Toll-like Receptor 4 and *CD14* Polymorphisms in Ankylosing Spondylitis: Evidence of a Weak Association in Finns

JENNIFER J. POINTON, KAY CHAPMAN, DAVID HARVEY, ANNE-MARIE SIMS, LINDA BRADBURY, KARI LAIHO, MARKKU KAUPPI, KALEVI KAARELA, JAAKKO TUOMILEHTO, MATTHEW A. BROWN, and B. PAUL WORDSWORTH

ABSTRACT. ***Objective.*** To investigate the association of *CD14* and *Toll-like receptor (TLR4)* with ankylosing spondylitis (AS).

Methods. A promoter variant in *CD14* and 2 coding polymorphisms in *TLR4* were investigated in UK and Finnish families with AS and in a UK case-control study. A metaanalysis of published *TLR4* and *CD14* studies was performed.

Results. In the Finnish study the *CD14*-260bp T variant showed an association (p = 0.006), and the common 2-marker *TLR4* haplotype showed a weak association (global p = 0.03), with AS. No associations were seen in the UK based studies or in the metaanalyses.

Conclusion. *CD14* and *TLR4* showed an association with AS in the Finns only. (First Release July 15 2008; J Rheumatol 2008;35:1609–12)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS GENETIC STUDIES IMMUNE SYSTEM

Ankylosing spondylitis (AS) is a chronic inflammatory arthritis. *HLA-B27* is a major genetic component, but a metaanalysis of 3 whole genome linkage scans suggested several additional linkage regions that include the *TLR4* and *CD14* genes[1]. TLR4 and CD14 are components of the innate immune response; TLR4 is a receptor for bacterial lipopolysaccharide (LPS) when bound to LPS-binding protein and CD14.

CD14 is in the 250kb region at 5q31.3 associated with inflammatory bowel disease (IBD)[2], proximal to the region linked to AS at 5q34[1]. The association of the promoter polymorphism *CD14*–260bp (rs2569190 C→T) (also designated -159bp) with IBD is variable[3,4]. It is associated with progression of reactive arthritis to chronic spondyloarthropathy in Finnish women[5] but not with AS[6]. The *TLR4* locus at 9q33.1 is within a linkage region for AS[1]. Two non-synonymous single nucleotide polymorphisms (nsSNP), D299G (rs4986790, A→G) and T399I (rs4986791, C→T) of *TLR4*, have been studied in IBD and AS with variable results[3,4,6-9]. IBD is commonly associated with AS and at least 1 genetic susceptibility factor is common to both diseases[10]. We therefore sought to clarify the associations of these genes with AS.

MATERIALS AND METHODS

Our study was approved by the research ethics committee boards in Finland and the UK (MREC project number 98/5/23). Participants gave informed consent prior to enrolment and all fulfilled the modified New York criteria for AS.

Finnish and UK AS families, cases, and controls. Details of the Finnish and UK families are shown in Table 1. The UK probands (n = 522) were used in the *CD14*-260bp case control study and were compared to 516 sex-matched controls. In the UK *TLR4* study, the probands and 430 additional sporadic cases (n = 952) were compared with control data from the 1958 British birth cohort (n = 1472) generated by the Wellcome Trust Case Control Consortium (WTCCC) under award 076113[11]. The list of investigators who contributed to the data is available from www.wtccc.org.uk. The Finnish study was family based only, as an independent control population of the size needed to generate the necessary statistical power was unavailable.

From the University of Oxford Institute of Musculoskeletal Sciences, Botnar Research Centre, Oxford, UK; Diamantina Institute for Cancer, Immunology and Metabolic Medicine (DI), University of Queensland, Brisbane, Australia; Rheumatism Foundation Hospital, Heinola; Department of Musculoskeletal Medicine, Medical School, University of Tampere, Tampere; National Public Health Institute; University of Helsinki, Helsinki; and South Ostrobothnia Central Hospital, Seinäjoki, Finland.

Funded by a program grant from the Arthritis Research Campaign (UK).

J.J. Pointon, DPhil, Research Assistant; K. Chapman, PhD, Botnar Research Fellow; D. Harvey, MSc, Graduate Student; B.P. Wordsworth, MB, FRCP, Professor of Rheumatology, University of Oxford Institute of Musculoskeletal Sciences, Botnar Research Centre; A-M Sims, DPhil, Research Assistant; L. Bradbury, MSc, Research Nurse, Diamantina Institute for Cancer, Immunology and Metabolic Medicine; M. Kauppi, MD, PhD, Specialist in Internal Medicine and Rheumatology and Senior Lecturer; K. Kaarela, MD, PhD, Chief Rheumatologist, Rheumatism Foundation Hospital, Heinola; K. Laiho, MD, PhD, Senior Rheumatologist, Rheumatism Foundation Hospital, Heinola and Department of Musculoskeletal Medicine, Medical School, University of Tampere; J. Tuomilehto, MD, PhD, Professor of Public Health, National Public Health Institute, University of Helsinki, and South Ostrobothnia Central Hospital; M.A. Brown, MD, FRACP, Professor of Immunogenetics, University of Oxford Institute of Musculoskeletal Medicine and Diamantina Institute.

Address reprint requests to Dr. J.J. Pointon, University of Oxford Institute of Musculoskeletal Sciences, Botnar Research Centre, Headington, Oxford, OX3 7LD, UK. E-mail: jennyp@well.ox.ac.uk

Accepted for publication March 14, 2008.

Table 1. The structure of the Finnish and UK families with ankylosing spondylitis. The Finnish sample comprised families and sporadic cases recruited from the Rheumatism Foundation Hospital, Heinola, Finland.

Family Structures	
Finnish	
Total number of families	300
Total number of sporadic cases	88
Families with 1 parent	92
Families with 2 parents (includes 10 families with an affected parent)	74
Families with sibs (1–5) (includes 20 families with at least 1 affected sib)	206*
Families with offspring of proband (includes 1 affected offspring)	15**
Sex ratio of probands (M:F)	2.1:1
Total affected	405
UK	
Parent case trios (includes 48 affected parents)	300
Multicase families	222
Families with 1 parent	72
Families with 2 parents (includes 12 families with an affected parent)	82
Families with 1 affected sib	222
Families with 2 or more affected sibs	29
Families with affected offspring of proband	17
Other affected relatives	4
Sex ratio of probands (M:F)	1.8:1

* Includes families with one or both parents and with offspring. ** Includes families with one parent. UK patients and families were recruited through attendance at Nuffield Orthopaedic Centre (Oxford, UK); from the Royal National Hospital for Rheumatic Disease (Bath, UK) AS Database; in response to public appeals; and referral from UK rheumatologists.

Laboratory methods. SNP were genotyped by restriction enzyme digestion of polymerase chain reaction products. Amplification and digest conditions are available on request. The *CD14*-260bp genotyping was replicated using SNPlex technology (Applied Biosystems, Warrington, UK) and 15 per cent of the *TLR4* genotypes were repeated to check for consistency.

Statistical methods. All genotypes were checked for Hardy Weinberg equilibrium. Mendelian inconsistencies were checked with the program PedCheck v1.1[12]. In the Finnish and UK families, transmission disequilibrium testing (TDT) of single markers and 2-marker haplotypes was carried out using the program TRANSMIT v2.5[13]. For each analysis 1000 bootstrap simulations were performed to calculate an empirical p value for association robust to linkage since many families had more than 1 affected offspring.

A case control analysis was performed on the UK sample. For the *CD14*-260bp SNP, probands were compared to controls using the chi-square test. Analysis of *TLR4* variants was done using genotype data generated by the WTCCC on the probands and additional sporadic cases using the chi-square test. Two-marker haplotype analysis of the *TLR4* variants was performed with the program WHAP (http://pngu.mgh.harvard.edu/~purcell/whap/)[14]. The analysis had 99% power under both dominant and log-additive models to detect an odds ratio (OR) of 2.5, assuming a population risk of AS of 0.01% and a risk allele frequency of 5% at a significance level of 0.05 using Quanto v1.2.3 (http://hydra.usc.edu/gxe).

We undertook a metaanalysis of *TLR4* association studies, including the findings from both this and previously published studies[7-10], by the Mantel-Haenszel test for fixed effects using StatsDirect software (http://www.statsdirect.com, England: StatsDirect Ltd., 2005). All published studies of *TLR4* variants in Caucasians with AS were included in the analysis. A similar analysis for the *CD14*-260bp SNP was performed using the current UK data and that of van der Paardt, *et al*[6].

RESULTS

Our results are shown in Table 2. We detected an association between AS and the *CD14*-260bp T allele in the Finnish families (global p = 0.006) that retained significance when transmission to female probands was considered (global p = 0.03). No association was seen between *CD14*-260bp and AS in the UK study (see Table 2) or in the metaanalysis of this study and that of van der Paardt, *et al*[6] [p = 0.97, OR = 0.99 (95% confidence interval [CI] = 0.9-1.2)].

In the Finnish families there was small over-transmission of the D299/T399 (AC) *TLR4* haplotype (global p = 0.03) but in the UK sample there were no associations of single- or 2-marker haplotypes with AS. No associations were observed between AS and *TLR4* in the metaanalysis of all published studies in Caucasians (Figure 1).

Table 2. Single- and 2-marker haplotype analysis of *CD14* and *TLR4* single-nucleotide polymorphisms in UK and Finnish families and UK-based case control studies.

SNP or Haplotype	Population	Study Design	Statistical Test	M.A.F.	Haplotype Frequency	p	Haplotype	Haplotype p
CD14-260	UK	Family based	TRANSMIT	0.49		0.14		
CD14-260	UK	Case control	chi-square	0.49		0.12		
CD14-260	Finnish	Family based	TRANSMIT	0.38		**0.006**		
CD14-260	Finnish	Family based females only	TRANSMIT	0.38		**0.03**		
TLR4 D299G	UK	Case control	chi-square	0.05		0.28		
TLR4 T399I	UK	Case control	chi-square	0.05		0.3		
TLR4 D299G/T399I	UK	Case control	WHAP				AC	0.48
TLR4 D299G	Finnish	Family based	TRANSMIT	0.07		0.06		
TLR4 T399I	Finnish	Family based	TRANSMIT	0.07		0.15		
TLR4 D299G/T399I	Finnish	Family based	TRANSMIT		0.93	**0.03**	AC	**0.02**

MAF: minor allele frequency. p values, generated by the program TRANSMIT, are global and those generated by the chi-square test are the 2-tailed corrected values. p values < 0.05 are shown in bold.

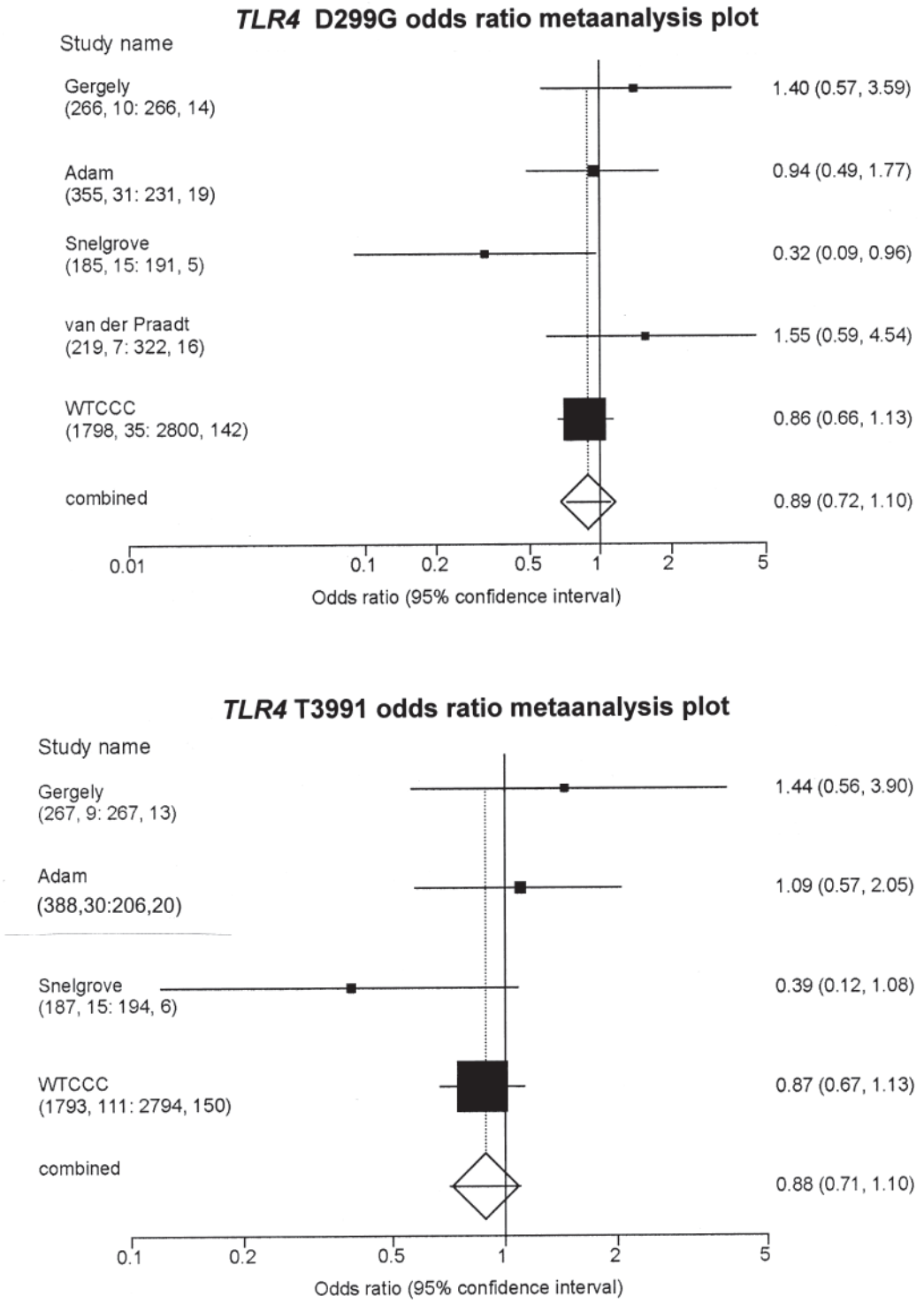

Figure 1. Forest plots for metaanalysis of *TLR4* variants in ankylosing spondylitis using Mantel-Haenszel test for fixed effects. The size of the black box is proportional to the percentage weight of each study in the analysis. Numbers under each study name are the numbers of each allele in cases and controls. Numbers on the right hand side of the plot are OR and 95% CI.

DISCUSSION

The weak associations between AS and both *CD14* and *TLR4* reported here in the genetically distinct Finnish population are interesting in view of the possible infective etiology of AS. These associations were not replicated in the UK-based studies or the metaanalyses. This could represent etiological heterogeneity, common in complex diseases, or be a false positive association in the smaller Finnish sample.

The minor allele frequency of the *CD14*-260bp SNP differs between Finns (0.38) and the UK (0.49) and Dutch (0.48) populations[6]; our frequency is similar to a previous estimate in Finns (0.39)[5]. The effect in the families with a female proband is consistent with the observation that women with the *CD14*-260bp T variant are more likely to progress to chronic spondyloarthropathy after reactive arthritis[5]. This effect requires independent confirmation as only 133 families fulfilled this criterion.

A role for *TLR4* in AS is plausible; it has a pivotal role in the innate immune response and it may be associated with IBD. The expression of TLR4 by peripheral blood cells is increased in patients with AS and correlates with the levels of other inflammatory markers[15]. However, the study of *TLR4* nsSNP and *CD14*-260bp promoter polymorphism combined with the metaanalyses presented here suggest that these genes do not have a major role in AS. Nonetheless, the role of the innate immune response in the pathogenesis of AS presents an exciting challenge and further studies in diverse populations will be of interest.

ACKNOWLEDGMENT

We are grateful to the many patients and physicians who contributed to the study and to the National Ankylosing Spondylitis Society for their assistance with recruitment.

REFERENCES

1. Carter KW, Pluzhnikov A, Timms AE, et al. Combined analysis of three whole genome linkage scans for ankylosing spondylitis. Rheumatology Oxford 2007;46:763-77.
2. Rioux JD, Silverberg MS, Daly MJ, et al. Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. Am J Hum Genet 2000;66:1863–70.
3. Gazouli M, Mantzaris G, Kotsinas A, et al. Association between polymorphisms in the Toll-like receptor 4, CD14, and CARD15/NOD2 and inflammatory bowel disease in the Greek population. World J Gastroenterol 2005;11:681–5.
4. Torok HP, Glas J, Tonenchi L, Mussack T, Folwaczny C. Polymorphisms of the lipopolysaccharide-signaling complex in inflammatory bowel disease: association of a mutation in the Toll like receptor 4 gene with ulcerative colitis. Clin Immunol 2004;112:85–91.
5. Repo H, Anttonen K, Kilpinen SK, et al. CD14 and TNFα promoter polymorphisms in patients with acute arthritis. Special reference to development of chronic spondyloarthropathy. Scand J Rheumatol 2002;31:355-61.
6. van der Paardt M, Crusius JB, de Koning MH, et al. No evidence for involvement of the Toll-like receptor 4 (TLR4) A896G and CD14-C260T polymorphisms in susceptibility to ankylosing spondylitis. Ann Rheum Dis 2005;64:235-8.
7. Adam R, Sturrock RD, Gracie JA. TLR4 mutations (Asp299Gly and Thr399Ile) are not associated with ankylosing spondylitis. Ann Rheum Dis 2006;65:1099–101.
8. Gergely Jr P, Blazsek A, Weiszhar Z, Pazar B, Poor G. Lack of genetic association of the Toll-like receptor 4 (TLR4) Asp299Gly and Thr399Ile polymorphisms with spondylarthropathies in a Hungarian population. Rheumatology Oxford 2006;45:1194–6.
9. Snelgrove T, Lim S, Greenwood C, et al. Association of Toll-like receptor 4 variants and ankylosing spondylitis: a case-control study. J Rheumatol 2007;34:368-70.
10. Wellcome Trust Case Control Consortium & The Australo-Anglo-American Spondylitis Consortium. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet 2007;39:1329-37.
11. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-79.
12. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998;63:259-66.
13. Clayton D. A generalization of the transmission/disequilibrium test for uncertain haplotype transmission. Am J Hum Genet 1999;65:1170–7.
14. Purcell S, Daly MJ, Sham PC. WHAP: haplotype-based association analysis. Bioinformatics 2007:23:255-6.
15. Yang Z-X, Liang Y, Zhu Y, et al. Increased expression of Toll-like receptor 4 in peripheral blood leucocytes and serum levels of some cytokines in patients with ankylosing spondylitis. Clin Exp Immunol 2007;149:48-55.