Preferential Association of Interferon Regulatory Factor 5 Gene Variants with Seronegative Rheumatoid Arthritis in 2 Swedish Case-Control Studies

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ABSTRACT. *Objective.* Two interferon regulatory factor 5 (*IRF5*) gene variants were examined for association with rheumatoid arthritis (RA).

Methods. A total of 2300 patients with RA and 1836 controls were recruited from 2 independent RA studies in Sweden. One insertion-deletion polymorphism (CGGGG indel) and one single-nucleotide polymorphism (rs10488631) in the *IRF5* gene were genotyped and analyzed within RA subgroups stratified by rheumatoid factor (RF) and anticitrullinated peptide antibodies (ACPA).

Results. The CGGGG indel was preferentially associated with the RF-negative (OR 1.29, $p = 7.9 \times 10^{-5}$) and ACPA-negative (OR 1.27, $p = 7.3 \times 10^{-5}$) RA subgroups compared to the seropositive counterparts. rs10488631 was exclusively associated within the seronegative RA subgroups (RF-negative: OR 1.24, $p = 0.016$; ACPA-negative: OR 1.27, $p = 4.1 \times 10^{-3}$).

Conclusion. Both the CGGGG indel and rs10488631 are relevant for RA susceptibility, especially for seronegative RA. (First Release Aug 1 2011; J Rheumatol 2011;38:2130–2; doi:10.3899/jrheum.110322)

Key Indexing Terms:

RHEUMATOID ARTHRITIS
GENETIC ASSOCIATION STUDY

INTERFERON REGULATORY FACTOR 5
SERONEGATIVE

Rheumatoid arthritis (RA) is a common chronic inflammatory debilitating disease of the joints. Two serological factors, rheumatoid factor (RF) and anticitrullinated peptide antibodies (ACPA), are considered standard markers for RA diagnosis¹. Studies have shown that seropositive and seronegative RA are 2 independent subgroups with pronounced differences in disease progression, severity, and treatment response, as well as molecular pathophysiology².

The interferon regulatory factor 5 gene (*IRF5*) is one of the few genetic factors that is primarily associated with seronegative RA³. *IRF5* encodes a key transcription factor in the type I interferon (IFN) pathway. Studies have indicated that the *IRF5* gene harbors 2 independent haplotypes associated with

autoimmune disorders⁴, the first tagged by a 5-bp insertion-deletion polymorphism (CGGGG indel) with either 3 or 4 copies of the repeat unit, located in the 5' region of the *IRF5* gene. The second independent signal is from the 3' region of the *IRF5* gene, tagged by the single-nucleotide polymorphism (SNP) rs10488631. Interestingly, for systemic lupus erythematosus (SLE) and primary Sjögren's syndrome, the 5' and 3' variants show comparable association signals; whereas for inflammatory bowel diseases and multiple sclerosis it is the 5' variants that dominate the disease risk⁵.

We investigated the CGGGG indel and the rs10488631 SNP for association with RA or RA serological subgroups.

MATERIALS AND METHODS

Subjects. RA patients from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study were residents in the southern and central part of Sweden, and control subjects were selected from the Swedish population registry. The Northern Sweden Rheumatoid Arthritis (NSRA) study included RA patients from the 4 northernmost counties of Sweden, and controls were recruited from the Medical Biobank of Northern Sweden. Serological status for RF and ACPA was determined as described^{6,7}. Written or verbal consent was obtained from all subjects. The ethics committees of the Karolinska Institutet or Umeå University Hospital approved the respective studies. Table 1 provides additional information on the study subjects.

Genotyping. The CGGGG indel and the rs10488631 SNP were genotyped as described^{4,5}. The call rates for the CGGGG indel and rs10488631 were over 90%. No discrepant genotypes were observed upon repeated genotyping of ~10% of the samples for both markers, nor was there any deviation from Hardy-Weinberg equilibrium.

Statistical analysis. PLINK software (Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; <http://pngu.mgh.harvard.edu/~plink/>) was used for all statistical analyses.

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Supported by research grants from the Swedish Research Council for Medicine and Health, grant no. A028001 and 90318501.

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Accepted for publication May 26, 2011.

Table 1. Clinical characteristics of patients with RA and controls from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) and Northern Sweden Rheumatoid Arthritis (NSRA) studies.

Characteristic	EIRA	NSRA
Participants, n (case:control)	2455 (1530:925)	1681 (770:911)
Age, yrs (case:control)*	51.4 ± 12.3 (50.9 ± 12.5:52.1 ± 11.8)	57.3 ± 13.1 (55.2 ± 14.1:59.1 ± 11.8)
Female, % (case:control)	71.4 (70.6:72.8)	70.6 (67.8:73.0)
RF-negative cases, %	33.8	25.3
ACPA-negative cases, %	38.9	31.4

* Age represented as mean ± SD. RA: rheumatoid arthritis; RF: rheumatoid factor; ACPA: anti-citrullinated peptide antibodies.

vard.edu/~purcell/plink/) was applied for Hardy-Weinberg equilibrium tests and association analysis.

RESULTS

When the whole set of patients was analyzed, the CGGGG indel was significantly associated with RA in the EIRA study and marginally associated with RA in the NSRA study (Table 2). As for other autoimmune diseases, the 4× allele was the risk allele. Stratified analysis supported the preferential associations of the CGGGG indel in the seronegative RA subgroups (RF-negative_{comb}: OR 1.29, $p = 7.9 \times 10^{-5}$; ACPA-negative_{comb}: OR 1.27, $p = 7.3 \times 10^{-5}$) compared to the seropositive counterparts. For the C allele of the rs10488631 SNP, the only consistent association signals in both the EIRA and NSRA studies, confirmed by a combined analysis, were

from the ACPA-negative RA subgroups (ACPA-negative_{comb}: OR 1.27, $p = 4.1 \times 10^{-3}$).

DISCUSSION

Although we have previously reported several 5' variants of the *IRF5* gene in RA susceptibility³, the most promising functional candidate in this region is the CGGGG indel reported here. Its 4× allele carries an additional CGGGG repeat unit compared to the nonrisk allele; this allows more binding of the transcription factor SP1, which has been shown to cause elevated *IRF5* expression⁴. However, the risk for ACPA-negative RA conferred by the CGGGG indel and the 5' SNP rs3807306 (which was reported previously as the most strongly associated SNP) is highly similar (OR_{CGGGG indel} = 1.27; OR_{rs3807306} = 1.28)³. Another variant of functional potential in this region

Table 2. Allelic association analysis of *IRF5* gene variants and RA.

		EIRA				NSRA				Combined [†]			
		Frequency		p*	OR (95% CI)	Frequency		p*	OR (95% CI)	Frequency		p*	OR (95% CI)
		Case (n)	Control (n)			Case (n)	Control (n)			Case (n)	Control (n)		
CGGGG indel (4× allele)	All RA	0.49 (1496)	0.44 (924)	8.8×10^{-4}	1.22 (1.09–1.37)	0.48 (763)	0.45 (802)	0.10	1.12 (0.98–1.29)	0.48 (2259)	0.44 (1726)	2.9×10^{-4}	1.18 (1.08–1.29)
	RF+	0.48 (935)	0.44 (924)	0.020	1.17 (1.03–1.33)	0.47 (562)	0.45 (802)	0.36	1.08 (0.92–1.25)	0.47 (1497)	0.44 (1726)	0.018	1.13 (1.02–1.24)
	RF–	0.50 (476)	0.44 (924)	1.0×10^{-3}	1.30 (1.11–1.52)	0.52 (192)	0.45 (802)	0.021	1.30 (1.04–1.63)	0.51 (668)	0.44 (1726)	7.9×10^{-5}	1.29 (1.14–1.46)
	ACPA+	0.47 (916)	0.44 (924)	0.026	1.16 (1.02–1.32)	0.47 (491)	0.45 (802)	0.23	1.10 (0.94–1.29)	0.47 (1407)	0.44 (1726)	0.014	1.13 (1.03–1.25)
	ACPA–	0.51 (579)	0.44 (924)	2.7×10^{-4}	1.31 (1.13–1.52)	0.50 (228)	0.45 (802)	0.085	1.20 (0.97–1.48)	0.50 (807)	0.44 (1726)	7.3×10^{-5}	1.27 (1.13–1.43)
rs10488631 (C allele)	All RA	0.15 (1390)	0.14 (904)	0.21	1.12 (0.94–1.32)	0.17 (767)	0.14 (892)	8.3×10^{-3}	1.29 (1.07–1.55)	0.16 (2157)	0.14 (1796)	0.015	1.17 (1.03–1.33)
	RF+	0.14 (867)	0.14 (904)	0.69	1.04 (0.86–1.26)	0.18 (568)	0.14 (892)	8.3×10^{-3}	1.31 (1.07–1.61)	0.15 (1435)	0.14 (1796)	0.016	1.14 (1.00–1.31)
	RF–	0.17 (445)	0.14 (904)	0.036	1.27 (1.02–1.58)	0.17 (191)	0.14 (892)	0.21	1.21 (0.90–1.64)	0.17 (635)	0.14 (1796)	0.016	1.24 (1.04–1.48)
	ACPA+	0.14 (852)	0.14 (904)	0.72	1.04 (0.85–1.26)	0.17 (496)	0.14 (892)	0.043	1.25 (1.01–1.54)	0.15 (1348)	0.14 (1796)	0.16	1.11 (0.96–1.28)
	ACPA–	0.16 (538)	0.14 (904)	0.041	1.25 (1.01–1.54)	0.18 (226)	0.14 (892)	0.022	1.37 (1.05–1.81)	0.17 (764)	0.14 (1796)	4.1×10^{-3}	1.27 (1.08–1.50)

EIRA: Epidemiological Investigation of Rheumatoid Arthritis; NSRA: Northern Sweden Rheumatoid Arthritis study; RF: rheumatoid factor; ACPA: anticitrullinated peptide antibodies. * 2-sided chi-square tests; < 0.05 considered statistically significant. [†] Combined analysis of the EIRA and NSRA studies was achieved by pooling genotype data. This strategy was supported by chi-square-based homogeneity tests, which did not show any significant differences in allele distributions for CGGGG indel or rs10488631 between studies.

is the rs10954213 SNP, which may create a functional polyadenylation site and influence the stability of *IRF5* messenger RNA. In addition, the rs2004640 SNP has been reported to create an alternative splice site in *IRF5*⁸, and the existence of additional functional variants cannot be excluded. As for the 3' variants of the *IRF5* gene, no biological hypothesis for their functions has been proposed. However, the C allele of the rs10488631 SNP has been reported to associate with enhanced *IRF5* expression independently of the CGGGG indel⁹. Our study demonstrated that the association pattern of the *IRF5* gene variants in RA, and more notably with seronegative RA, resembles the patterns observed for SLE and primary Sjögren's syndrome, in which both the 5' and 3' variants are associated with and confer comparable risk to the disease^{4,5}.

The preferential association of the *IRF5* gene variants with seronegative RA has also been reported in other populations. In a Spanish RA case-control study, the 5' SNP rs2004640, rs752637, and rs10954213 and the SNP rs10488631 were found to have stronger associations with ACPA-negative RA¹⁰. A study with French and Western European RA family trios reported the risk effects of the 3 SNP rs3757385, rs2004640, and rs10954213, where the signals were also stronger in the RF-negative RA subgroup¹¹. Meanwhile, contradictory results were observed from a Euro-American and a Korean RA case-control study. The former study reported that the 3' SNP rs10488631 was exclusively associated with ACPA-positive RA¹²; and the latter detected association of the 5' SNP rs2004640 in the ACPA-positive RA subgroup only¹³. Low power due to the sample sizes, lower prevalence and higher clinical heterogeneity of seronegative RA, varying risk allele frequencies and genetic heterogeneity among populations may account for these inconsistent observations.

Indeed, although seropositive and seronegative RA seem to be comparable in heritability¹⁴, their distinct genetic etiologies have been addressed in genome-wide association studies and other large-scale studies¹⁵. Evidence on genetic factors shared by these 2 RA subgroups is also accumulating. These findings emphasize the different, but related, pathogenic mechanisms underlying seropositive and seronegative RA.

ACKNOWLEDGMENT

We thank Annika Ahlford, Sofia Nordman, Ann-Christin Wiman, and Torbjörn Öst for assistance with genotyping, and the members of the Epidemiological Investigation of Rheumatoid Arthritis group for aiding with data collection.

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