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 [9q343, 9]. 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-acetylgalactosamine and N-acetylgalactosaminylamine. M-ficolin binds GlcNAc bearing neoglycoproteins, N-acetylgalactosaminylamine and sialyl-N-acetylgalactosaminylamine, 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 (ACPA) 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 longstanding 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
**FCN1 polymorphisms in rheumatoid arthritis**

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

<table>
<thead>
<tr>
<th></th>
<th>FCN1</th>
<th>FCN2</th>
<th>FCN3</th>
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</thead>
<tbody>
<tr>
<td>rs2989727</td>
<td>9q34</td>
<td>(Prom2)rs7865453</td>
<td>1p35.3</td>
</tr>
<tr>
<td>Promoter A/G</td>
<td>rs1071583 A/G</td>
<td>Promoter A/C</td>
<td>IVS5 C/G</td>
</tr>
<tr>
<td>RA patients (population 1) Allele 1</td>
<td>0.33</td>
<td>0.91</td>
<td>0.002</td>
</tr>
<tr>
<td>Allele 2</td>
<td>0.67</td>
<td>0.90</td>
<td>0.003</td>
</tr>
<tr>
<td>Controls (population 1) Allele 1</td>
<td>0.42</td>
<td>0.85</td>
<td>0.043</td>
</tr>
<tr>
<td>Allele 2</td>
<td>0.58</td>
<td>0.78</td>
<td>0.176</td>
</tr>
<tr>
<td>RA patients (population 2) Allele 1</td>
<td>0.33</td>
<td>0.91</td>
<td>0.059</td>
</tr>
<tr>
<td>Allele 2</td>
<td>0.67</td>
<td>0.73</td>
<td>0.059</td>
</tr>
<tr>
<td>Controls (population 2) Allele 1</td>
<td>0.36</td>
<td>0.73</td>
<td>0.017</td>
</tr>
<tr>
<td>Allele 2</td>
<td>0.64</td>
<td>0.74</td>
<td>0.017</td>
</tr>
<tr>
<td>RA patients (pooled) Allele 1</td>
<td>0.39</td>
<td>0.33</td>
<td>0.32</td>
</tr>
<tr>
<td>Allele 2</td>
<td>0.64</td>
<td>0.74</td>
<td>0.12</td>
</tr>
<tr>
<td>Controls (pooled) Allele 1</td>
<td>0.39</td>
<td>0.33</td>
<td>0.32</td>
</tr>
<tr>
<td>Allele 2</td>
<td>0.64</td>
<td>0.74</td>
<td>0.12</td>
</tr>
<tr>
<td>Pooled OR</td>
<td>0.58</td>
<td>0.73</td>
<td>0.16</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(1.12–1.67)</td>
<td>(1.11–1.66)</td>
<td>(1.01–1.14)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.002</td>
<td>0.043</td>
<td>0.059</td>
</tr>
</tbody>
</table>

A C>T change at position 6424 and a G>T change in position 6359 (rs32469544).

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

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/ml, 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-LIATM 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, α = 0.007, this study had sufficient power (β = 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 (1.25–3.70), P = 0.002 overall] (Table 2). Analysis of agreement between SNPs suggested linkage between rs2989727 and rs1071583 in both populations (ρ = 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 0.970 (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.
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.
- FCN1 SNPs can be used in the prediction models for RA.

Acknowledgements

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References