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Concise Report

Polymorphisms in the ficolin 1 gene (*FCN1*) are associated with susceptibility to the development of rheumatoid arthritis

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Objectives. We investigated the possible association of rheumatoid arthritis (RA) with single nucleotide polymorphisms (SNP) within the ficolin (*FCN)* genes. Two SNPs in the *FCN1* gene, four SNPs in the *FCN2* gene and one SNP in the *FCN3* gene were studied.
Methods. The SNPs within the *FCN* genes were detected by an experimental INNO-LiPA methodology (Innogenetics, Belgium) in a population consisting of 338 RA patients and 595 controls. The significant SNPs were further evaluated in two subpopulations and related to carriage of the human leukocyte antigen-shared epitope (HLA-SE), rheumatoid factor (RF) and the presence of anti-citrullinated protein/peptide antibodies (ACPA).
Results. Two SNPs in the *FCN1* gene were significantly associated with RA: the A allele rs2989727 was significantly increased in RA patients (67%) compared with controls (60%) ($P=0.002$). Also, the frequency of the G allele of rs1071583 was increased in RA patients (68%) compared with controls (61%) ($P=0.003$). Analysis of agreement between SNPs suggested strong linkage between rs2989727 and rs1071583. Carriage of a *FCN1* SNP was independent of carriage of the HLA-SE, RF status and ACPA positivity.
Conclusions. We describe two linked SNPs in the *FCN1* gene that are associated with the development of RA.

Key words: Rheumatoid arthritis, Ficolin, Polymorphisms.

Introduction

The lectin pathway of complement activation is a key mechanism for the mammalian acute phase response to infection and involves carbohydrate recognition by mannose-binding lectin (MBL) and ficolins. The major role of these pattern-recognition molecules (PRMs) is related to the first-line defence against potential pathogens by recognition of a specific spectrum of glycoproteins that is crucial in maintaining the self *vs* non-self discrimination. Besides this, some of the PRMs may also exhibit endogenous functions in homeostasis including the removal of apoptotic cells [1].

So far, three different ficolin proteins have been characterized in human, each encoded by a separate gene. L-ficolin (also known as ficoline/P35 or hucolin) is encoded by the *FCN2* gene [9q34.3]. H-ficolin (Hakata Ag) is encoded by the *FCN3* gene localized on chromosome 1 and is, together with L-ficolin, secreted in the serum [2]. The third ficolin (M-ficolin) is encoded by the *FCN1* gene located also on chromosome 9q34. Little is known about M-ficolin, but Liu *et al*. [3] proved that M-ficolin is a secretory protein from neutrophils and monocytes in peripheral blood and type II alveolar epithelial cells. Especially, L-ficolin binds to clinically important micro-organisms while H-ficolin mainly binds *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine. M-ficolin binds GlcNAc bearing neoglycoproteins, *N*-acetylgalactosamine and sialyl-*N*-acetyllactosamine, suggesting recognition of microbial carbohydrate residues. It has also been demonstrated that each of the ficolin types act by activation of the lectin pathway to exert their innate immunity capacities [2–6].

The aims of the present study were to investigate whether there is an association of the development of rheumatoid arthritis (RA) with polymorphisms in any of the three described *FCN* genes. We also evaluated dependency with three other RA-associated biomarkers: carriage of the human leukocyte antigen-shared epitope (HLA-SE), the presence of anti-citrullinated protein/peptide antibodies (ACPAs) and rheumatoid factor (RF).

Patients and methods

Patients

The study sample (338 RA patients and 595 controls) was derived from two populations.

Population 1 (pop1) consisted of 194 patients with long-standing RA (median disease duration of more than 9 yrs). Radiological and serological data of these patients have previously been described [7, 8]. Those RA patients were compared with a control population of 205 healthy individuals.

Population 2 (pop2) was derived from a consecutive cohort of 1003 patients with a new diagnostic problem, with RA in the differential diagnosis [9, 10]. After 1 yr, patients were classified as RA ($n=144$) and RA was excluded in 629 non-RA patients. Serological data of this cohort were previously described. All RA patients and a random sample of 390 non-RA patients were used in the current study. Serum antibody results could be obtained in 150 RA patients of pop1 and in 520 patients (including 142 RA patients and 378 non-RA patients) of pop2. All RA patients fulfilled the ACR classification criteria for RA [11].

All participants gave informed consent and the study was approved by the local ethical committee.

FCN determination

Several DNA variants, both in coding and non-coding sequences, were analysed.

FCN1. A G>A variant at position −1981 in the promotor region (rs2989727) and a A>G variant at position 7919 in exon 9 (rs1071583).

FCN2. A A > C variant at position −64 (rs7865453), an A>G variant in position −4 (ss32469537) of the promoter and in exon 8

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TABLE 1. Allele frequencies and allelic ORs in the pooled population

		FCN1		*FCN2*				*FCN3*
		9q34		9q34				1p35.3
		rs2989727 Promoter G/A	rs1071583 Exon 9 A/G	(Prom2)rs7865453 Promoter A/C	(Prom3) -4AtoG Promoter A/G	T236M Exon 8 C/T	rs7851696 Exon 8 G/T	rs3813800 IVS5 C/G
RA patients (population1)	Allele 1	0.33	0.31	0.91	0.71	0.70	0.91	1.00
	Allele 2	0.67	0.69	0.09	0.29	0.30	0.09	0.00
Controls (population 1)	Allele 1	0.42	0.41	0.85	0.78	0.77	0.84	0.98
	Allele 2	0.58	0.59	0.15	0.22	0.23	0.16	0.02
RA patients (population 2)	Allele 1	0.33	0.33	0.91	0.72	0.68	0.89	1.00
	Allele 2	0.67	0.67	0.09	0.28	0.32	0.11	0.00
Controls (population 2)	Allele 1	0.39	0.38	0.89	0.73	0.73	0.89	1.00
	Allele 2	0.61	0.62	0.11	0.27	0.27	0.11	0.00
RA patients (pooled)	Allele 1	0.33	0.32	0.91	0.71	0.69	0.90	1.00
	Allele 2	0.67	0.68	0.09	0.29	0.31	0.10	0.00
Controls (pooled)	Allele 1	0.40	0.39	0.88	0.74	0.74	0.87	0.99
	Allele 2	0.60	0.61	0.12	0.26	0.26	0.13	0.01
Pooled OR		1.37	1.36	0.72	1.16	1.28	0.75	0.18
(95% CI)		(1.12–1.67)	(1.11–1.66)	(0.53–0.99)	(1.04–0.94)	(1.01–1.14)	(0.55–1.01)	(0.02–1.39)
P-value		0.002	0.003	0.043	0.176	0.019	0.059	0.017

a C>T change at position 6424 and a G>T change in position 6359 (ss32469544).

FCN3. An SNP localized in IVS5 at position 3836 [C > G (rs3813800)].

Genotyping was carried out by a multi-parameter assay allowing simultaneous detection of the relevant DNA variants in the three *FCN* genes using reverse-hybridization technology (Innogenetics). Briefly, specific probes designed to hybridize with their complementary sequences amplified from the target DNA are coated as dots on nitrocellulose strips. The hybridized probes are visualized as coloured dots and can be interpreted visually. Primers were developed for selective amplification of the relevant genomic DNA sequences.

Detection of autoantibodies

RF was determined by the latex fixation test (Difco Lab, Detroit, MI, USA). A cut-off at a 95% specificity level (25 U/m, sensitivity = 45%) was previously defined [7].

ACPAs were detected by a research line immunoassay (LIA) containing the synthetic citrullinated peptide referred to as pepA (INNO-LIA™ RA, Innogenetics) [7, 10, 12]. This test had a specificity of ≥98.5% and a sensitivity of 63.6% in an independent cohort [7].

HLA-SE (HLA-shared epitope) typing by INNO-LiPA (line probe assay) technology

DNA was extracted from whole blood samples and amplified using the INNO-LiPA HLA-DRB1 or -DRB decoder amplification kits (Innogenetics). HLA typing was performed with the INNO-LiPA HLA-DRB1 or -DRB decoder kits (Innogenetics), according to the manufacturer's instructions. The amino acid sequences QRRAA, QKRAA and RRRAA at positions 70–74 constitute the RA HLA-SE sequence. Patients were classified into two groups according to the inheritance of zero *vs* one or two copies of the HLA-SE.

Statistical methods

Odds ratios (ORs), 95% confidence intervals (CIs) and chi-square statistics or Fisher exact tests were calculated in 2 × 2 tables. Gamma with its 95% CI, as a measure of ordinal correlation, was calculated in N × M tables. Logistic regression models were fit and predicted probability plots were calculated as previously described [9]. Dependency between markers was evaluated in the pooled cohort by calculating a common OR (Mantel–Haenszel common OR controlled for diagnosis) and Mann–Whitney testing for continuous parameters. The Hardy–Weinberg equilibrium was evaluated in the control populations. Analyses were performed using SPSS 12.0 (Chicago, IL, USA). A *P*-value of <0.05 was considered as statistical significant except for the single nucleotide polymorphism (SNP) selection, where a *P*-value of <0.007 was used as a way to handle multiple comparisons. Given the 338 RA patients and 595 controls, $\alpha = 0.007$, this study had sufficient power ($\beta = 0.8$) to detect ORs of less than 1.8 for threats with a prevalence of <0.8 in controls.

Results

Selection of the associated SNPs

For the selection of the significantly associated SNPs, allele frequencies, allelic ORs and *P*-values were calculated in the pooled population (Table 1). The pre-set significance level for SNP selection resulted in a selection of two SNPs in the *FCN1* gene: the *FCN1* rs2989727 and the *FCN1* rs1071583 SNP. Further analysis of controls suggested Hardy–Weinberg equilibrium was present for all SNPs.

Further analysis of the FCN1 genotypes

Carriage of at least one A allele of rs2989727 was associated with RA [OR = 2.23 (1.22–4.06) in pop1; OR = 2.40 (1.23–4.68) in pop2; OR = 2.24 (1.45–3.46), $P < 0.001$ overall] (Table 2). Carriage of at least one G allele of rs1071583 was associated with RA [OR=2.08 (1.14–3.81) in pop1 and OR = 1.92 (1–3.68) in pop2; OR = 1.94 (1.257–3.00), $P = 0.002$ overall] (Table 2). Analysis of agreement between SNPs suggested linkage between rs2989727 and rs1071583 in both populations ($\gamma = 0.999$, $P < 0.001$).

Independency of the FCN1 SNPs and the HLA-SE, RF and ACPA

No dependency between the HLA-SE and carriage of the two *FCN1* SNPs could be observed [common OR = 0.965 (0.658–1.416) and 0.976 (0.660–1.443)]. Similarly, no dependency of *FCN1* carriage and ACPA [common OR = 1.056 (0.496–2.251) and 1.149 (0.544–2.427)] or the RF [common OR = 1.264 (0.662–2.412) and 1.020 (0.533–1.951)] could be demonstrated.

TABLE 2. Gene frequencies of the 2 FCN1 SNPs

		Genotype	rs2989727	OR (GGvsAG/AA)	Genotype	rs1071583	OR (AA *vs* AG/GG)
Population 1	RA	GG	0.09	2.23 (1.22–4.06)	AA	0.09	2.08(1.14–3.81)
		AG	0.48		AG	0.44	
		AA	0.43		GG	0.46	
	Control	GG	0.19		AA	0.18	
		AG	0.47		AG	0.47	
		AA	0.35		GG	0.36	
Population 2	RA	GG	0.08	2.40(1.23–4.68)	AA	0.09	1.92(1.00–3.68)
		AG	0.49		AG	0.48	
		AA	0.43		GG	0.43	
	Control	GG	0.17		AA	0.15	
		AG	0.44		AG	0.45	
		AA	0.38		GG	0.40	
Overall	RA	GG	0.09	2.24 (1.45–3.46)	AA	0.09	1.94 (1.257–3.00)
		AG	0.48		AG	0.46	
		AA	0.43		GG	0.45	
	Control	GG	0.18		AA	0.16	
		AG	0.45		AG	0.46	
		AA	0.37		GG	0.38	

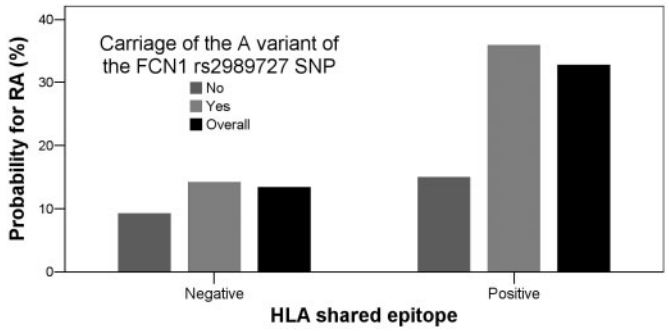

FIG. 1. Probability for RA in the function of the HLA-SE and FCN1 status. The black bars indicate the overall probability for RA in function of the HLA-SE status. The other bars (red and blue in the online version) indicate the eventual added value of *FCN1* testing to HLA testing. The figures for the FCNrs rs2989727 or the *FCN1* rs1071583 were very similar. The probability for RA was adjusted for the *a priori* change for RA given in [9].

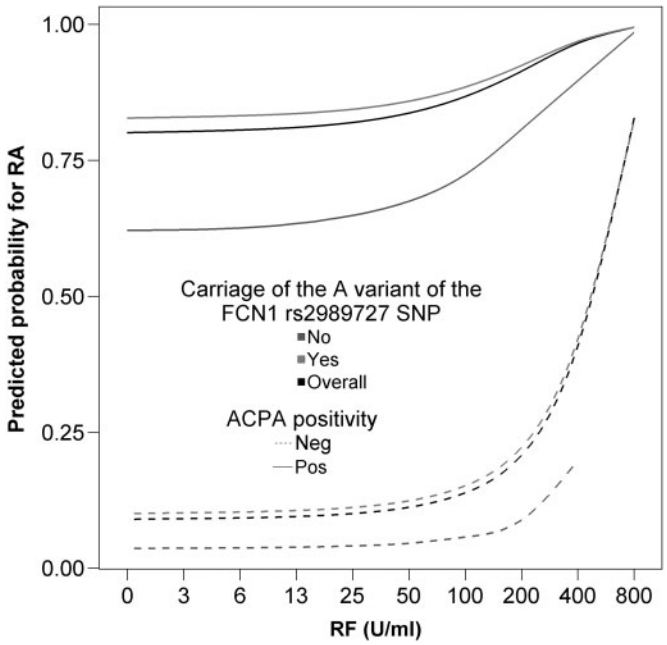

FIG. 2. Plots of the predicted probabilities in function of RF titres, ACPA testing and *FCN1* testing. The black line indicates the added value of ACPA testing to RF testing as already shown in [9]. The other lines (red and blue in the online version) indicate the eventual added value of *FCN1* testing to combined RF and ACPA testing. The figures for the FCNrs rs2989727 or the *FCN1* rs1071583 were almost identical. Dashed lines indicate ACPA negativity, full lines indicate ACPA positivity. The predicted probability plots were calculated by means of logistic regression.

Potential additional value of FCN1 testing to HLA-SE testing or serological testing in a prediction model for RA in pop2

Logistic regression demonstrated that *FCN1* testing contributed independently to HLA-SE testing in predicting RA [OR = 2.383 (95% CI 1.451–3.912, $P = 0.001$) for carriage of the A variant of the rs2989727 SNP and OR = 2.05 (95% CI 1.252–3.353, $P = 0.004$) for carriage of the G variant of the rs1071583 SNP. Similarly, *FCN1* testing contributed independently to serological testing (ACPA positivity and RF as a continuous variable) OR = 2.581 (95% CI 1.289–5.166, $P = 0.007$) for carriage of the A variant of the rs2989727 SNP and OR = 2.307 (95% CI 1.144–4.655, $P = 0.020$) for carriage of the G variant of the rs1071583 SNP] (Figs 1 and 2). Interaction terms were non-significant and removed from the models.

Discussion

We describe for the first time an association between carriage of a SNP in the *FCN1* gene and the susceptibility for RA: the G/A variant of the rs2989727 SNP and the A/G variant of the rs1071583 SNP. Seven candidate SNPs in three different FCN genes were studied in a population of 338 RA and 595 controls, allowing selecting of two FCN1 SNPs. This population consisted of two subpopulations and the similar ORs demonstrated in both subpopulations increase the robustness of our findings. However, further confirmation of these associations is needed, especially in populations with different genetic backgrounds.

We also demonstrated that carriage of a *FCN1* SNP is independent of carriage of the HLA-SE, RF and ACPA positivity. Although the ORs were rather small to make the test useful as a diagnostic tool for clinical practice, the independency with the HLA-SE and the RA-associated serum markers triggered to explore a multi-parameter prediction model. Figures 1 and 2 show that *FCN1* determination has a significant additional value to HLA-SE or combined ACPA and RF testing. The figures suggest that the additional positive predictive value of carriage of the A variant of the rs2989727 SNP or the G variant of the rs1071583 SNP is limited but that the absence of the A variant of the

rs2989727 SNP or the G variant of the rs1071583 SNP has some additional value to exclude the diagnosis of RA.

The physiological meaning of our findings remains speculative, as little is known about the role of M-ficolins [3, 13]. One of the key characteristics of the PRMs in innate immunity lies in the first line defence, in particular, the specific recognition of invading micro-organisms and the discrimination between self and non-self. One can hypothesize that the DNA variations might result in an altered capacity to recognize self and non-self. Other contributing mechanisms may be related to the capacity of clearing apoptotic cells [3, 13].

To conclude, we describe two linked SNPs in the *FCN1* gene that are associated with the development of RA.

Rheumatology key messages

- Two linked SNPs in the *FCN1* gene are associated with RA.
- *FCN1* SNPs are independent from RF, anti-CCP and the HLA-SE.
- *FCNI* SNPs can be used in the prediction models for RA.

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