CONCISE REPORT

Polymorphism at position +896 of the toll-like receptor 4 gene interferes with rapid response to treatment in rheumatoid arthritis

K Kuuliala, A Orpana, M Leirisalo-Repo, H Kautiainen, M Hurme, P Hannonen, M Korpela, T Möttönen, L Paimela, K Puolakka, A Karjalainen, H Repo

Ann Rheum Dis 2006;**65**:1241–1243. doi: 10.1136/ard.2006.055137

The aim of this study was to determine whether the +896 A→G substitution of the Toll-like receptor 4 (*TLR4*) gene, causing the Asp299→Gly change in the extracellular domain of *TLR4*, influences treatment response in recent-onset rheumatoid arthritis. 169 patients with rheumatoid arthritis were genotyped from the Finnish Rheumatoid Arthritis Combination Therapy trial, in which they were treated either with only one disease-modifying antirheumatic drug (DMARD) with or without prednisolone (single group), or with three DMARDs and prednisolone (combination group). Patients homozygotic for the wild-type +896A allele were compared with those having the polymorphic G allele in terms of early clinical response (at 6 months) by the 28-joint Disease Activity Score (DAS28). 1 of 20 (5%; (95% confidence interval (CI) 1 to 5)) patients of the single group with *TLR4*+896AG or GG and 29 of 67 (43%; (95% CI 31 to 56)) patients with AA were in remission ($p=0.001$). DAS28 of the single group with *TLR4*+896AG or GG was higher than with AA ($p=0.019$). In the combination group, remission rates and DAS28 values were comparable between the genotypes. The polymorphic *TLR4*+896G allele may impair treatment response to single DMARD treatment in recent-onset rheumatoid arthritis.

Rheumatoid arthritis is a chronic, systemic inflammatory disease that targets synovial joints and may lead to preterm work disability.¹ Five-year results of the Finnish Rheumatoid Arthritis Combination Therapy (FIN-RACo) trial suggested that to maintain work ability, patients with early rheumatoid arthritis should reach remission during the first 6 months of disease-modifying antirheumatic drug (DMARD) treatment.² Markers that predict such response to DMARD treatments are needed.

Immunopathogenesis of rheumatoid arthritis may include innate immune system activation by an unknown exogenous or endogenous antigen, resulting in phagocyte activation and production of pro-inflammatory cytokines, and, subsequently, recruitment of adaptive immunity.³ For instance, Toll-like receptor 4 (*TLR4*) and *CD14* are components of a plasma membrane receptor complex mediating phagocyte activation by bacterial lipopolysaccharide⁴ and endogenous ligands such as heat shock proteins, fibronectin and hyaluronate fragments.⁵ Polymorphic diversity in the receptor complex components may modulate the magnitude of inflammatory response. *TLR4*+896A→G has been associated with lipopolysaccharide hyporesponsiveness⁶ and various inflammatory conditions,⁷ and *CD14* -159C→T with altered *CD14* expression with aberrant regulation of inflammation and increased risk of cardiovascular events.⁴

We hypothesised that the *TLR4*+896 and *CD14* -159 polymorphisms, which probably have a role in the inflammatory response, may affect the clinical response to inflammation-alleviating DMARD treatment in patients with rheumatoid arthritis. The polymorphisms were determined in patients with early rheumatoid arthritis taking part in the FIN-RACo trial, treated with a single DMARD or a combination of DMARDs.⁸

METHODS

Patients

In all, 195 native Finns with recent-onset rheumatoid arthritis (disease duration <2 years) were randomised in the FIN-RACo trial to receive either single (initially sulfasalazine with or without prednisolone) or combination (sulfasalazine, methotrexate, hydroxychloroquine and prednisolone) treatment.⁸ Intra-articular glucocorticoid injections were allowed according to the attending physician's judgement. For our study, blood samples were available from 169 patients (87 single and 82 combination). Clinical assessment data at baseline and at 3 and 6 months were used for calculating 28-joint Disease Activity Score (DAS28).⁹

Nucleic acid analysis

DNA for *TLR4*+896 determination was isolated by a salting out procedure and the polymorphism was analysed using a 5'-nuclease assay. DNA for *CD14* -159 determination was isolated using QIAamp DNA blood midi kit (Qiagen, Hilden, Germany), and the polymorphism was analysed by cycle minisequencing.¹⁰

Statistical analysis

Significance between groups was evaluated by t test, permutation test (Monte Carlo p value) or χ^2 test. Repeated measures were analysed using generalising estimating equation models with exchangeable correlation structure. Multivariate logistic regression with bootstrap estimate of variance was used to estimate odds ratios (ORs) and their 95% confidence intervals (CIs).

RESULTS

Baseline demographic (age, sex, disease duration, number of patients positive for rheumatoid factor and *CD14* -159T allele frequency) and clinical (erythrocyte sedimentation rate, numbers of swollen and tender joints, pain, patient's and physician's assessment by Visual Analogue Scale and DAS28) characteristics were comparable in the *TLR4*+896AA ($n=132$) and AG/GG ($n=37$) groups (data not shown).

Abbreviations: DAS28, 28-joint Disease Activity Score; DMARD, disease-modifying antirheumatic drug; FIN-RACo, Finnish Rheumatoid Arthritis Combination Therapy; *TLR4*, toll-like receptor 4

Figure 1 Proportion with 95% CI of patients in 28-joint Disease Activity Score (DAS28) remission after treatment for 6 months. Combi, combination treatment; Single, single treatment.

The corresponding characteristics (with *TLR4* +896G allele frequency) according to the *CD14* -159 genotype were also comparable (data not shown). The effect of *TLR4* +896G allele dose could not be evaluated, as only one of the participants was a GG homozygote.

In logistic regression analysis, combination treatment, *TLR4* +896AA versus AG/GG genotype and DAS28 at baseline were the only important predictors of remission at 6 months (table 1). DAS28 remission occurred less often among patients with *TLR4* +896AG or GG than among those with AA, and this difference was significant in the single group ($p = 0.001$; fig 1). Patients of the single group achieved DAS28 remission 8.7 times (95% CI 1.3 to 59.6) and patients of the combination group 1.5 times (95% CI 0.8 to 2.7) more often if they were *TLR4* +896AA homozygotes.

Table 1 Logistic regression analysis for the odds to reach DAS28 remission ($DAS28 < 2.6$) after 6 months of treatment

Variable at the beginning of the study	OR (95% CI)*	p Value
Combination strategy†	2.52 (1.18 to 5.38)	0.017
Age (years)	0.98 (0.94 to 1.02)	0.32
Female sex	0.53 (0.24 to 1.20)	0.13
Rheumatoid factor present	1.19 (0.51 to 2.77)	0.69
<i>CD14</i> -159CT or TT	0.82 (0.38 to 1.80)	0.63
<i>TLR4</i> +896AG or GG	0.21 (0.07 to 0.64)	0.006
DAS28	0.52 (0.33 to 0.82)	0.005
Disease duration (months)	1.01 (0.94 to 1.08)	0.78

DAS28, 28-joint Disease Activity Score; TLR, Toll-like receptor.

*Bootstrap estimate of variance.

†Reference single strategy.

The effect of the *CD14* -159 and *TLR4* +896 genotype combination was not significant ($OR = 0.15$, $p = 0.90$), and was therefore excluded from the model.

Treatment response, evaluated by decrease in DAS28 values (table 2), was significantly diminished in patients having the *TLR4* +896G allele in the single group ($p = 0.019$), but not in the combination group ($p = 0.31$), and the number of intra-articular corticosteroid injections per patient was higher among those with the G allele in the single group (median (interquartile range) 8 (4, 12) v 5 (1, 11); $p = 0.047$), but not in the combination group (3 (0, 10) v 3 (0, 6); $p = 0.10$).

No significant differences were found in DAS28 remission rate (table 1) and treatment response (data not shown) between the *CD14* -159 genotypes, and the effect of *CD14* -159 and *TLR4* +896 genotype combination on the results was not significant ($OR = 0.15$, $p = 0.90$).

DISCUSSION

Of the patients with early rheumatoid arthritis treated with a single DMARD (starting with sulfasalazine) for 6 months, patients with the *TLR4* +896AG or GG genotype achieved DAS28 remission less often and required more intra-articular corticosteroid injections than patients with the wild-type AA genotype. This observation was not evident in patients treated simultaneously with three DMARDs and prednisolone. These data suggest the novel idea that the *TLR4* +896 minor allele is associated with poor response to a single DMARD treatment in early rheumatoid arthritis. According to the current recommendations, treatment of early rheumatoid arthritis is started with one or more of the traditional DMARDs, because they are inexpensive and not necessarily inferior to recently introduced biological response modifiers.¹¹ However, to maintain the patients' working capacity optimally, treatment should result in rapid remission, preferably in 6 months.² Results of our study raise the question of

Table 2 Effects* of the *TLR4* +896 genotype on 28-joint Disease Activity Score values at baseline, and at 3 and 6 months of treatment

	Genotype		p Value
	AA mean (95% CI)	AG or GG mean (95% CI)	
Single treatment			
Baseline	5.60 (5.35 to 5.84)	5.92 (5.43 to 6.41)	0.019†
3 months	3.48 (3.14 to 3.83)	4.16 (3.44 to 4.87)	0.25
6 months	2.98 (2.66 to 3.30)	3.89 (3.41 to 4.38)	0.096
Combination treatment			
Baseline	5.50 (5.27 to 5.72)	5.47 (5.11 to 5.83)	0.002
3 months	2.83 (2.50 to 3.17)	3.20 (2.44 to 3.95)	0.31†
6 months	2.50 (2.16 to 2.84)	2.95 (2.26 to 3.65)	0.90

*Generalised estimating equation with exchangeable correlation structure.

†Effect of genotype overtime.

whether the initial treatment of patients with early rheumatoid arthritis having the *TLR4* +896 minor allele should comprise a combination of DMARDs.

The biological mechanisms underlying this finding may include insufficient response to exogenous or endogenous ligands by *TLR4* with 299Gly (caused by *TLR4* +896A→G), an aberration that a single DMARD may not be able to overcome. However, this study can only be regarded as hypothesis-generating, because the sample size was quite small and so far there are no publications supporting the finding, and because the exact role of 299Gly in *TLR4* expression or signalling is still under investigation.⁶

Previous studies have looked for genetic¹² and biochemical¹³ markers that reliably predict the outcome of treatment for rheumatoid arthritis. If confirmed in larger studies, the *TLR4* +896 polymorphism may become a part of such a profile. Consequently, the chances of achieving early remission and maintaining working capacity would be maximised, whereas the chances of high costs and loss of productivity would be minimised.

In conclusion, the results of this preliminary study raise the possibility that the polymorphic *TLR4* +896G allele may interfere with treatment response to a single DMARD in early rheumatoid arthritis. If confirmed in other studies, this polymorphism may provide a predictive marker suitable for identifying patients requiring early combination DMARD treatment.

ACKNOWLEDGEMENTS

We thank the other members of the FIN-RACo trial group: Jari Ahonen, Harri Blåfield, Sinikka Forsberg, Claes Friman, Per Franzen, Marianne Gripenberg-Gahmberg, Markku Hakala, Mikko Hakola, Tapani Helve, Kirsti Ilva, Heikki Julkunen, Pentti Järvinen, Oili Kaipainen-Seppänen, Kalevi Koota, Juhani Koski, Riitta Luosujärvi, Reijo Luukkainen, Martti Nissilä, Heikki Piirainen, Ilpo Pälvimäki, Kaisa Vuori and Urpo Yli-Kerttula, and also Sinikka Repo-Koskinen, Eija Späre and Eija Hämäläinen for skilful technical help.

Authors' affiliations

K Kuuliala, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland

A Orpana, Department of Clinical Chemistry and Medical Genetics, Laboratory Diagnostics, Helsinki University Central Hospital, Helsinki

M Leirisalo-Repo, Department of Medicine, Division of Rheumatology, Helsinki University Central Hospital

H Kautiainen, Rheumatism Foundation Hospital, Heinola, Finland

M Hurme, Department of Microbiology and Immunology, University of Tampere Medical School, and Tampere University Hospital, Tampere, Finland

P Hannonen, Department of Medicine, Jyväskylä Central Hospital, Jyväskylä, Finland

T Möttönen, Department of Medicine, Division of Rheumatology, Turku University Central Hospital, Paimio Hospital, Paimio, Finland

L Paimela, Orton Hospital, Invalid Foundation, Helsinki

K Puolakka, Department of Medicine, Lappeenranta Central Hospital, Lappeenranta, Finland

A Karjalainen, Department of Internal Medicine, Oulu University Hospital, Oulu, Finland

H Repo, Department of Medicine, Division of Infectious Diseases, Helsinki University Central Hospital

Funding: This study was supported by grants from Finska Läkaresällskapet to Krista Kuuliala and Helsinki University Hospital Research funds to HR.

Competing interests: None.

Ethics approval: The ethics committees in all 18 participating hospitals approved the study protocol.

Correspondence to: K Kuuliala, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, PO Box 21 (Haartmaninkatu 3), FIN-00014 Helsinki, Finland; krista.anttonen@helsinki.fi

Accepted 28 March 2006

Published Online First 10 April 2006

REFERENCES

- 1 **de Croon EM**, Sluiter JK, Nijssen TF, Dijkmans BA, Lankhorst GJ, Frings-Dresen MH. Predictive factors of work disability in rheumatoid arthritis: a systematic literature review. *Ann Rheum Dis* 2004;**63**:1362–7.
- 2 **Puolakka K**, Kautiainen H, Möttönen T, Hannonen P, Korpela M, Hakala M, *et al*. Early suppression of disease activity is essential for maintenance of work capacity in patients with recent-onset rheumatoid arthritis: five-year experience from the FIN-RACo trial. *Arthritis Rheum* 2005;**52**:36–41.
- 3 **Arend WP**. The innate immune system in rheumatoid arthritis. *Arthritis Rheum* 2001;**44**:2224–34.
- 4 **Arroyo-Espiguero R**, Avanzas P, Jeffery S, Kaski JC. CD14 and toll-like receptor 4: a link between infection and acute coronary events? *Heart* 2004;**90**:983–8.
- 5 **Radstake TRDJ**, Roelofs MF, Jenniskens YM, Oppers-Walgreen B, van Riel PL, Barrera P, *et al*. Expression of Toll-like receptors 2 and 4 in rheumatoid synovial tissue and regulation by proinflammatory cytokines interleukin-12 and interleukin-18 via interferon- γ . *Arthritis Rheum* 2004;**50**:3856–65.
- 6 **Arbour NC**, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, *et al*. *TLR4* mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000;**25**:187–91.
- 7 **Schröder NW**, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. *Lancet Infect Dis* 2005;**5**:156–63.
- 8 **Möttönen T**, Hannonen P, Leirisalo-Repo M, Nissilä M, Kautiainen H, Korpela M, *et al*. Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomised trial. *Lancet* 1999;**353**:1568–73.
- 9 **Prevoo ML**, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;**38**:44–8.
- 10 **Repo H**, Anttonen K, Kilpinen SK, Palotie A, Salven P, Orpana A, *et al*. *CD14* and *TNF α* promoter polymorphisms in patients with acute arthritis. *Scand J Rheumatol* 2002;**31**:355–61.
- 11 **Bingham SJ**, Buch MH, Tennant A, Emery P. The impact of escalating conventional therapy in rheumatoid arthritis patients referred for anti-tumour necrosis factor- α therapy. *Rheumatology* 2004;**43**:364–8.
- 12 **Criswell LA**, Lum RF, Turner KN, Woehl B, Zhu Y, Wang J, *et al*. The influence of genetic variation in the *HLA-DRB1* and *LTA-TNF* regions on the response to treatment of early rheumatoid arthritis with methotrexate or etanercept. *Arthritis Rheum* 2004;**50**:2750–6.
- 13 **Kuuliala A**, Leirisalo-Repo M, Möttönen T, Hannonen P, Nissilä M, Kautiainen H, *et al*. Serum soluble interleukin-2 receptor predicts early remission in patients with recent-onset rheumatoid arthritis treated with a single disease-modifying antirheumatic drug. *Clin Exp Rheumatol* 2005;**23**:243–6.