



## A functional polymorphism of TIR-domain-containing adaptor protein is not associated with axial spondyloarthritis

Tineke Cantaert, Millicent A Stone, Mariette Terborg, Rebecca Mogg, Niek de Vries, Anthony G Wilson, Paul-Peter Tak and Dominique Baeten

*Ann Rheum Dis* published online 23 Dec 2007;  
doi:10.1136/ard.2007.082784

---

Updated information and services can be found at:  
<http://ard.bmj.com/cgi/content/abstract/ard.2007.082784v2>

---

*These include:*

### Rapid responses

You can respond to this article at:  
<http://ard.bmj.com/cgi/eletter-submit/ard.2007.082784v2>

### Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

---

### Notes

---

**Online First** contains unedited articles in manuscript form that have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Online First articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Online First articles must include the digital object identifier (DOIs) and date of initial publication.

---

To order reprints of this article go to:  
<http://journals.bmj.com/cgi/reprintform>

To subscribe to *Annals of the Rheumatic Diseases* go to:  
<http://journals.bmj.com/subscriptions/>

## **A functional polymorphism of TIR-domain-containing adaptor protein is not associated with axial spondyloarthritis**

Tineke Cantaert<sup>1</sup>, Millicent A. Stone<sup>2,3</sup>, Mariëtte ter Borg<sup>1</sup>, Rebecca Mogg<sup>2</sup>, Niek De Vries<sup>1</sup>, Anthony G. Wilson<sup>4</sup>, Paul P. Tak<sup>1</sup>, Dominique Baeten<sup>1</sup>

<sup>1</sup>Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, The Netherlands. <sup>2</sup>Royal National Hospital Rheumatic Diseases, University of Bath, UK. <sup>3</sup>University of Toronto <sup>4</sup>School of Medicine & Biomedical Sciences, Royal Hallamshire Hospital, University of Sheffield, UK.

Corresponding author: Dominique Baeten, MD, PhD, Clinical Immunology and Rheumatology, F4-218, Academic Medical Center/University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. [D.L.Baeten@amc.uva.nl](mailto:D.L.Baeten@amc.uva.nl)

Keywords: spondyloarthritis, TLR signalling, polymorphism

## **Abstract**

**Objective:** A genetic variant of the TLR2/4 adaptor protein TIRAP (SNPC539T) was identified in a UK and in several African populations. The heterozygous genotype of this SNP has been associated with protection from severe infections. This allele results in an attenuated response to bacterial pathogens. As an exaggerated innate immune response to pathogens has been implicated in spondyloarthritis (SpA) pathogenesis, we analyzed if the heterozygous C/T genotype was underrepresented in axial SpA compared to healthy controls.

**Methods:** 204 axial SpA patients and 175 population-matched controls were included. SNP C539T was determined with a sequence specific PCR and direct sequencing.

**Results:** The frequency of the haplotypes was similar in cases and controls (87% for C and 13% for T in both groups). The C/T genotype, which attenuates TLR signalling, was not underrepresented in cases versus controls (19% in controls versus 24% in cases,  $p=0.44$ ). The T/T genotype, was slightly lower in cases than in controls, although this was not significant (3.4% in controls versus 1% in cases,  $p=0.15$ ). Within the cases, there were no differences in disease phenotype or activity between patients with the C/C or C/T genotype.

**Conclusion:** This study did not show significant associations of SNP S180L of the TLR2/4 adaptor protein TIRAP with axial SpA.

## Introduction

Spondyloarthritis (SpA) is a prevalent form of chronic inflammatory arthritis. Whereas the exact pathophysiology remains unknown, the prevention of SpA-like diseases in HLA-B27 transgenic rats when kept in germ-free conditions (1) and the induction of the reactive arthritis subtype of SpA by gastrointestinal infections suggest an important role for bacterial triggers. The absence of viable microbes in the joint and the inefficacy of antibiotic treatment suggest that an abnormal immune response to the bacteria rather than the infection itself is important (2). Extensive histopathological studies (3) support the notion that the innate rather than the acquired arm of the immune system may be involved in this exaggerated inflammatory response.

Toll-like receptors (TLR) are pattern recognition receptors of the innate immune system that recognise a wide variety of microbial molecules. Binding of these ligands leads to a complex signalling cascade resulting in the transcription of proinflammatory genes. The selective increase of TLR2 and TLR4 in peripheral SpA (4), the involvement of TLR4 in experimental Chlamydia-induced arthritis (5), and the potential association of TLR4 polymorphisms with ankylosing spondylitis (AS) (6, 7) suggest that this pathway may also be involved in SpA. Of interest for chronic inflammation, TLRs are not only stimulated by microbial ligands but also by self motifs such as heat shock proteins and hyaluronic acid, thereby leading to sterile inflammation (8). A failure to tightly control the intensity or duration of TLR signalling may therefore contribute to the severity and persistence of chronic inflammatory diseases such as SpA (9, 10).

One key molecule involved in the regulation of TLR2/4 signalling is MyD88-adaptor like (Mal, encoded by the gene TIRAP). Recently, heterozygous carriage of a genetic variant of Mal (SNP C539T, Serine180Leucine) was shown to be associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis in different UK and African populations (11). This SNP leads to an amino acid substitution (Serine to Leucine) in Mal which attenuates TLR2/4 signalling and thereby protects against excessive inflammation. As the control of excessive TLR-mediated inflammatory responses could also be important in SpA, we investigated if Mal the 539T allele was underrepresented in SpA compared to healthy controls.

## Materials and methods

### *Patients*

The study included 204 AS patients with proven sacroiliitis on classical X-ray or MRI and 175 matched controls from the UK (12). In the patient cohort, the median age was 51 (40-60) years and the median disease duration 19 (11-29) years. 76.5% were male and 91% were HLA-B27 positive. The median disease activity as assessed by the BASDAI score was 4.8 (3.0-6.8). All patients and controls were white Caucasian subjects who gave written informed consent to participate in the study as approved by the local Ethics Committee.

### *Sequence-specific primer PCR*

DNA was extracted from peripheral blood cells according to standard procedures. 50-100 ng DNA was amplified using the T3-thermocycler (Biometra, Goettingen, Germany; 34 cycles, hybridisation temperature of 58°C) using sequence-specific primers were designed with the 3' end complementary to SNP C539T in order to detect the C and T variants (forward-C primer = CACCATCCCCCTGCTGTC; forward-T primer = CACCATCCCCCTGCTGTT) and a reverse primer = GATACAAACCCCGACAGCC. PCR products were analysed on a 1% agarose gel stained with ethidium bromide.

### *Direct Sequencing*

All samples containing the rare allele were confirmed by direct sequencing. Genomic DNA was amplified using forward primer 5'-TATAGTGTCCGAGCTGTGCC-3' and reverse primer 5'-GATACAAACCCCGACAGCC-3'. Sequencing reaction was performed with 3-10 ng PCR product according to the manufacturer's instructions (Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Foster City, California, USA) and analysed on the 3730 DNA analyser (Applied Biosystems).

### *Statistics*

Based on the available UK data (11), the cohort size was calculated in order to detect with 80% power an allele frequency difference of 2% and a genotype frequency difference of 12%. Differences in genotype and haplotype frequencies were calculated with chi-square test. The magnitude of the association was expressed as odds ratio with 95% confidence interval compared to the Ser/Ser genotype. Comparisons between groups were performed with the Mann-Whitney U test. A two sided p value <0.05 was considered statistically significant.

## **Results**

### *Mal S180L haplotypes in axial SpA*

A total of 389 samples (204 cases and 175 controls) were analyzed by SNP specific PCR and, in case of inconclusive PCR, by direct sequencing. 90 samples showed a clear band in the T-specific PCR (Figure 1), which was confirmed by direct sequencing. In controls, the frequency of the C allele was 86.9% (304/350) and of the T allele 13.1% (46/350). In axial SpA, the frequencies were similar with 87.2% (356/408) for the C allele and for 12.7% (52/408) for the T allele.

### *Mal SNP C539T genotypes in axial SpA*

Both in cases and in controls, the distribution of the genotypes followed the Hardy-Weinberg equilibrium. The expected genotype frequencies were 132/175 (75.7%) C/C, 40/175 (22.6%) C/T, and 3/175 (1.7%) T/T for controls and 155/204 (76%) C/C, 45/204 (22%) C/T, and 4/204 (2%) T/T for cases. As shown in Table 1, the occurrence of the C/T genotype was not different in cases (48/204 or 23.5%) versus controls (34/175 or 19.4%) (p=0.44). The T/T genotype was slightly lower in cases than in controls (2/204 or 1% versus 6/175 or 3.4%) but this did not reach statistical significance (p=0.15) as this study was powered to detect significant differences in the C/T genotype rather than in the T/T genotype.

### *Disease phenotype and activity in Mal SNP C539T C/T versus C/C genotype in axial SpA*

Without affecting susceptibility, the Mal SNP C539T may still affect the phenotype and/or activity of axial SpA. In an explorative analysis, we compared patients with C/T versus those with C/C for gender distribution, HLA-B27 status, age at onset of symptoms, age at diagnosis, peripheral joint disease, as well as BASDAI and serum C-reactive protein levels at time of blood sampling. We could not detect any difference in demographic or clinical features in patients with the C/C genotype compared to patients with the C/T genotype (table 2)

## Discussion

As the Mal SNP C539T has been shown to possibly restrain TLR signalling (11), we hypothesized that this polymorphism may be underrepresented in SpA and thereby contributes to the putative excessive inflammatory response to bacteria and/or self-molecules. In this pilot study, we could not confirm this hypothesis as the frequency of the alleles was statistically identical in axial SpA and healthy individuals and, more importantly, there was no difference in the C/T genotype between cases and controls.

However, several issues should be taken into consideration interpreting these results. Firstly, as we powered the study to detect similar differences for the allele frequencies as described in previous studies (11), we can not exclude weaker associations that can only be detected in larger cohorts. This is of particular relevance for the T/T genotype, which appears to be slightly underrepresented in the cases. It should also be noted that the allele frequencies in the control groups were slightly different in our study and the previous report (11). Secondly, the functional outcome of TLR2/4 triggering is probably related to the functional interaction of different SNPs rather than uniquely to Mal SNP C539T. The study of potential interactions with independent Mal polymorphisms such as SNP rs671492 (13) or TLR polymorphisms (6,7) remains of major interest in SpA. Thirdly, the SNP C539T may affect the phenotype rather than susceptibility to axial SpA. A preliminary analysis of the phenotype and cross-sectional disease activity failed to reveal clear differences between axial SpA with C/T versus C/C genotype. However, as our study was not designed to assess this question, more detailed longitudinal analyses remain warranted. Finally, our study assessed only axial SpA and not other subtypes of SpA. Follow-up studies will have to assess other SpA subtypes as bacterial triggers and TLR signalling have mainly been related to gut and peripheral joint inflammation (1, 4, 5). Of interest with regard to the association between SpA and gut inflammation, a genetic analysis of TLR pathways demonstrated a modest contribution of Mal polymorphisms in inflammatory bowel disease (14).

## Acknowledgements

Dr. Derek Gordon Associate Professor of Statistical Genetics, Rutgers University for the sample size calculations. This work was partially supported by a Human Immunology Research Grant of the Dana Foundation and by the European Community's FP6 funding. This publication reflects only the author's views. The European Community is not liable for any use that may be made of the information herein.

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in ARD and any other BMJPG products and sublicences such use and exploit all subsidiary rights, as set out in our licence (<http://ARD.bmjournals.com/fora/licence.pdf>).

## References

1. Taurog JD, Maika SD, Satumtira N, Dorris ML, McLean IL, Yanagisawa H, et al. Inflammatory disease in HLA-B27 transgenic rats. *Immunol Rev* 1999;169:209-23.
2. Inman RD. Mechanisms of disease: infection and spondyloarthritis. *Nat Clin Pract Rheumatol* 2006;2(3):163-9.
3. De Rycke L, Kruithof E, Vandooren B, Tak PP, Baeten D. Pathogenesis of spondyloarthritis: insights from synovial membrane studies. *Curr Rheumatol Rep* 2006;8(4):275-82.
4. De Rycke L, Vandooren B, Kruithof E, De Keyser F, Veys EM, Baeten D. Tumor necrosis factor alpha blockade treatment down-modulates the increased systemic and local expression of Toll-like receptor 2 and Toll-like receptor 4 in spondylarthropathy. *Arthritis Rheum* 2005;52(7):2146-58.
5. Zhang X, Glogauer M, Zhu F, Kim TH, Chiu B, Inman RD. Innate immunity and arthritis: neutrophil Rac and toll-like receptor 4 expression define outcomes in infection-triggered arthritis. *Arthritis Rheum* 2005;52(4):1297-304.
6. van der Paardt M, Crusius JB, Garcia-Gonzalez MA, Dijkmans BA, Pena AS, van der Horst-Bruinsma IE. Susceptibility to ankylosing spondylitis: no evidence for the involvement of transforming growth factor beta 1 (TGFB1) gene polymorphisms. *Ann Rheum Dis* 2005;64(4):616-9.
7. Snelgrove T, Lim S, Greenwood C, Peddle L, Hamilton S, Inman R, et al. Association of toll-like receptor 4 variants and ankylosing spondylitis: a case-control study. *J Rheumatol* 2007;34(2):368-70.
8. Ulevitch RJ. Therapeutics targeting the innate immune system. *Nat Rev Immunol* 2004;4(7):512-20.
9. Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* 2005;5(6):446-58.
10. Sacre SM, Andreakos E, Kiriakidis S, Amjadi P, Lundberg A, Giddins G, et al. The Toll-like receptor adaptor proteins MyD88 and Mal/TIRAP contribute to the inflammatory and destructive processes in a human model of rheumatoid arthritis. *Am J Pathol* 2007;170(2):518-25.
11. Khor CC, Chapman SJ, Vannberg FO, Dunne A, Murphy C, Ling EY, et al. A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. *Nat Genet* 2007;39(4):523-8.
12. Marinou I, Montgomery DS, Dickson MC, Binks MH, Moore DJ, Bax DE, et al. The interferon induced with helicase domain 1 A946T polymorphism is not associated with rheumatoid arthritis. *Arthritis Res Ther* 2007;9(2):R40.
13. De Jager PL, Franchimont D, Waliszewska A, Bitton A, Cohen A, Langelier D, et al. The role of the Toll receptor pathway in susceptibility to inflammatory bowel diseases. *Genes Immun* 2007;8(5):387-97.

**Table 1:** Numbers and percentages of individuals with the different genotypes of Mal SNP C539T. P values were calculated using Chi-square test or \* Fisher's exact test. Odds ratio's were compared to the C/C genotype.

SNP C539T Genotype	Controls (n = 175)	Cases (n = 204)	P value	Odds ratio (95% CI)
C/C	135 (77.2 %)	154 (75.5 %)	0.89	1
C/T	34 (19.4 %)	48 (23.5 %)	0.44	1.23 (0.75-2.03)
T/T	6 (3.4 %)	2 (1 %)	0.15*	0.29 (0.06-1.47)

**Table 2:** Disease phenotype and severity in Mal SNP C539T C/T versus C/C genotype in SpA. Data are presented as median values (interquartile range) or as percentage positive patients (gender, HLA-B27, peripheral joint disease). Age at onset of symptoms and age at diagnosis are expressed in years. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and serum C-reactive protein (CRP) levels were assessed cross-sectionally at the time of blood sampling for genotyping.

	SNP C539T Genotype		2 sided p value
	C/C N=154	C/T N=48	
Gender (male)	80%	79.1%	0.91
HLA B27 positive	89.5%	94.8	0.31
Age at onset of symptoms	19 (16-23)	20.5 (16.5-24.5)	0.59
Age at diagnosis	28 (22.5-38)	28 (21-34)	0.23
Peripheral joint disease	18%	21%	0.77
BASDAI	4.9 (3-6.9)	4.3 (2.2-6.6)	0.23
CRP (mg/L)	9 (5-19)	9 (6-29)	0.38

**Figure Legend:**

**Figure 1:** Analysis of Mal SNP C539T by PCR. DNA was amplified with either C or T specific forward primers and the specific PCR products were analyzed on a 1% agarose gel stained with ethidium bromide. Sample 1 has the T/T genotype, sample 2 the C/T genotype and sample 3 the C/C genotype.

