h)	56.2 ± 32.9	54.3 ± 39.0	0.757	57.0 ± 32.4	53.7 ± 36.3	0.454	57.2 ± 32.3	54.3 ± 35.5	0.478	56.9 ± 32.6	52.1 ± 37.5	0.378
CRP (mg/L)	11.90 (5.11, 42.65)	11.80 (4.39, 60.00)	0.892	12.30 (5.43, 49.88)	10.35 (3.84, 34.95)	0.146	12.30 (5.29, 45.80)	10.70 (4.39, 43.30)	0.521	12.55 (5.30, 46.18)	8.31 (3.72, 43.00)	0.128

Anti-CCP, anti-cyclic citrullinated peptide antibody; CRP, C-reactive protein; CXCR5, CXC chemokine receptor 5; DAS, disease activity score; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; RF, rheumatoid factor.

Data are expressed as mean ± standard deviation (SD), frequency (percentage), or median (25^{th} and 75^{th} percentiles), where appropriate. The Mann-Whitney *U* test and Student's *t*-test were applied for quantitative data analyses as approprite. The χ^2 test was applied for categorical data if not stated otherwise.

[a] Osteoporosis-related data were only collected in 266 RA patients. Logistic regression, adjusting for age and sex, was used to assess the association between osteoporosis and CXCR5 polymorphisms.

Correlations of CXCR5 Polymorphisms and Laboratory Characteristics of RA

We compared the levels of total bilirubin, several liver enzymes, and lipids in the sera of RA patients and healthy controls with different CXCR5 polymorphism genotypes (Figure 2). None of these laboratory parameters showed significantly different distributions among controls with or without CXCR5 polymorphism. However, in RA patients, TBIL was significantly lower in the CA+AA genotype carriers than in the carriers of the CC genotype of rs630923 (p=0.027). Similarly, TC and LDL-c were significantly lower in the rs3922 AG+GG genotype carriers (p=0.015 for TC and p=0.024 for LDL-c). Furthermore, ALP was lower in the rs630923 CA+AA genotype carriers (p=0.013) and rs676925 CG+GG genotype carriers (p=0.015).

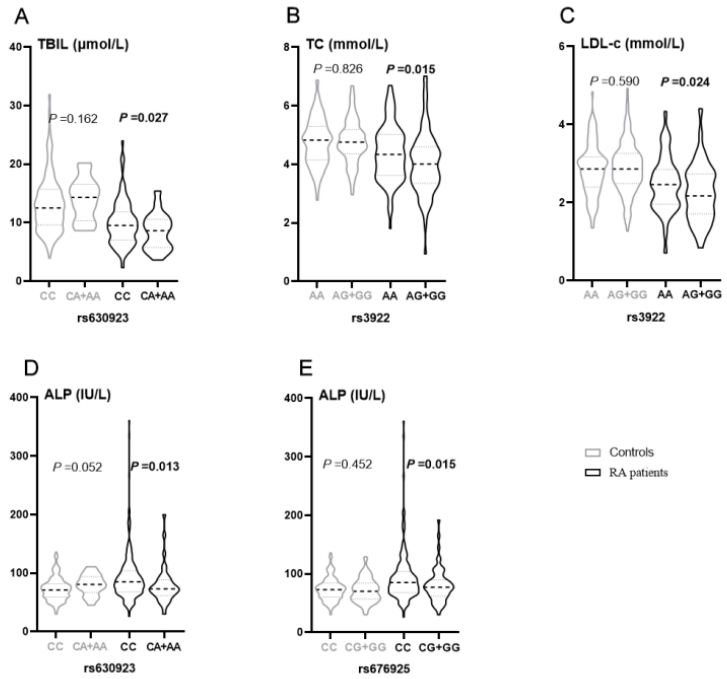

Figure 2. Distribution of multiple laboratory tests' serum levels in different CXC chemokine receptor 5 (CXCR5) single nucleotide polymorphism genotypes. The dashed lines represent medians and interquartile ranges.
ALP, alkaline phosphatase; LDL-c, low-density lipoprotein cholesterol; RA, rheumatoid arthritis; TBIL, total bilirubin; TC, total cholesterol.

DISCUSSION

RA is a polyetiological disease that develops as a result of the combined influence of exogenous and endogenous causes.[6] The genetic risk loci identified so far only account for approximately half of the total heritability of RA.[31] Therefore, finding other risk variants may aid our understanding of RA pathophysiology.

Proper interplay between CXCR5 and its ligand CXCL13 is the impetus for Tfh and B cells to migrate to secondary lymphoid organs and format lymphoid follicles, which is a prerequisite for humoral immunity.[11,12] Aberrant Tfh cells and CXCR5 activity are involved in RA susceptibility and disease progression.[14–17] However, to the best of our knowledge, no association study has ever focused on CXCR5 gene polymorphisms and the risk of RA. Therefore, in this study, we first reported that the rs630923 A allele was associated with a significantly higher risk of RA, while the rs497916 T and rs3922 G alleles may have a protective effect on RA development. Similarly, individuals with the ATGG haplotype, which contains the risky rs630923 A allele, were more likely to develop RA; however, CTGC and CTGG, which contain protective alleles (T allele of rs497916 and G allele of

rs3922) and no risk allele, may decrease RA risk. Concomitantly, in the RA group, we found that different genotypes were significantly associated with specific laboratory values, including RF, TBIL, TC, LDL-c, and ALP.

Rs630923 is located in the CXCR5 gene promoter, where the alternate alleles of a SNP may result in either formation or abolition of a binding site for specific transcription factors and therefore play piloting roles in regulating gene expression.[32] Mitkin et al. found that the A allele of rs630923 could create a functional binding site for the transcription factor myocyte enhancer factor 2C and was correlated with reduced activity of the CXCR5 promoter.[18] Furthermore, the rs630923 A allele was predicted to alter NFκB binding and could thus potentially inhibit CXCR5 transcription as well.[19] However, decreased expression is not equivalent to suppressed function. Bentebibel et al. reported that the CD4+ T cells expressing low levels of CXCR5 secreted larger amounts of IL-21 and IL-10 and induced proliferation and differentiation of naïve B cells into Ig-producing cells more efficiently than those with high CXCR5 expression.[33] The information derived from the aforementioned studies and our own suggest that in RA-susceptible individuals, rs630923 might affect CXCR5 expression in Tfh cells, thereby enhancing the ability of Tfh cells to help B cells produce autoantibodies, ultimately leading to disease. Several previous studies have indicated that rs630923 was related to a reduced risk of MS in Caucasians.[18,19] Although both RA and MS are chronic inflammatory diseases, they affect entirely different target tissues, namely, synovial membranes and glia, respectively. Therefore, we consider that the underlying etiopathogenesis of RA and MS differ, which may mean that rs630923 has distinct roles in different diseases. Additionally, the discrepancies between our results and those of others may be partly attributed to race and genotyping method variations.

Using a dual luciferase report assay, Duan et al. confirmed that the rs3922 mutation homozygous (GG genotype) was associated with higher levels of CXCR5 than the AG+AA genotypes.[21] In our study, the rs3922 G allele played a protective role in RA. This is similar to the scenario with rs630923; specifically, individuals carrying genotypes thougnt to be associated with increasing CXCR5 expression may be at a lower risk for RA, and vice versa. Therefore, we assumed that if any polymorphisms in the CXCR5 gene could affect its own protein expression, they would promote Tfh-dependent B cell maturation and autoantibody production. However, using a flow cytometry assay, Duan et al. found that rs676925 could not affect CXCR5 expression levels.[21] We speculated that, because of this, rs676925 may not affect CXCR5 function and RA susceptibility. Regarding rs497916, no studies of its relationship with CXCR5 expression have been performed. Owing to the linkage disequilibrium between rs497916 and rs3922, we hypothesized that rs3922 might be the genuine RA susceptibility locus.[34] Rs497916 is coinherited with rs3922, which makes the former seem to be related to lower RA risk as well. Functional studies are necessary to confirm this hypothesis.

Six common haplotypes were identified in this study. There are three haplotypes containing the protective alleles of rs497916 and rs3922; of these, the CTGC and CTGG were associated with a reduced risk of RA. However, because of the additional effect of the rs630923 risk allele, ATGC resulted in the carriers being more likely to develop RA. These findings support the results of the relationship between each individual SNP and RA susceptibility. Furthermore, we assumed that the inhibitory effect of rs630923 on the expression of CXCR5 might exceed the beneficial effects of rs3922 and rs497916.

RA is characterized by the overproduction of autoantibodies such as RF and anti-CCP; therefore, these laboratory values have been incorporated into the classification criteria.[24] Furthermore, the presence of RF is related to higher disease activity.[27] We observed that RF levels were significantly higher in RA patients with the rs497916 CC genotype than in those with the CT+TT genotypes, which may also be attributable to the T allele of rs497916, which could relatively weaken Tfh cell function and decrease autoantibody production. RF could lead to immune complex formation in the joints, complement activation, and subsequent recruitment and activation of inflammatory leukocytes.[35] The accumulation of RF exacerbates disease progression and promotes the occurrence of RA tosome extent. These results suggest that CXCR5 polymorphisms are associated with RA disease activity. Unexpectedly, higher DAS28-ESR and DAS28-CRP levels were present in rs3922 protective allele carriers. We hypothesize that these correlations might have been affected by medication. Genetic biomarkers are not only capable of identifying susceptible individuals but also therapy selection and monitoring of response to therapy.[36] Additionally, subgroup analyses of RA

patients according to their treatment status in follow-up studies are necessary. This would help to explore the associations between CXCR5 genetic polymorphisms and the treatment response of RA patients and to reduce the direct impact of drugs on disease activity.

Trends in various laboratory indicators between healthy controls and RA patients were partly confirmed in this study. For instance, in RA patients, serum bilirubin decreased, total cholesterol and LDL-c levels decreased in developing RA; such decreases were unlikely to be due solely to lipid-lowering treatment, and ALP levels were elevated in both the serum and synovial fluid of RA patients.[37-39] Our results also raise the possibility that genetic variations in CXCR5 could influence the levels of these laboratory indicators in RA patient serum (Figure 2), which might be attributable to the intricate interplay among CXCR5 expression, Tfh cell activation, and RA inflammation; however, these findings require further validation because of the small sample size.

In conclusion, this is the first study to investigate the association between CXCR5 polymorphisms and RA susceptibility. Rs630923 was associated with a significantly elevated risk of RA; however, it was observed that rs497916 and rs3922 may protect against RA development. These findings increase the possibility of identifying RA-susceptible individuals based on genetic markers. Further exploration of the relationships between SNPs in CXCR5 and RA disease activity, and the mechanism leading to laboratory values exhibiting significantly different levels within distinct CXCR5 genotypes, is warranted.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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