NLRP3 AND CARD8 POLYMORPHISMS INFLUENCE HIGHER DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

POLIMORFIZMI NLRP3 I CARD8 UTIČU NA POJAČANU AKTIVNOST BOLESTI U REUMATOIDNOM ARTRITISU

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Summary

Background: The activation of NLRP3-inflammasome may contribute to inflammatory processes in rheumatoid arthritis (RA). Functional polymorphisms in the genes coding for its components NLRP3 and CARD8 were associated with a proinflammatory phenotype. Our aim was to investigate the influence of these polymorphisms on RA susceptibility and disease activity at the time of diagnosis and after six months of treatment.

Methods: A group of 128 RA patients treated with methotrexate and 122 healthy controls were genotyped for NLRP3 rs35829419 (p. Q705K) and CARD8 rs2043211 (p. C10X) polymorphisms.

Results: RA susceptibility was not influenced by the investigated polymorphisms or their interaction. The investigated polymorphisms explained 8% of variability in DAS28 at the time of diagnosis. Carriers of NLRP3 rs35829419 or CARD8 rs2043211 polymorphisms had significantly higher DAS28 at the time of diagnosis (p=0.003; p=0.022; respectively). Polymorphic CARD8 rs2043211 TT genotype was also associated with higher DAS28 after six months of treatment (p=0.033).

Conclusions: Genetic variability of inflammasome components may contribute to higher disease activity at the time of diagnosis and after 6 months of methotrexate treatment in RA patients. Better understanding of the immunological mechanisms behind a more active course of RA may suggest novel treatment approaches in a subset of patients with a proinflammatory phenotype.

Keywords: polymorphism, rheumatoid arthritis, inflammasome, BIRC5, NLRP3

Kratak sadržaj

Uvod: Aktivacija NLRP3-inflamazoma može pojačati zapaljenske procese u reumatoidnom artritisu (RA). Funkcionalni polimorfizmi u genima koji kodiraju za njegove komponente NLRP3 i CARD8 dovedeni su u vezu sa proinflamatornim fenotipom. Naš cilj bio je da istražimo uticaj ovih polimorfizama na podložnost RA-u i aktivnost bolesti u vreme dijagnoze i posle šest meseci.

Metode: U grupi od 128 obolelih od RA-a koji su lečeni metotreksatom kao i kod 122 zdravih kontrolnih ispitanika urađena je genotipizacija za polimorfizme NLRP3 rs35829419 (p. Q705K) i CARD8 rs2043211 (p. C10X).

Rezultati: Na podložnost RA-u nisu uticali istraživani polimorfizmi niti njihova interakcija. Istraživani polimorfizmi objašnjavaju 8% varijabilnosti u DAS28 u vreme dijagnoze. Nosioci polimorfizama NLRP3 rs35829419 ili CARD8 rs2043211 imali su značajno viši DAS28 u vreme dijagnoze (p=0,003, odnosno p=0,022). Polimorfni genotip CARD8 rs2043211 TT takođe je bio povezan sa višim DAS28 posle šest meseci lečenja (p=0,033).

Zaključak: Genetska varijabilnost komponenti inflamazoma može dovesti do pojačane aktivnosti bolesti u vreme dijagnoze i posle šest meseci lečenja metotreksatom kod obolelih od RA-a. Bolje razumevanje imunoloških mehanizama koji stoje iza aktivnijeg toka RA-a moglo bi ukazati na nove pristupe u lečenju u podskupu pacijenata sa proinflamatornim fenotipom.

Ključne reči: polimorfizam, reumatoidni artritis, inflamazom, BIRC5, NLRP3

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Abbreviations: CARD8 – caspase recruitment domain-containing protein 8; DAS28 – Disease Activity Score examined in 28 joints; NLRP3 – NACHT, LRR and PYD domains-containing protein 3; RA – rheumatoid arthritis.

Introduction

The heterogeneity of response observed with rheumatoid arthritis (RA) therapeutics may suggest the existence of differing underlining molecular etiology, albeit presenting as the same disease (1). To achieve better treatment response, more knowledge of the underlying mechanisms and predictive understanding of the response are needed (2, 3). An underlying mechanism that may contribute to the RA pathology, joints inflammation and cartilage destruction *in vivo* is activation of the NACHT, LRR and PYD domains-containing protein 3 (NLRP3)-inflammasome (4). Its activation results in the caspase-1 mediated cleavage of pro-IL-1β into active IL-1β, a proinflammatory cytokine (5). The nonsynonymous gain-of-function polymorphism *NLRP3* rs35829419 (p. Q705K) leads to an overactive NLRP3 inflammasome (6), while caspase recruitment domain-containing protein 8 (*CARD8*) rs2043211 (p. C10X) polymorphism encodes truncated protein, which does not inhibit the NF-κB stimulation of inflammatory response (7). It was suggested that interaction between *NLRP3* rs35829419 and *CARD8* rs2043211 increases RA susceptibility (8) and that *CARD8* rs2043211 increases early-stage and long-term disease activity (7), but the results are inconclusive (9).

In this study, we investigated the association of *NLRP3* and *CARD8* polymorphisms with RA susceptibility and disease activity at the time of diagnosis and after six months of methotrexate (MTX) treatment.

Patients and Methods

We performed a retrospective study in RA patients who were older than 18 years, of Central Caucasian origin and initially treated with MTX monotherapy for at least six months at the Department of Rheumatology, University Medical Centre, Ljubljana, Slovenia. Control group included Slovenian healthy blood donors between 18 and 65 years.

The study was approved by the Republic of Slovenia National Medical Ethics Committee and was carried out according to the Declaration of Helsinki. All patients gave their written informed consent to participate in the study.

Demographic and clinical data on RA patients were obtained from the patients' medical records: data on disease duration before beginning of treatment and Disease Activity Score examined in 28 joints (DAS28) assessed at the time of diagnosis (baseline) (10) and after 6 months of treatment.

Genomic DNA was isolated from peripheral blood leukocytes and genotyped for *NLRP3* rs35829419 and *CARD8* rs2043211 using a fluorescence-based competitive allele-specific real time polymerase chain reaction (KASPar assay) according to the manufacturer's instructions (KBiosciences, Herts, UK).

Median was used to present the central tendency with the 25th to 75th percentile range as a measure of variability. Chi-square test was used to assess Hardy-Weinberg equilibrium (HWE) and to compare proportions between groups. Logistic regression was used to assess the influence of interaction between polymorphisms on RA susceptibility. Mann-Whitney U or Kruskal-Wallis H test were used to estimate the differences in DAS28 between genotypes under an additive (*NLRP3*, *CARD8*) or recessive (*CARD8*) genetic model. Because baseline DAS28 was normally distributed, we used a multivariate generalized linear model to analyse the influence of interaction between polymorphisms on baseline DAS28. Multivariate linear regression was performed to test for the association between polymorphisms and DAS28 with adjustment for clinical factors (gender, age and disease duration before beginning of treatment). Adjusted R square was used to evaluate the contribution of both polymorphisms to the variability in baseline DAS28 among RA patients. All significance tests were two-tailed and conducted at the 0.05 level of significance. Analyses were performed using R (11) and SPSS.

Results

Patient characteristics

In total, 128 RA patients (102 females and 26 males) with a median (25–75% range) age of 56.9 (45.5–65.3) years and 122 controls (85 females and 43 males) that were 53 (47–60) years old were included in the study. Gender and age did not differ significantly between RA patients and controls (p=0.068, p=0.065, respectively). In RA patients disease duration before beginning of treatment was 2.8 (0–15) weeks. Baseline DAS28 was 5.1 (4.3–5.9) and DAS28 after 6 months of treatment was 3.1 (2.2–4.6).

### Association of NLRP3 and CARD8 on RA susceptibility

All the patients and controls were genotyped for *NLRP3* rs35829419 and *CARD8* rs2043211 polymorphisms. In total, 114 (89%) RA patients were homozygous for wild type *NLRP3* CC genotype, while 14 (11%) carried one polymorphic allele. Among the controls, 105 (86%) were carriers of *NLRP3* CC genotype while 14 (11%) carried one polymorphic allele.

Altogether 49 (38.3%) RA patients were homozygotes for the wild type *CARD8* A allele, 64 (50%) were heterozygotes and 15 (11.7%) carried two polymorphic T alleles. Among the controls, 54 (44.3%) were carriers of *CARD8* AA genotype, 57 (46.7%) were heterozygotes and 11 (9%) were homozygotes for polymorphic T allele. Allele frequencies for *NLRP3* as well as for *CARD8* were in Hardy-Weinberg equilibrium (p=0.660, p=0.272, respectively).

Table I The influence of *NLRP3* and *CARD8* polymorphism on DAS28 at the time of diagnosis and after 6 months of treatment.

Genotype		N (%)	DAS28 at diagnosis		DAS28 after 6 months of treatment[a]	
			Median (range)	p-value	Median (range)	p-value
NLRP3 rs35829419	CC CA AA	114 (89.1) 14 (10.9) 0 (0)	5.00 (4.27–5.83) 6.00 (5.26–7.15) NA	0.003[b]	3.00 (2.19–4.60) 3.10 (2.35–4.95) NA	0.666[b]
CARD8 rs2043211	AA AT TT	49 (38.3) 64 (50) 15 (11.7)	5.00 (4.30–5.95) 5.05 (4.13–5.71) 5.80 (5.10–6.40)	0.049 [b] 0.022 [c]	3.00 (2.00-4.30) 2.60 (2.12–4.35) 4.25 (2.50–6.10)	0.100[b] 0.033[c]

[a] – information on DAS28 is missing for 6 patients; [b] – additive genetic model; [c] – recessive genetic model; NA – not applicable.
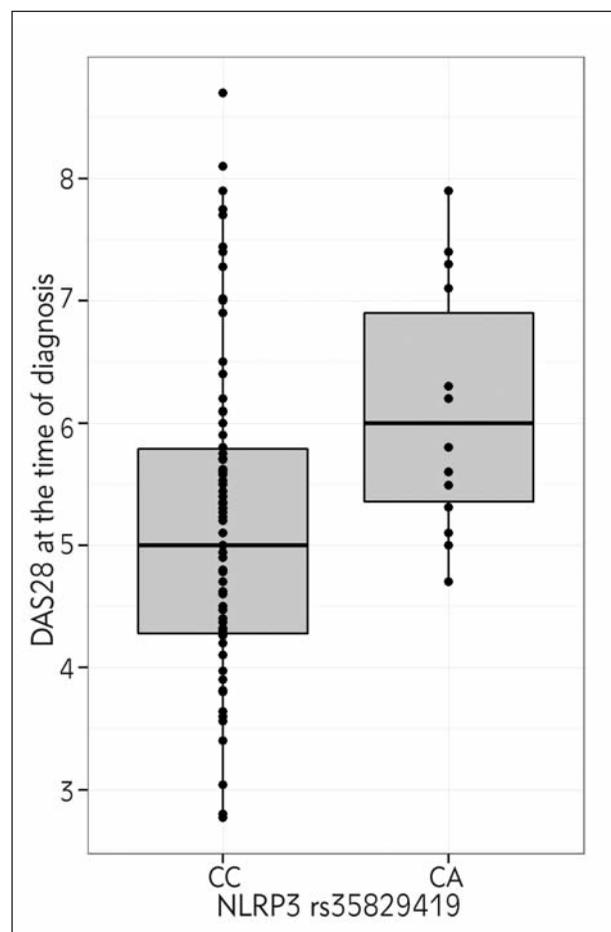
Figure 1 Association between NLRP3 rs35829419 and DAS28 at the time of diagnosis.

The allele frequencies of *NLRP3* and *CARD8* polymorphism did not differ significantly between RA patients and controls (p=0.472, p=0.571, respectively). The interaction between these polymorphisms was also not significantly associated with RA susceptibility (p=0.936; OR=0.910; 95%CI: 0.093–8.40).

Association of NLRP3 and CARD8 and DAS28

Among RA patients, no homozygotes for polymorphic *NLRP3* AA genotype were found, however, carriers of CA genotype had significantly higher baseline DAS28 than the carriers of wild type genotype CC (p=0.003) (*Table I, Figure 1*). This association remained highly significant after adjustment for clinical factors (p=0.007; B=0.906; 95% CI: 0.248–1.565). Homozygotes for polymorphic *CARD8* T allele had marginally higher baseline DAS28 in the additive model but highly significant in the recessive model (p=0.049; p=0.022, respectively) (*Table I, Figure 2*). The association in the recessive model remained significant after adjustment for clinical factors (p=0.037; B=0.690; 95%CI: 0.042–1.338). *NLRP3* and *CARD8* (recessive model) explained altogether 8% of the variability in baseline DAS28 (p=0.006; B=0.925; 95%CI: 0.274–1.577 and p=0.029; B=0.704; 95%CI: 0.072–1.337).

After 6 months of MTX treatment, only *CARD8* showed association with higher DAS28 under the recessive model (p=0.033) (*Table I, Figure 2*). This association remained significant after adjustment for clinical factors (p=0.026; B=0.986; 95%CI=0.120–1.852).
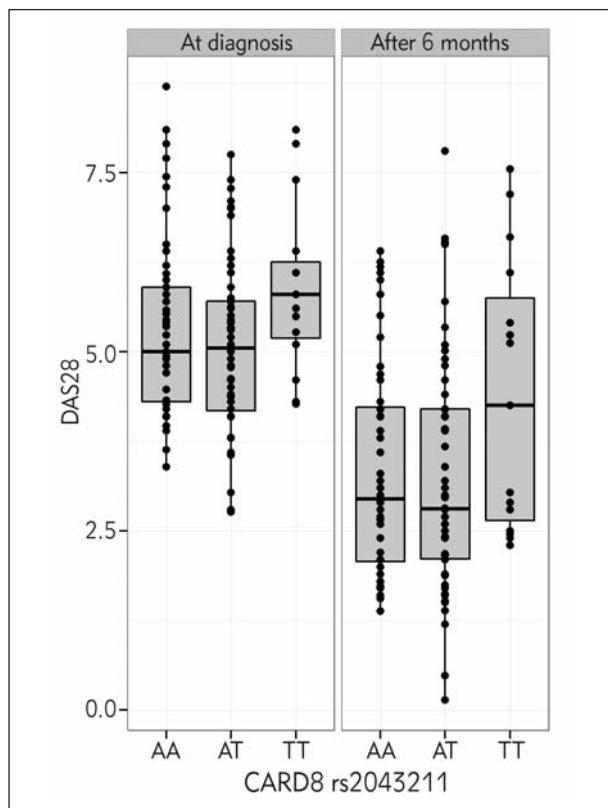
Figure 2 Association between *CARD8* rs2043211 and DAS28 at the time of diagnosis and after 6 months of treatment.

Discussion

We report for the first time on the influence of *CARD8* rs2043211 and *NLRP3* rs35829419 polymorphisms on a more active course of disease at the time of diagnosis in RA patients. *CARD8* rs2043211 remained associated with DAS28 also after 6 months of RA treatment.

In agreement with previous reports, we did not observe any association between *NLRP3* rs35829419 or *CARD8* rs2043211 and RA susceptibility (7, 9, 12, 13). Moreover, we did not observe any influence of the interaction between both investigated polymorphisms on RA susceptibility, contrary to a previous report (8). It was suggested that 50% to 60% of RA susceptibility can be explained by genetic variation. Major genetic risk was observed within HLA-DRB1 genes, and the second largest was observed within PTPN22, with the effect size of ~1.8 (14), while smaller effects were observed for other genetic variants. In our study, we can assure with 80% of probability that the detected risk factor was greater than 1.7. This is probably too high to detect the genetic variants that influence RA susceptibility, so we could report false negative results.

In our study, heterozygotes for *NLRP3* rs35829419 polymorphism had significantly higher baseline DAS28. This could be due to the *NLRP3* gain-of-function polymorphism, which leads to an overactive NLRP3 inflammasome (6), and probably to increased activation of IL-1β (4). Homozygosity for the polymorphic *CARD8* rs2043211 T allele strongly influenced DAS28 at the time of diagnosis and after 6 months of treatment. Our results are in agreement with the study that reported the influence of *CARD8* rs2043211 on more severe disease course in long-lasting RA (7). Truncated CARD8 resulting from *CARD8* rs2043211 may reduce its control over the NF-κB signalling pathway and thus lead to a more active transcriptional factor and an amplification of the inflammatory processes (7). The NF-κB is essential for the expression of both inflammatory cytokines and tissue destructive enzymes in RA (15, 16). The contribution of NLRP3 and CARD8 to active RA was also shown in the gene expression study. Namely, NLRP3 was upregulated and CARD8 was downregulated in the PBMCs of RA patients prior to receiving infliximab therapy in comparison to healthy controls. However, *NLRP3* rs35829419 or *CARD8* rs2043211 polymorphisms were not associated with baseline expression levels (9).

Our study had some limitations as retrospective data collection could have introduced biases and additional data on the presence of erosions at the beginning of disease and after 6 months of treatment were not available but would be very informative. Although matched cased control analysis would be more appropriate for the analysis of susceptibility to RA, our controls were selected so that they did not significantly differ from cases in gender and age. Our study was also not biased by genetic heterogeneity since all the patients were recruited in a geographic area with an ethnically homogeneous population (17).

Conclusion

This study offers further evidence about the role of inflammasome in RA. Future investigations on bigger cohorts are needed to confirm our results. Investigated polymorphisms may influence more active inflammation and more active course of RA at the time of diagnosis and in early treatment, which may be due to increased activation of NLRP3-inflammasome. Genetic profile of the patient may reveal some of the immunological mechanisms and may provide a clue towards more effective treatment schemes.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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