

**The severity of ankylosing spondylitis and responses to anti-tumour necrosis factor biologics are not influenced by the tumour necrosis factor receptor polymorphism incriminated in multiple sclerosis**

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## **ABSTRACT**

Genetic polymorphism (*rs1800693*) of *TNFRSF1A* (type 1 tumour necrosis factor receptor) encodes a potentially anti-inflammatory soluble truncated form of the p55 receptor, which is associated with predisposition to multiple sclerosis but protection against ankylosing spondylitis (AS). We analysed 2917 UK Caucasian cases by linear and logistic regression for associations of *rs1800693* with disease severity assessed by the Bath Ankylosing Spondylitis measures of disease activity and function (BASDAI, BAS-G and BASFI) and/or responses to anti-TNF therapy. In contrast to predictions, *rs1800693* GG homozygotes actually had significantly worse BASDAI (mean 4.2, 95%CI 4.0-4.5) than AA homozygotes (mean 3.8, 95%CI 3.7-4.0) in both the unadjusted (difference=0.4, p=0.006) and adjusted analyses (difference=0.2 to 0.5, p=0.002 to 0.04 depending on the adjustment model). We found no evidence that *rs1800693* predicted functional status (BASFI) or global disease scores (BAS-G), and it exerted no influence on either the intention to treat with or efficacy of anti-TNF treatment.

## **INTRODUCTION**

Ankylosing spondylitis (AS) is a highly heritable spondyloarthropathy that affects up to 0.66 per cent of western European populations. [1] Genome-wide association studies suggest that more than 100 loci contribute susceptibility to AS.[2, 3] The potentially life-changing effects of the more severe forms of the disease are largely due to spinal inflammation and fusion. Work related disability is common [4] and mortality rates are increased, particularly in men with persistently active inflammation.[5] The severity of AS is also partly genetic but this is not well characterised.[6, 7] A pivotal role of the pro-inflammatory cytokine tumour necrosis factor (TNF) in AS is clearly demonstrated by the efficacy of anti-TNF biologics.[8]The prognosis for individuals with AS has improved greatly over the past decade with the introduction of these drugs for those with more severe forms of the disease. [3, 9] Genes almost certainly influence the response to anti-TNF biologics but this has not been investigated systematically in AS. In contrast, there is some evidence from other inflammatory conditions that responses to anti-TNF treatment are at least in part under genetic control.[10, 11]

*TNFRSF1A* encodes the p55 tumour necrosis factor receptor 1 (TNFR1), one of the two receptors through which TNF signals.[12] Associations between AS and *TNFRSF1A* have previously been reported in Caucasians and Chinese.[13, 14] The common *TNFRSF1A* single nucleotide polymorphism (SNP) *rs1800693* has potentially important functional consequences. The “G” allele results in exon 6 skipping, a premature stop codon and a truncated soluble form of TNFR1 that can bind TNF, potentially inhibiting its activity.[15] The G allele strongly increases the risk of multiple sclerosis (MS) [16] but is associated with protection against AS.[2] These opposite genetic associations mirror the contrasting effects of anti-TNF medications in these two conditions; anti-TNF therapy is highly effective for AS [8] but may precipitate or worsen MS.[17]

We predicted that the soluble truncated TNFR1 from the “MS-risk” G allele at *rs1800693* could have similar anti-inflammatory effects to anti-TNF biologics by binding and neutralising TNF in vivo. Since anti-TNF medication reduces disease severity in AS, we investigated whether the *rs1800693* G allele might (1) be associated with lower disease activity/severity, (2) reduce the requirement for anti-TNF therapy, and/or (3) influence the efficacy of anti-TNF therapy in AS.

## **RESULTS AND DISCUSSION**

After allowing for failed *TNFRSF1A* genotyping (67/2917 cases) there were 2850 cases for analysis (84.5% HLA-B27+). Genotype groups at *rs1800693* did not significantly differ in age, gender, disease duration, HLA-B27, age of onset, proportion on biologics or proportion with iritis, psoriasis, joint swelling or inflammatory bowel disease (IBD) (Table 1).

### **BASDAI**

Linear regression analyses (Table 2) showed a small but statistically significant difference in BASDAI between the GG (4.2, 95%CI 4.0-4.5) and AA genotypes (3.8, 95%CI 3.7-4.0), although the absolute difference in BASDAI was small and in the opposite direction from predictions. This remained significant after adjustment for age, gender, disease duration, HLA-B27 status and biologic use. It also remained significant after the inclusion of all covariates that showed a significant association with BASDAI (Table 2). Neither the addition of joint swelling as a covariate, nor sequential removal of adjustment variables affected these results. We estimate that the difference of 0.4 between the groups was the minimum difference that we could have detected (at 80% power and  $\alpha=0.05$ ).

### **BASFI and BAS-G**

There was no statistically significant association between the *rs1800693* genotype and BASFI in unadjusted analyses (GA mean BASFI 4.2 95%CI 4.0-4.4 vs AA 4.1 95%CI 3.9-4.3 regression coefficient (RC) 0.1,  $p=0.4$ ; GG mean BASFI 4.4 95%CI 4.0-4.7 vs. AA RC=0.3,  $p=0.1$ ,  $n=2774$ ). After partial adjustment for age, gender, disease duration, biologic use and HLA-B27 status there was a small but significant difference between the GG and AA groups (GA vs. AA RC=0.11 95%CI -0.1-0.4,  $p=0.4$ ; GG vs. AA RC=0.4 95%CI 0.04-0.8,  $p=0.03$ ,  $n=2070$ ) but this was not apparent in models adjusted for all covariates significantly associated with BASFI (GA vs. AA RC=-0.03 95%CI -0.2-0.1,  $p=0.7$ ; GG vs. AA RC=0.05 95%CI -0.2-0.3,  $p=0.7$ ,  $n=1957$ , adjusted for age, gender, disease duration, current biologic treatment, BASDAI, BAS-G, IBD, psoriasis, HLA-B27 status) (Supplementary table 1).

Similar results were found for BAS-G (Supplementary table 2). There was no significant difference between genotype groups in unadjusted analyses (GA mean BAS-G 4.3 95%CI 4.1-4.6 vs. AA 4.2 95%CI 4.0-4.3 RC=0.2,  $p=0.1$ ; GG mean BAS-G 4.3 95%CI 4.0-4.6 RC=0.15,  $p=0.4$ ). After partial adjustment for age, gender, disease duration, biologic use and HLA-B27 status there was a marginally significant difference between the GG and AA groups (GA vs. AA RC=0.19 95%CI -0.06-0.4,  $p=0.1$ ; GG vs. AA RC=0.36 95%CI 0.01-0.7,  $p=0.05$ ,  $n=2056$ ) but again this disappeared in more fully adjusted models (GA vs. AA RC=0.04 95%CI -0.1-0.2,  $p=0.6$ ; GG vs. AA RC=-0.03 95%CI -0.3-0.2  $p=0.8$ ,  $n=2022$ , adjusted for gender, NSAID use, BASFI, BASDAI, iritis, psoriasis, disease duration, age, HLA-B27 status).

### **Anti-TNF therapy**

No significant difference was apparent in the intention to treat AS with anti-TNF therapy between the various *rs1800693* genotype groups in unadjusted (intended biologic treatment GG  $n=84/340$ , 24.7% vs AA  $209/1014$ , 20.6% OR 1.2,  $p=0.2$ ) analyses. We have fitted a predictive model for intention to treat with anti-TNF therapy including *TNFRSF1A* genotype, age, gender, NSAID use, BASFI, BAS-G, psoriasis and IBD as covariates. The sensitivity of this model was 12.7% and the specificity was 97.2%. There was no significant difference in the intention to treat with anti-TNF therapy between genotype groups in this adjusted analysis. (Supplementary table 3)

Focusing on the 538 cases on biologic treatment, there were no significant differences in patient-rated treatment efficacy between rs1800693 genotypes in either the unadjusted or adjusted analyses.

None of our predictions about the potential influence of rs1800693 on disease severity or response to treatment in AS were confirmed in these analyses. There was actually a small but statistically significant association between the rs1800693 GG genotype (putatively AS-protective) and disease activity measured by BASDAI, which was robust to adjustment for a number of confounding factors. However, this is contrary to the expected direction since our *a priori* hypothesis was that carriers of the rs1800693 G allele would actually have less severe AS because the resulting soluble truncated TNFR1 would bind and neutralise TNF. BASDAI is an imperfect measure of purely current AS disease activity because it is also influenced by chronic changes in the axial skeleton. For the current study BASDAI is probably a less reliable indicator of disease severity than BASFI because it is considerably less heritable (Heritability estimates: BASDAI 0.49 vs BASFI 0.76).[3] The observation that the rs1800693 genotype did not affect BASFI or BAS-G together with the observation that any association with BASDAI was contrary to expectations constitutes convincing evidence that rs1800693 does not influence disease severity in AS .

In two studies of rs1800693 in MS no association was apparent with disease severity or age of onset.[18, 19] In contrast, in one of these studies another SNP, rs4149584, which results in an amino acid change in TNFRSF1A, was associated with reduced severity of attacks but not with recovery from attacks or recurrence within one year.[20]

In our study, the absolute size of the difference (0.4) in mean BASDAI between genotype groups (GG 4.2 vs AA 3.8) was relatively small. However, this difference spans the threshold for starting anti-TNF treatments in the UK which is set at a BASDAI of 4[21], meaning that the influence on treatment prescription could be larger. Overall no statistically significant effects of rs1800693 genotype on other possible markers of disease severity (intention to treat with anti-TNF, BASFI or BAS-G) were found. Although in partially adjusted analyses for BASFI and BAS-G there were marginally statistically significant differences between the GG and AA groups, these disappeared in the more fully adjusted model suggesting an effect of a confounding variable which was then adjusted for. Our cross-sectional study has several limitations. The use of patient questionnaires can result in incorrect/missing data and data entry errors. We used BASDAI as our primary measure of disease severity as this is a commonly used clinical measure, but ideally our study would have benefited from the application of more objective measures, such as radiology or metrology scores. In conclusion, these data exclude a clinically significant effect from rs1800693 on disease severity in AS or an influence on the efficacy of anti-TNF therapy.

## **MATERIALS AND METHODS**

### ***Participants***

We genotyped 2917 UK Caucasians satisfying either the modified New York criteria for AS or the ASAS imaging criteria for axial spondyloarthritis.[22, 23] Following a written informed consent (COREC 06/Q1606/139 and OXREC B 07/Q1605/35: this ethics includes all the questionnaires we used to collect that information), a structured questionnaire was used to obtain demographic information, medications and a visual analogue scale (VAS, 0-10) of the efficacy of treatment. Disease severity was

assessed using the BASDAI (Bath AS Disease Activity Index), BASFI (Bath AS Functional Index) and BAS-G (Bath AS-Global) instruments, which have previously been shown to reflect heritable severity traits in AS.[7]

### ***Genotyping***

Participants were genotyped for *rs1800693* and for the HLA-B27 tagging SNP *rs4349859* as previously described.[14]

### ***Statistical methods***

All statistical analyses were carried out using IBM SPSS version 22.0. Descriptive statistics for groups were compared using the Chi-squared test for categorical variables and one-way ANOVA for continuous variables. Genotypes at *rs1800693* (AA, GA, GG) were indicator-coded for use as the independent variable in linear regression analyses. Linear regression was performed for continuous outcome variables and logistic regression for categorical outcome variables. All analyses used AA as the reference group. Complete case analyses were performed for each outcome. The primary outcome variable was BASDAI. Secondary outcome variables were BASFI and BAS-G and intention to treat with anti-TNF therapy. Age, gender, disease duration, HLA-B27 status and use of biologic treatments were specified pre-hoc as covariates in linear regression analyses.[8, 24-26] Individual covariates were then included sequentially in a regression analysis with genotype and a further model built containing only these significant covariates. A prediction model was built for intention to treat with anti-TNF therapy using logistic regression, with covariates of *rs1800693* genotype, age, gender, BASDAI, BASFI, BAS-G, psoriasis and IBD. To exclude data entry errors limits were applied to age, disease duration, BASDAI, BASFI and BAS-G to exclude impossible values. Since missing data relating to the joint swelling component of the BASDAI questionnaire could indicate either absence of joint swelling or not having completed that question, analyses were performed with and without this covariate. Assumptions of linear regression were tested by examining histograms and normal P-P plots of residuals and scatterplots of standardised predicted values against standardised residuals. Tolerance and variation inflation factor (VIF) were examined as measures of collinearity; all values for tolerance were >0.4 and all VIF values were <2.5. Variables were additionally sequentially removed from BASDAI analysis.

### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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	AA	GA	GG	p	Missing data (% of all participants)
N	1115	1349	386	-	2.3
Mean Age $\pm$ standard deviation (SD)	55.1 $\pm$ 13.5	54.6 $\pm$ 13.4	54.3 $\pm$ 13.0	0.6	3.5
% male	72.7	71.7	76.6	0.2	3.3
Disease duration (years, mean $\pm$ SD)	28.9	29.4	28.3	0.5	6.6
BASDAI (mean $\pm$ SD)	3.8 $\pm$ 2.3	4.0 $\pm$ 2.3	4.2 $\pm$ 2.4	0.02	2.7
BASFI (mean $\pm$ SD)	4.1 $\pm$ 2.9	4.2 $\pm$ 2.7	4.4 $\pm$ 2.8	0.3	2.8
BAS-G (mean $\pm$ SD)	4.2 $\pm$ 2.7	4.4 $\pm$ 2.6	4.3 $\pm$ 2.6	0.3	3.6
Age of onset (mean $\pm$ SD)	25.9 $\pm$ 10.9	25.1 $\pm$ 10.5	26.0 $\pm$ 11.4	0.2	5.5
% reported biologic use at recruitment	19.5	19.7	21.6	0.7	4.0
% with IBD	11.7	11.4	10.2	0.8	6.3
% with psoriasis	18.5	19.5	16.9	0.5	3.5
% with at least one episode of iritis	45.7	47.7	42.5	0.2	3.4
% reporting unexplained joint swelling	76.8	75.2	75.4	0.6	-

**Table 1:** Descriptive statistics for *rs1800683* genotype groups. Missing data for % biologic use refers to % of all participants missing data on medication use. Missing data cannot be provided for unexplained joint swelling (see methods).

	Unadjusted (n=2776)			Partially adjusted (n=2070) <sup>a</sup>			Adjusted including B27 <sup>b</sup> (n=2023)			Adjusted excluding B27 <sup>c</sup> (n=2482)		
	RC (95%CI)	p	R <sup>2</sup>	RC (95%CI)	p	R <sup>2</sup>	RC (95%CI)	p	R <sup>2</sup>	RC (95%CI)	p	R <sup>2</sup>
<b>GA</b>	0.2 (-0.01 to 0.4)	0.06	0.003	0.2 (0.002 to 0.4)	0.05	0.04	0.1 (-0.03 to 0.2)	0.1	0.6	0.05 (-0.07 to 0.2)	0.4	0.6
<b>GG</b>	0.4 (0.1 to 0.7)	0.006		0.5 (0.2 to 0.8)	0.002		0.2 (0.01 to 0.4)	0.04		0.2 (0.04 to 0.4)	0.02	

**Table 2:** Results of unadjusted and adjusted linear regression analyses with BASDAI as outcome. All analyses are relative to the reference AA genotype. The regression coefficient represents the mean difference in BASDAI relative to the AA group (for multiple regression assuming that other variables in the analysis are constant). P values refer to significance of that group relative to the AA group. RC is the regression coefficient. R<sup>2</sup> is the coefficient of determination, a measure of goodness of fit of the model.

<sup>a</sup>=adjusted for age, gender, disease duration, HLA-B27 status and current biologic use. <sup>b</sup>=adjusted for all significantly associated covariates (gender, disease duration, HLA-B27 status, psoriasis, NSAID use, iritis, BASFI, BAS-G, current biologic use). <sup>c</sup>=adjusted for everything in model b other than HLA-B27 status.