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The CARD8 p.C10X polymorphism associates with the inflammatory activity in early rheumatoid arthritis

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ABSTRACT

Objectives: CARD8 and NLRP3 are constituents of the inflammasome, which regulates interleukin 1β production. We evaluated the influence of polymorphisms in CARD8 and NLRP3 on rheumatoid arthritis (RA) susceptibility and severity.

Methods: CARD8 p.C10X and NLRP3 p.Q705K genotypes were assessed in >500 controls and early RA patients from northern Sweden. The patients were monitored regularly during 2 years. The 28 joint disease activity score (DAS28) and its separate components were compared across genotypes.

Results: Patients with ≥1 variant allele in CARD8 (CARD8-X) had increased DAS28, tender joint count and erythrocyte sedimentation rate during the 2 year follow-up, despite receiving disease-modifying anti-rheumatic drugs to a higher extent. CARD8-X was significantly overrepresented among patients who received anti-TNF therapy during the first two years. CARD8 and NLRP3 genotypes did not influence radiological joint damage or associate with an increased susceptibility.

Conclusions: Carriage of CARD8-X associates with a worse disease course in early RA.
INTRODUCTION

The management of early rheumatoid arthritis (RA) has undergone substantial changes during the last decades, where the strategy of pharmaceutical therapy is now characterized by early aggressive treatment with an increasing number of available substances [1]. This improves the disease course but good predictors are still essential in order to individualize the therapy for each patient. The genetic basis of the disease, which is substantial in terms of susceptibility, may provide some prognostic information, in particular regarding HLA-DRB1 alleles. However, no genetic marker of disease progression has so far reached a wider clinical use for prognostic purposes.

Since the discovery in 2002, the interest in the cytoplasmic, Interleukin-1β (IL-1β) regulating protein complex called the inflammasome, has increased rapidly. The association of its genetic variants with auto inflammatory syndromes and deregulated IL-1β production is now well established [2], and strong indications of a substantial role in modulating adaptive immune responses are emerging [3, 4]. Assembly of the inflammasome proteins (i.e. NLRP3, ASC and CARD8), caused by e.g., urate crystals, adenosine triphosphate or Toll-like receptor ligation, enables the activation of caspase 1 and thereby cleavage of proIL-1β into bioactive IL-1β. Although the inflammasome clearly plays a major role in many conditions characterized by deregulated innate immunity, its possible role in diseases such as RA, where adaptive immune responses are more prominent, remains to be clarified. We recently found an epistatic association regarding p.Q705K (previously reported as Q703K) in NLRP3 (previously called CIAS1) and p.C10X in CARD8 (also known as TUCAN), and ACPA-positive RA and a more severe disease course [5]. Also, Fontalba et al found the CARD8 p.C10X polymorphism to influence nuclear factor κB (NF-κB) transcriptional activity and to be associated with RA severity [6]. There are several conceivable pathways in RA
pathophysiology where CARD8 could play a significant role; In addition to its involvement in the IL 1β-regulating inflammasome [7], CARD8 is an inhibitor of NF-κB [8, 9]. The activation and translocation of NF-κB to the nucleus induces gene expression of e.g., tumor necrosis factor alpha (TNF), pro-IL1β, and matrix metalloproteinases, i.e. prototypic mediators of inflammation and tissue degradation in RA [10]. Furthermore, several of the disease-modifying substances used in RA possess NF-κB inhibiting properties to various extents, and more profound NF-κB suppression significantly reduces disease severity in murine models of RA [10].

In a previous paper we described an epistatic relation between NLRP3 and CARD8 that increased susceptibility and predicted a worse disease course in early RA patients from south-eastern Sweden [5]. Also, the CARD8 p.C10X associated with RA severity in two groups of Spanish RA patients [6]. In this study the influence of NLRP3 p.Q705K and CARD8 p.C10X was evaluated on susceptibility and disease progression in a well-characterized cohort of early RA patients in northern Sweden.

PATIENTS AND METHODS

Subjects

A total of 560 patients (32% males and 68% females) with early RA (duration of symptoms <12 months), fulfilling at least four of the seven American College of Rheumatology (ACR) criteria for RA [11], were consecutively included in the study. The patients, of whom none were included in the previous study [5], were assessed clinically on regular basis (baseline, and after 6, 12, 18 and 24 months) and Disease Activity Score (DAS28) was calculated as previously presented [12]. The mean (± SD) age at inclusion was 54.4(± 14.1) years and
duration of symptoms was 6.6±2.7 months. During the study period 84.6 % (n=451/533) of the patients were treated with disease-modifying-anti-rheumatic drug(s) (DMARDs) and 46.7 % (n=252/540) of the patients received oral corticosteroids during the 2 years. Within the first 2 years 40 (7.3%) patients were prescribed anti-TNF therapy, and during the complete follow-up time 85 patients had been treated with TNF inhibitors. Anterior-posterior radiographs of the hands, wrists and feet, obtained at baseline (n=171) and after 2 years (n=146), were graded blind according to the Larsen score [13]. Rheumatoid factor (RF), analyzed by routine methods, was positive in 71.7% of the patients, and 67.2% had anti-CCP2 antibodies (Diastat kit from Axis-Shield Diagnostics, Dundee, UK; cut off value 5 units/mL) at inclusion. HLA-DRB1* 0404/0401 and PTPN22 genotyping were performed as previously described [12].

A total of 568 controls from the Medical Biobank of northern Sweden were randomly selected and matched for sex and age within a range of 5 years and the distribution of males and females were 26.3% and 73.7%, respectively [12]. The Regional Ethics Committee at the University Hospital at Umeå approved the study protocol and all participants gave their written informed consent.

Genotyping

DNA was extracted from EDTA-treated whole blood using a standard method. The p.C10X polymorphism in CARD8 (c.30T>A, rs2043211) and p.Q705K in CIAS1 (c.2107C>A, rs35829419) were analysed by TaqMan® assays (ID C__25648615_10 and C__11708080_1, respectively) according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA). Genotyping of both polymorphisms were successful in 543 patients.

Statistical analysis

The Chi-square test was used for testing categorical data between groups. ANOVA for repeated measurements was used to compare data collected at several time points. Odds Ratio
(OR) was calculated with 95% confidence interval (CI). All P-values refer to two-sided tests and a P-value \( \leq 0.05 \) was considered statistically significant. The calculations were performed using SPSS package (SPSS for Mac 13.0: SPSS Inc. Chicago, IL, USA). The occasional missing data points were considered to occur at random and, in such cases the last value was brought forward. Overall, this was used at 6.7% of the occasions, e.g. at 6 months in 11.7%, 8.2% at 12 months, 2.6% at 18 months, and 2.7% at 24 months. Each value was only brought forward once.

**RESULTS**

The genotype and allele distribution of the *CIAS1* and *CARD8* among both patients and controls were in agreement with the Hardy-Weinberg equilibrium. The distributions of genotypes or alleles between patients and controls revealed no significant differences (Table 1). Nor were there any differences in distribution in individuals stratified for presence or absence of anti-citrullinated protein antibodies, rheumatoid factor, shared epitope or carriage of the T variant of the protein tyrosine phosphatase non-receptor 22 (data not shown).
Table 1. Frequency distribution (% of total of each group) of the p.Q705K polymorphism of NLRP3 and the p.C10X polymorphism of CARD8 in patients with early RA and matched controls.

<table>
<thead>
<tr>
<th></th>
<th>CARD8-CC</th>
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<th>CARD8-CX</th>
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<th>CARD8-XX</th>
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<td></td>
<td>Controls</td>
<td>Patients</td>
<td>Controls</td>
<td>Patients</td>
<td>Controls</td>
<td>Patients</td>
</tr>
<tr>
<td>NLRP3-QQ</td>
<td>191 (33.6)</td>
<td>197 (36.3)</td>
<td>237 (41.7)</td>
<td>223 (41.1)</td>
<td>62 (10.9)</td>
<td>61 (11.2)</td>
</tr>
<tr>
<td>NLRP3-QK</td>
<td>24 (4.2)</td>
<td>21 (3.9)</td>
<td>37 (6.5)</td>
<td>36 (6.6)</td>
<td>15 (2.6)</td>
<td>4 (0.7)</td>
</tr>
<tr>
<td>NLRP3-KK</td>
<td>2 (0.4)</td>
<td>1 (0.2)</td>
<td>0</td>
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</table>

Patients carrying CARD8-X had significantly higher DAS28 (p=0.02), ESR (p=0.004) and tender joint count (p=0.02) than CARD8-CC patients during the 2 year follow-up as tested by ANOVA for repeated measurements (figure 1). The difference in swollen joint count was not statistically significant (p=0.284). There were no significant differences concerning Larsen score at baseline, the radiological progression during the 24 months or the score at 24 months after stratification for carriage of CARD8-X (data not shown). There were no associations between NLRP3 and disease activity measures, regardless of CARD8 genotype.

Overall, CARD8-X patients were treated with DMARDs and oral corticosteroids to a higher extent (detailed in supplementary table 1, available online only). However, significant difference was only reached at 12 months for both DMARDs (p= 0.04) and corticosteroids (p= 0.04) (89.0% vs. 81.2%, and 40.8% vs. 30.3%, respectively). Within the first 2 years of disease 40 patients were prescribed a TNF inhibitor, and 30 of them carried CARD8-X (X²= 4.14 p=0.042) e.g. 9.2% of those with CARD8-X and 4.6% without carriage of CARD8-X. During the whole follow-up time (mean ± SD) 6.2 ± 2.8 years, 58 of the 85 treated with a
TNF inhibitor had CARD8-X (68.2) compared with 270 of 462 (58.4%) without a TNF inhibitor (p= 0.09).

Fig 1. Disease activity measures over time in early RA patients in relation to CARD8 genotype. a) DAS28, b) ESR, c) Tender joint count (TJC), d) Swollen joint count (SWJ).

DISCUSSION

In this prospective cohort of patients with early RA, we find that the presence of at least one variant allele of CARD8 is associated with a more active disease during the two year follow-up. The differences in DAS28, ESR, and TJC occurred despite the fact that these patients were more aggressively treated with DMARDs and oral corticosteroids. Furthermore, initiation of TNF blocking therapy within 2 years was significantly more common among patients with CARD8-X, probably reflecting the rheumatologists’ response to increased disease activity measures. This is in line with our previous finding in another early RA cohort, where CARD8-X was strongly associated with the initiation of anti-TNF therapy within 5
years [5], although we do not confirm an increased risk of developing RA or an additional effect of \textit{NLRP3} p.Q705K on disease activity.

The A allele of \textit{CARD8} predicts a stop codon at position 10, prematurely terminating the protein, and this rather profound change could have consequences for the protein’s function in both inflammasome-mediated processes and NF-κB suppression. This may be the case even in the presence of alternate isoforms of the protein caused by transcription downstream of the polymorphism, as recently described [14]. No functional studies have been published regarding p.C10X and inflammasome-mediated IL-1β production, and it has been suggested that CARD8, as compared to NLRP3 and ASC, may have only limited influence on this process [7]. On the other hand, the inhibitory effect of CARD8 on NF-κB activity is better known [8, 9], and it has also been shown that this mechanism is influenced by p.C10X [6]. Thus, we hypothesize that the dysregulated proinflammatory immune response in early RA is enhanced via defect inhibition of NF-κB by CARD8-X.

Despite the significant differences regarding disease activity, structural joint damage did not appear to be influenced by p.C10X. One explanation to this discrepancy is that the correlation between disease activity and radiological progression is only partial, and that p.C10X might not play a major role in the latter. Other reasons could be that the more aggressive pharmacotherapy among CARD8-X patients attenuated differences in radiological progression, or that differences occur only after longer follow-up.

We conclude that carriage of CARD8-X predicts a more severe early disease course in RA, and that these patients are more likely to be in need of early TNF blocking therapy. It remains to be determined whether these effects are mediated via defect inhibition of either NF-κB, the inflammasome/IL-1β pathway, or both.
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Competing interests

None.

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