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The chromosome 16q region associated with ankylosing spondylitis includes the candidate gene tumour necrosis factor receptor type 1-associated death domain (*TRADD*)

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Abstract

Objective—To replicate and refine the reported association of ankylosing spondylitis (AS) with two non-synonymous single nucleotide polymorphisms (nsSNPs) on chromosome 16q22.1.

Methods—Firstly, 730 independent UK patients with AS were genotyped for rs9939768 and rs6979 and allele frequencies were compared with 2879 previously typed historic disease controls. Secondly, the two data sets were combined in meta-analyses. Finally, 5 tagging SNPs, located between rs9939768 and rs6979, were analysed in 1604 cases and 1020 controls.

Results—The association of rs6979 with AS was replicated, $p=0.03$, OR=1.14 (95% CI 1.01 to 1.28), and a trend for association with rs9939768 detected, $p=0.06$, OR=1.25 (95% CI 0.99 to 1.57). Meta-analyses revealed association of both SNPs with AS, $p=0.0008$, OR=1.31 (95% CI 1.12 to 1.54) and $p=0.0009$, OR=1.15 (95% CI 1.06 to 1.23) for rs9939768 and rs6979, respectively. New associations with rs9033 and rs868213 ($p=0.00002$, OR=1.23 (95% CI 1.12 to 1.36) and $p=0.00002$ OR=1.45 (95% CI 1.22 to 1.72), respectively, were identified.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Anglia and Oxford Multicentre Research Ethics Committee, St John's, Thorpe Road, Peterborough, UK.

Contributors JJP, DH, TK, LHA, CF, JDR, MHW, MMW, MAB and BPW were responsible for the concept, design and execution of the experiments. JJP, MAS, RDS, JDR, MHW, MMW, MAB and BPW were responsible for interpretation of data and drafting the article. JJP, DH, TK, LHA, CF, MAS, RDS, JDR, MHW, MMW, MAB and BPW were responsible for revising it critically for important intellectual content. JJP and BPW were responsible for final approval of the version to be published.

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Conclusions—The region on chromosome 16 that has been replicated in the present work is interesting as the highly plausible candidate gene, tumour necrosis factor receptor type 1 (TNFR1)-associated death domain (*TRADD*), is located between rs9033 and rs868213. It will require additional work to identify the primary genetic association(s) with AS.

INTRODUCTION

Ankylosing spondylitis (AS) is a polygenic inflammatory arthritis of the axial skeleton.¹ Apart from *HLA-B27*, genome-wide linkage scans suggest several regions of linkage, including 16q22.1.² Subsequently, a limited genome-wide association study (GWAS) of approximately 14 500 non-synonymous single nucleotide polymorphisms (nsSNPs) in 1000 AS cases and 1500 controls, undertaken by the Wellcome Trust Case Control Consortium and Triple A (Australo-Anglo-American) Spondyloarthritis Consortium (WTCCC/TASC), provided preliminary evidence for several associations,³ that were replicated in British, North American and Spanish studies.^{3–6}

Two positively associated SNPs ($p \leq 0.01$) (rs9939768 and rs6979) lying within approximately 0.5 Mb of each other on 16q22.1 identified this region for further investigation; rs9939768 is in *EXOC3L* and rs6979 is in *RLTPR*, neither of which has an obvious role in AS. *RLTPR* expression is downregulated in psoriatic skin but is not genetically associated with psoriasis.⁷ However, the region contains tumour necrosis factor receptor type 1 (TNFR1)-associated death domain (*TRADD*)⁸ and CCCTC-binding factor (*CTCF*), both plausible functional candidates for AS. *TRADD* interacts with the death domain of TNFR1 and is required for intracellular signalling leading to nuclear factor (NF) κ B activation and apoptosis.⁹ *CTCF* is a transcriptional insulator implicated in the regulation of major histocompatibility complex (MHC) class II gene expression,¹⁰ a function potentially relevant to AS since CD4-positive cells are involved in animal models of spondyloarthropathy.¹¹

Validation of the results from GWAS is essential because false positives are common. In this study, we genotyped a new patient sample for rs9939768 and rs6979 and compared the allele frequencies to historic disease controls previously typed by the WTCCC.³ We performed meta-analyses using these new data combined with published data. Finally we genotyped several new SNPs in the region and tested them for association with AS.

MATERIALS AND METHODS

Patients and controls

All patients with AS were either members of the National Ankylosing Spondylitis Society (UK), attendees at the Nuffield Orthopaedic Centre (Oxford, UK) or referrals from rheumatologists in the UK. All patients fulfilled the modified New York criteria for the diagnosis of AS and were British Caucasians (grandparents from UK or Ireland). The study was approved by the research ethics committee board in the UK (MREC project number 98/5/23). DNA was prepared from blood by standard methods, following informed consent. The patient replication sample was 730 AS cases. Control data came from 3 sources: (1) data from 2879 individuals with either multiple sclerosis, breast cancer or autoimmune thyroid disease from the WTCCC/TASC GWAS³; (2) for the assessment of rs9033 and rs868213, control data were available from the 1958 British birth cohort (58BC) (deposited by P Deloukas, Wellcome Trust Sanger Institute, Cambridge, UK; <http://www.b58cgene.sgul.ac.uk/>, October 2008) on 1400 and 1423 individuals, respectively; and (3) from 1020 healthy British Caucasian blood donors and spouses of patients with osteoarthritis. This study also uses genotyping data from 1000 AS cases and 1500 (58BC) controls generated by WTCCC/TASC under award 076113.³ The list of investigators who contributed to the data is available from <http://www.wtccc.org.uk>.

Genotyping

SNP genotyping performed by the WTCCC/TASC was performed with the Infinium I assay (Illumina, Cambridge, UK).³ Additional tagging SNPs were identified from the HapMap (<http://www.HapMap.org>) and genotyped either by iPLEX (MassArray, Sequenom, San Diego, California, USA) (SNPs rs13312727, rs9939768, rs9924876, rs8051891 and rs6979) or KASPar technology (KBiosciences, Hoddesdon, Herts, UK) (SNPs rs9033 and rs9924876). Genotyping cluster plots were manually examined.

Statistical analysis

SNPs were tested for adherence to Hardy–Weinberg equilibrium. Allele frequencies of cases and controls were compared by contingency tables using the Cochran–Armitage test of trend. Meta-analysis was performed using the Mantel–Haenszel test for fixed effects odds ratio (OR) using the StatsDirect statistical package (<http://www.statsdirect.com/>). Power calculations were made with Quanto (<http://hydra.usc.edu/gxe/>) using a gene-only log additive model, a disease prevalence of 0.004 and significance level of 0.05¹²; r^2 was calculated with LDmax¹³ for data reported here and with Haploview V.4.1 for HapMap data.¹⁴

RESULTS

Case-control association testing

Study A: replication of WTCCC/TASC nsSNPs and meta-analysis—We replicated the association of AS with rs6979 ($p=0.03$, OR=1.14 (95% CI 1.01 to 1.28)) and demonstrated a trend towards association with rs9939768 ($p=0.06$, OR=1.25 (95% CI 0.99 to 1.57)). No significant linkage disequilibrium (LD) was observed between rs9939768 and rs6979 ($r^2=0.003$ and $r^2=0.015$ for HapMap and WTCCC/TASC data, respectively). To increase the statistical power, we combined these data with the results from the WTCCC/TASC GWAS in a meta-analysis which revealed stronger associations for these SNPs with AS (rs6979 $p=0.0009$, OR=1.15 (95% CI 1.06 to 1.23); rs9939768 $p=0.0008$, OR=1.31 (95% CI 1.12 to 1.54)), as shown in table 1. The Cochran Q statistic, a measure of heterogeneity, for both SNPs was >0.05 , which indicates that the studies are combinable; I^2 , a measure of inconsistency, cannot be calculated for analyses combining only two studies. Power calculations were made using the numbers of cases and controls, allele frequencies and OR shown in table 1. Our current replication study for rs6979 and rs9939768 had $\geq 58\%$ power to detect an OR of 1.25 (a typical genetic effect size), but this increased to $\geq 84\%$ in the meta-analyses, see table 1.

Study B: analysis of additional SNPs at 16q21.1—We genotyped 5 new tagging SNPs that were not included in the WTCCC/TASC study (rs9033, rs13312727, rs868213, rs9924876 and rs8051891) in 1604 AS cases and 1020 additional non-overlapping ethnically matched controls. The results are shown in table 1. For SNPs rs9033 and rs868213 control data from the 58BC were combined with our control data to increase the study power. SNPs rs9033 and rs868213 showed strong associations with AS ($p=0.00002$, OR=1.23 (95% CI 1.12 to 1.36), $p=0.00002$, OR=1.45 (95% CI 1.22 to 1.72), respectively). Significant LD was observed between markers rs13312727 and rs868213 ($r^2=0.35$) and LD between all the other markers $r^2<0.092$. For HapMap data, $r^2<0.02$ between all SNPs except between rs13312727 and rs868213 where $r^2=0.36$. In the controls, rs8051891 was monomorphic and very rare in the cases so it was not possible to perform a meaningful analysis and the findings for rs13312727 and rs9924876 were not significant.

Three additional nsSNPs (rs3868142, rs8052655 and rs9972635), in the region bounded by rs9939768 and rs6979, were genotyped in the WTCCC/TASC GWAS (see table 1) and are not associated with AS. An LD plot with the relative positions of all SNPs and associated genes is shown in figure 1.

DISCUSSION AND CONCLUSIONS

We have replicated the association between rs6979 and AS and demonstrated a trend for association with rs9939768 in a new independent data set. Combining data by meta-analysis provides strong evidence for an association of this region of chromosome 16, which is already implicated by linkage studies.¹⁵ We have genotyped several new SNPs in cases and controls and have identified two strong associations with AS (rs9033 and rs868213) with excellent statistical power (table 1), in part because additional controls were identified from the 58BC. These SNPs are independently associated with AS since there is no LD between them ($r^2=0.015$). The functional candidate gene *TRADD* is located between them (figure 1). Three other SNPs (rs13312727, rs9924876 and rs8051891), including one in *TRADD* (rs13312727), were also genotyped; they have low minor allele frequencies (MAFs) and their apparent lack of association with AS could reflect lack of statistical power. Three additional nsSNPs, in the region bounded by rs9939768 and rs6979 (rs3868142, rs8052655, rs9972635) had been genotyped by WTCCC/TASC but are not associated with AS and are also underpowered.

Examination of the LD in the region, using HapMap data¹⁶ and including only SNPs with MAF >5%, shows that the SNPs in this study are in a region with six major LD blocks. SNPs rs9033, rs13312727, rs9939768, rs868213, rs9924876 and rs8051891 are in one large block, rs3868142 and rs8052655 are in the adjoining block and rs9972635 and rs6979 lie between blocks 5 and 6 (see figure 1). HapMap lists 328 SNPs between rs9033 and rs6967. Of these, 170 are monomorphic in Europeans and 141 have a MAF of 0.1 or less. Of the remaining 17 SNPs, 1 tags 5 others, 2 tag 2 others each, leaving 8 with useful MAFs in a region of about 0.5 Mb, making this region difficult to study.

The associated SNPs span a region that contains plausible candidate genes. *TRADD* is of particular interest because of its interactions with TNFR1 and Toll-like receptors 3 and 4.¹⁷ It therefore plays a crucial role in the regulation of NF κ B signalling and the regulation of proinflammatory cytokines. A second gene, *CTCF*, could have a role in AS since recent evidence suggests a role in the expression of MHC class II genes.¹⁰

We have replicated the findings of the WTCCC for SNPs on chromosome 16p22.1 and have identified two additional independently associated SNPs. To achieve better discrimination of the associated genotypes it will be necessary to undertake further large studies using all the available informative SNPs in the region. This study highlights the necessity of robust replication of whole genome associations and the need for still larger sample sizes to generate sufficient statistical power to detect small genetic effects reliably.

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Figure 1.

The relative positions of the single nucleotide polymorphisms (SNPs) in this study and genes in this region of chromosome 16 are indicated. Dark blocks are indicative of regions of high linkage disequilibrium (LD) as measured by D' .

Table 1
Association testing of single nucleotide polymorphisms (SNPs) in the region of the *EXOC3L* gene

SNP	Study	Chromosome 16 position	MAF cases	MAF controls	MAF 58BC	p Value	OR (95% CI)	Cases (n)	Controls (n)	Power (%)
Study A										
rs9939768 C/G	WTCCC/TASC	67 219 107	0.085 (C)	0.063 (C)		0.004	1.38 (1.10 to 1.74)	921	1453	70
rs9939768 C/G	Replication vs three diseases	67 219 107	0.075 (C)	0.061 (C)		0.060	1.25 (0.99 to 1.57)	724	2871	40
rs9939768 C/G	Meta-analysis	67 219 107	0.080 (C)	0.061 (C)		0.0008	1.31 (1.12 to 1.54)	1645	4324	84
rs6979 C/T	WTCCC/TASC	67 691 668	0.51 (C)	0.47 (C)		0.01	1.16 (1.03 to 1.31)	922	1465	70
rs6979 C/T	Replication vs three diseases	67 691 668	0.51 (C)	0.48 (C)		0.03	1.14 (1.01 to 1.28)	725	2879	58
rs6979 C/T	Meta-analysis	67 691 668	0.51 (C)	0.48 (C)		0.0009	1.15 (1.06 to 1.23)	1647	4344	93
Study B										
rs9033 A/G	This study plus 58BC controls	67 181 999	0.48 (G)	0.43 (G)	0.43 (G)	0.00002	1.23 (1.12 to 1.36)	1345	2297	99
rs13312727 T/G	This study	67 188 443	0.03 (G)	0.03 (G)		0.70	1.07 (0.70 to 1.62)	1530	996	7
rs868213 A/G	This study plus 58BC controls	67 220 457	0.09 (G)	0.07 (G)	0.06 (G)	0.00002	1.45 (1.22 to 1.72)	1558	2423	99
rs9924876 G/A	This study	67 221 050	0.022 (A)	0.025 (A)		0.50	0.88 (0.57 to 1.36)	1502	961	10
rs8051891 G/A	This study	67 223 775	0.002 (A)	0 (A)		0.07	NA	1534	998	NA
Additional SNPs										
rs3868142 C/T	WTCCC/TASC	67 320 223	0.081 (T)		0.068 (T)	0.06	1.23 (0.98 to 1.55)	922	1466	46
rs8052655 G/A	WTCCC/TASC	67 409 180	0.048 (A)		0.041 (A)	0.60	1.09 (0.79 to 1.51)	922	1466	9
rs9972635 A/G	WTCCC/TASC	67 682 580	0.064 (G)		0.054 (G)	0.32	1.13 (0.87 to 1.48)	922	1466	16

SNPs with significant association are highlighted in bold. SNPs rs3848290 at position 67 040 335 bp and rs11558534 at position 67 867 739 bp are the next non-significant SNPs from the WTCCC/TASC study; they mark the limits of the associated region. SNP positions are taken from the Ensembl database (<http://www.ensembl.org>) (August 2009).

MAF, minor allele frequency; NA, not applicable; WTCCC/TASC, Wellcome Trust Case Control Consortium and Triple A Spondyloarthritis Consortium.