# Anti-tumour necrosis factor-alpha response associated with combined CD226 and HLA-DRB1*0404 haplotype in rheumatoid arthritis

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## Abstract

### Objective

*Predicting response to anti-tumour necrosis factor alpha (anti-TNFα) drugs at baseline remains an elusive goal in rheumatoid arthritis (RA) management. The purpose of this study was to determine if baseline genetic variants of PTPRC, AFF3, myD228, CHUK, MTHFR1, MTHFR2, CD226 and a number of KIR and HLA alleles could predict response to anti-TNF-α in rheumatoid arthritis patients.*

### Methods

*Peripheral blood samples were collected from 238 RA patients treated with anti-TNFα drugs. Genotyping was performed using biochip array technology by Randox Laboratories Ltd. and sequence specific polymerase chain reaction. Linear regression analysis was performed to investigate the role of these genotypes in predicting response to treatment, as defined by European League Against Rheumatism (EULAR) response classification and absolute change in disease activity score (DAS28).*

### Results

*Of 238 RA patients analysed, 50.4% received adalimumab, 29.7% received etanercept, 14.8% received infliximab, 3.4% certoluzimab and 1.7% golimumab. The MTHFR1 variant rs1801133 was significantly associated with the EULAR response, p=0.044. Patients with the HLA-DRB1*0404 allele displayed a significantly larger reduction in DAS28 compared to non-carriers (mean -2.22, -1.67 respectively, p=0.033). CD226 rs763361 was the only SNP variant significantly associated with ΔDAS28 (p=0.029)*

### Conclusion

*This study has investigated individual allele associations with reductions in DAS28 across a range of anti-TNFα treatments. A combined predictive model indicates that patients with the HLA-DRB1*0404 allele and without the CD226 rs763361 polymorphism exhibit the largest reduction in DAS28 after anti-TNF-α treatment.*

**Key words**

anti-TNF-α, rheumatoid arthritis, response prediction, genotype, polymorphism

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## Introduction

Anti-tumour necrosis factor alpha (anti-TNF-α) blocking agents are effective at reducing disease activity measures in approximately 70% of rheumatoid arthritis (RA) patients (1). As TNF is a key driver of joint inflammation, in those who respond, anti-TNF-α biologic drugs reduce immune cell infiltration into the joint and diminish joint destruction. Now that there are several classes of biologic drug available for severe RA, there is a pressing need to find a widely accepted test that will help the clinician predict response and select the most appropriate therapy for each patient (2). A number of studies have assessed potential clinical and biological predictors of response, but in many cases biomarkers that appeared to be promising were not confirmed as clinically relevant markers in further studies.

A lower baseline health assessment questionnaire score and the concurrent use of methotrexate are associated with a good response to anti-TNF-α treatment (1). A high body mass index, low baseline disease activity and smoker status have all been associated with reduced rates of response to anti-TNF-α drugs (3-5). The presence of rheumatoid factor and/or anti-citrullinated protein antibodies (ACPA) has been associated with response to TNF-inhibitors in some but not all studies (1, 5-7). A pilot study in 170 RA patients demonstrated how clinical factors could be combined with a serum biomarker (serum myeloid-related protein, MRP8/14) in a treatment algorithm capable of predicting anti-TNF-α response (8).

Many single nucleotide polymorphism studies have demonstrated associations with anti-TNF-α treatment response. Genetic variants of components which map to T cell function associate with response, including IRAK 3, which negatively regulates toll-like receptor (TLR) signalling, conserved helix-loop-helix ubiquitous kinase (CHUK) and myeloid differentiation primary response protein (MyD88), which activate or inhibit nuclear factor-κB (NF-kB) signalling (9, 10). MyD88 and CHUK were previously associated with etanercept response in RA patients (7). A contradictory study found no response association with genetic variants of CD226, AF3/FMR2 family, member 3 (AFF3) in addition to CHUK and MyD88 (11). Genetic variants of the mitogen-activated protein kinase (MAPK) signalling pathway components also showed associations with infliximab and adalimumab responders in RA (12).

The shared epitope susceptibility loci have also formed the focus of anti-TNFα response association studies. Studies of UK and Swedish RA patients indicate no clear association with response, though these studies generalise the various human leukocyte antigen (HLA) DRB1 haplotypes rather than studying allele specific associations (13-15). A study which focused on dissecting shared epitope phenotypes established that valine at position 11 of HLA-DRB1 is associated with improved response to anti-TNF-α therapy in RA (16).

In a previous pilot study of RA by our group, the presence of specific haplotypes of genes central to T cell function Killer cell immunoglobulin-like receptor 2DS2 (KIR2DS2) and HLA-C group 1/2 (homozygous), was observed as being associated with anti-TNF-α response (17). We therefore designed an expanded study to independently validate whether specific KIR and HLA haplotypes and several promising genetic variants could predict response to anti-TNF-α treatment in a cohort of biologic naive UK RA patients.

## Methods

*Patient selection*

The following inclusion criteria were used for the current study: 1). RA patients fulfilling ACR/EULAR diagnostic criteria (18), 2). Treated with anti-TNF-α treatment as part of routine clinical practice, 3). Fulfilled the BSR 2001 criteria for anti-TNF-α therapy, 4). Had a DAS28 score of >5.1 when originally assessed for treatment (before baseline), 5). Reached 6 months of follow-up. Patients who stopped anti-TNF-α temporarily during first six months or discontinued therapy prior to the 6 month follow up for reasons other than inefficacy were excluded. Two hundred and thirty eight eligible RA patients were

**Table I.** Patient characteristics by EULAR response class after six months of anti-TNF-α treatment.

| Cohort characteristics | Non-responders (n=59) | Moderate responders (n=87) | Good responders (n=92) | Combined (n=238) | *p*-value |
|---|---|---|---|---|---|
| Gender, female, n (%) | 44 (73.3) | 70 (80.5) | 66 (71.0) | 180 (75.0) | 0.32 |
| Age at baseline, years, median (IQR) | 53.6 (14.0) | 54.7 (15.5) | 53.1 (15.7) | 54.5 (15.5) | 0.94 |
| Disease duration, years | 11.5 (10.1) | 12.5 (9.9) | 11.4 (9.3) | 11.8 (9.7) | ns |
| Concurrent cDMARDs, n (%) | 51 (85.0) | 73 (83.9) | 82 (88.2) | 206 (85.8%) | 0.70 |
| DAS28 score at baseline, mean (SD) | 4.9 (1.3) | 5.9 (1.1) | 5.3 (1.1) | 5.4 (1.2) | <0.0001 |
| DAS28 score at outcome, mean (SD) | 5.3 (1.2) | 4.1 (0.8) | 2.1 (0.8) | 3.8 (1.6) | <0.0001 |
| Change in DAS28 score, mean (SD) | 0.4 (1.0) | -1.8 (0.8) | -3.1 (1.0) | -1.6 (1.6) | <0.0001 |
| baseline TJC, median (IQR) | 8.5 (9.8) | 12.0 (10.5) | 11.0 (10.3) | 10.0 (10.0) | 0.037 |
| baseline SJC, median (IQR) | 5.0 (7.8) | 8.0 (7.5) | 8.5 (7.3) | 8.0 (7.0) | 0.0007 |
| baseline CRP, median (IQR) | 5.7 (13.4) | 13.0 (25.0) | 6.7 (14.0) | 8.0 (19.7) | 0.043 |
| baseline HAQ, median (IQR), n | 2.0 (1.0), 45 | 1.8 (1.0), 55 | 1.9 (0.9), 57 | 1.9 (1.0), 157 | 0.92 |

Values are mean with standard deviation (SD) or interquartile range (IQR), number (n), or percentage (%), where indicated.
DAS28: 28 joint disease activity score; cDMARD: conventional disease-modifying anti-rheumatic drug; HAQ: health assessment questionnaire; CRP: C-reactive protein; SJC: swollen joint count; TJC: tender joint count; ns: not significant.

recruited from rheumatology biologic clinics at Altnagelvin Hospital, Londonderry and Musgrave Park Hospital, Belfast, Northern Ireland. Office for Research Ethics Committees Northern Ireland (ORECNI) approval (11/NI/0188) was obtained for the study. Informed consent was obtained for all participants in the study, allowing for publication of anonymised clinical data.

*Patient recruitment, sample collection, clinical information*

The study was supported by a patient advisory group who met regularly throughout the study to advise on study design, recruitment literature and results dissemination. Eligible patients were invited by mailed patient information sheets, a minimum of 48 hours before a routine care appointment. Additional blood samples were obtained from consenting patients who were either about to commence, or had been on an anti-TNF-α treatment in the past. Blood samples were processed by a silica based extraction kit to isolate genomic DNA (DNAEasy, Qiagen Inc.). DNA samples were aliquoted and stored at -80°C until analysis. Clinical and demographic information was extracted from medical records and clinic databases after written informed consent. Rheumatoid factor (RF; <30 IU/mL = negative, 30-50 IU/mL = weak positive, >50 IU/mL = positive) and anti-cyclic citrullinated peptide (anti-CCP; >10U/ml = positive, 7-10U/ml = equivocal, <7 U/ml = negative) tests were performed by turbidimetry and automated enzyme immunoassay, respectively. Disease activity was compiled for baseline and 6 months of treatment with anti-TNF-α, using DAS28-ESR (19). Following 6 months of treatment, the patients were assigned a moderate responder, good responder or non-responder status, according to EULAR criteria (20). The main demographic and clinical features of the patients are shown in Table I.

*Genotyping*

All genotyping was performed by custom Rheumastrat™ biochip array technology (Evidence Analyser™, Randox Laboratories Ltd.). Genotyping was confirmed by PCR-SSOP method (17). Positive controls of known KIR, HLA or single nucleotide polymorphism (SNP) genotype, were included in the typing procedure. DNA was typed for the presence or absence of previous response associated framework KIR genes: KIR2DS2 (activator) and KIR2DL2 (inhibitor). HLA-DRB1 typing was performed on the following shared epitope alleles: *03, *0101, *1001, *0401, *0104 and *0404. A modified version of the HLA-C typing method was used to define the HLA-C1 and C2 groups using probe C293 and C291, respectively (17).

Single nucleotide polymorphisms previously published as associated with therapeutic response and disease severity were typed for the following gene loci (in brackets): HLA-DR/BTNL2 (rs1980493), protein tyrosine phosphatase, receptor type C (PTPRC) (rs10919563), AFF3 (rs10865035), CD226 (rs763361), myD88 (rs7744), CHUK (rs11591741), methylenetetrahydrofolate reductase 1 (MTHFR1) (rs1801133), MTHFR2 (rs1801131) (7, 21-25).

*Statistical methods and analysis*

The study size was based on power calculations that indicated that an overall sample size of 150 patients across 3 groups would provide >80% power (at the conventional significance level alpha=0.05) to identify a genetic factor with a small effect size (Cohen's f-squared = 0.10).

The significance of the differences in proportions of responders and non-responders exhibiting a specific genotype was assessed using Fisher's exact test. For the numeric measures including DAS28 scores at baseline, DAS28 score changes at month 6 (ΔDAS28), Health Assessment Questionnaire scores at baseline, t-based statistics were used to assess the difference between two means, one-way ANOVA for multiple means, and Pearson correlation for assessing correlations between numerical variables. Whereas the normality assumptions underpinning those parametric tests above do not firmly hold, non-parametric counterparts of those techniques (*e.g.* Kruskal-Wallis test instead of one-way ANOVA) were applied (as indicated in Table I). In particular for the DAS28 scores at baseline, at month 6 and their difference (ΔDAS28), the normality assumptions were well supported by the data, hence validating the parametric

tests used and subsequent linear regression analyses. For the other numerical measures in Table I, Age, baseline TJC, SJC, CRP, and HAQ, the non-parametric Kruskal-Wallis tests were used, and median and interquartile range (IQR) instead of mean and standard deviation are reported. Using the software R, A series of systematic linear regression analyses were used to construct a most appropriate models consisting of significant predictors (detailed in the Results section). All tests were two-sided unless otherwise stated. Where applicable, Adjustments for multiple testing were made using Holm's method.

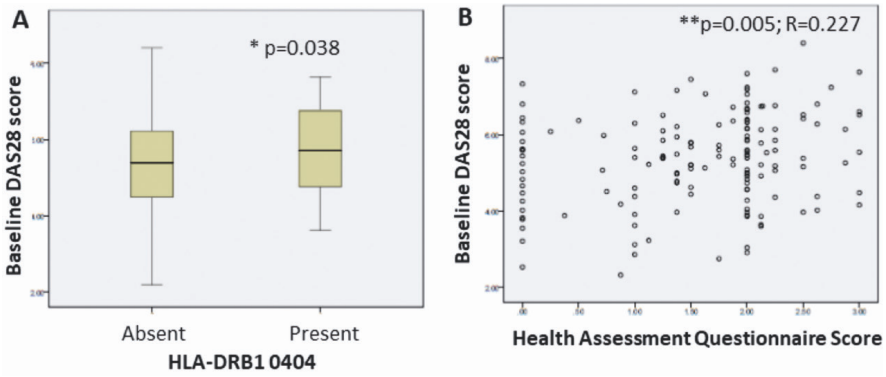


**Fig. 1.** Significant factors associated with baseline DAS28 score.
**A**: Box plot of baseline DAS28 scores for RA patients with the HLA-DRB1 0404 Allele absent or present. Central bar represents mean, outer box standard error and error bars standard deviation of grouping.
**B**: Dot plot correlation of baseline DAS28 scores for RA patients *vs*. baseline health assessment questionnaire score.

**Table II.** Association of baseline clinical factors with change in DAS28 score, over 6 months treatment.

| Factor | Group | Group No.s | Group % | Group Mean delta DAS28 | SD | *p*-value |
|---|---|---|---|---|---|---|
| Gender | Male | 59 | 24.8% | -1.70 | 0.08 | 0.659 |
| | Female | 179 | 75.2% | -1.81 | -0.03 | |
| Baseline cDMARD | No | 33 | 13.9% | -1.71 | 0.08 | 0.762 |
| | Yes | 205 | 86.1% | -1.80 | -0.01 | |

## Results

*Patient demographics*

There was no significant difference between the three response groups (*i.e.*, good, moderate, and non-responder) of patients with respect to the distribution of age ($p$=0.74), gender ($p$=0.32) or concomitant conventional DMARD use ($p$=0.70) (Table I study cohort characteristics). Baseline tender and swollen joint counts were significantly lower in non-responders, whereas baseline CRP was significantly elevated in moderate responders; both differences were statistically significant ($p$=0.027, $p$=8.5E-04, $p$=0.063, respectively). The mean change in DAS28 differed significantly between each EULAR response group, with non-responders at 0.4±1.0, moderate responders -1.8±0.8 and good responders -3.1±1.0 ($p$=4.10E-45). For patients who had laboratory data available, 149 or 73.8% of those tested were rheumatoid factor positive at the start of the study and 94 or 72.9% of those tested were anti-cyclic citrullinated peptide (anti-CCP) antibody positive.

238 patients received a combination of conventional DMARD (cDMARD) and anti-TNF-α drugs. Of the five anti-TNF-α drugs prescribed in the study population, adalimumab was prescribed for 119 (50.4%), etanercept for 70 (29.7%), infliximab for 35 (14.8%), certoluzimab for 8 (3.4%) and golimumab for 4 (1.69%) (Supplementary Table S1 of anti-TNF-α drugs prescribed across study cohort). There was no significant difference in treatment outcome between the five anti-TNF-α drugs when comparing patients who did or did not receive concurrent cDMARDs such as methotrexate (ANOVA, $p$=0.093).

*Baseline characteristics (gender, HAQ and genotype vs. baseline DAS28)*

Baseline DAS28 was found to be significantly higher in patients with HLA-DRB1*0404 haplotype ($p$=0.038; absent mean DAS28 5.30±1.235; present mean DAS28 5.75±1.151; Fig. 1A, Suppl. Table S2). Gender and baseline cDMARD did not exert a statistically significant effect on change in DAS28 over 6 months of treatment (ΔDAS28) in the study RA population (Table II). Since strength of association of a particular genotype with ΔDAS28 could be influenced by gender, baseline DAS28 and concurrent cDMARD use, adjustments considering these factors were necessary in later predictive model tests. As has been observed in previous studies, baseline DAS28 was significantly correlated with baseline HAQ (p0.005; R=0.227; n=154; Fig. 1B).

*Individual factor association with response to anti-TNF-α treatment*

Using the EULAR classification system none of the shared epitope alleles were strongly associated with response to anti-TNF-α (HLA-DRB1*0404 $p$=0.059, Suppl. Table S3). The MTHFR1 SNP was weakly associated with the EULAR response ($p$=0.044), but the CD226 SNP was not ($p$=0.202).

The associations between presence or absence of individual alleles on ΔDAS28 were investigated (Suppl. Table S4; Fig. 2). Notably, presence of the HLA-DRB1*0404 allele compared to absence was significantly associated with a larger reduction in DAS28 (mean -2.22 and -1.67 respectively, $p$=0.033). CD226 SNP was the only gene variant significantly associated with ΔDAS28 ($p$=0.029) (Suppl. Table S4). The mean reduction in DAS28 was greatest for the CD226 homozygous wild type TT variant (-2.35), compared to heterozygous

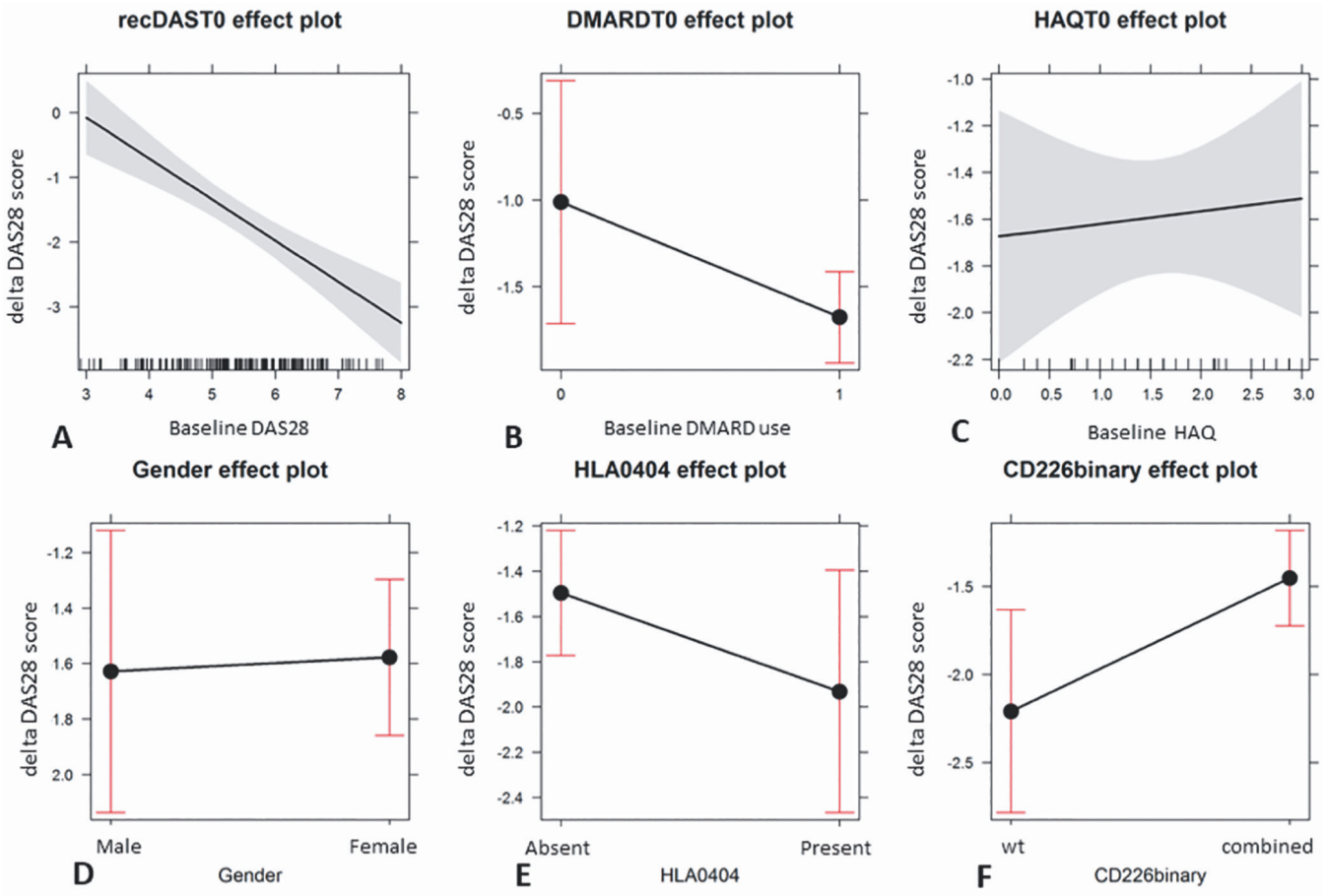


**Fig. 2.** Association between genetic and clinical factors and change in DAS28 score after 6 months treatment.
**A**: Association between baseline DAS28 score and delta DAS28.
**B**: Association between baseline cDMARD use and delta DAS28. 0: none used; 1: cDMARD used.
**C**: Association between baseline HAQ score and delta DAS28.
**D**: Association between gender and delta DAS28.
**E**: Association between HLA-DRB1 0404 genotypes and delta DAS28. 0, absent; 1, present.
**F**: Association between binary CD226 and delta DAS28. wt: wildtype TT; combined: CT and CC carriers.

carrier genotype TC (-1.57) and homozygous genotype CC (-1.85).

*Multi-factor regression models*

As previous reports indicate that ΔDAS28 could be influenced by gender, baseline DAS28 and concurrent cDMARD use, and HAQ, we thus investigated whether these four factors are significantly associated with ΔDAS28 in our cohort of patients (1). We fit a linear regression model using ΔDAS28 as dependent variable, and the four factors mentioned here as independent variables. A complicated full model with all possible pair-interaction terms was explored. However, with this 11-term model (one intercept term, 4 terms for individual variables, and 6 interaction terms), only the baseline term (recDAST0) would be significant (data not shown). We therefore removed the interaction terms with higher *p*-values, and started with only the interaction term between the two categorical variables (gender and concurrent cDMARD). However the gender-cDMARD interaction term was still found to be non-significant (*p*-value = 0.26), subsequently a simplified regression model without interaction terms was fitted. The results of this regression analysis are shown in Supplementary Table S5. As can be seen from table, gender ($p$=0.99) and HAQ ($p$=0.52) were not significantly associated with ΔDAS28. Both factors were excluded from all subsequent analysis. As expected, baseline DAS28 score was a highly significant predictor of response ($p$<0.001), while concurrent cDMARD therapy was only of borderline significance ($p$=0.051). Subsequently, baseline DAS28 was built into a base model, with cDMARD and all the genetic factors added individually to the base model to examine their association with ΔDAS28. Briefly, a series of linear regression models were fitted with baseline DAS28, plus one genetic factor or one other factor. The other factors considered here included RF status, anti-CCP status, the type of anti-TNF-α used and concurrent cDMARD use. Screening through all the genetic factors and the other listed factors, only CD226 was found to contribute significantly (at the level of alpha=0.05) to ΔDAS28 after adjusting the effect of baseline DAS28 score (Table III; Fig. 3B). The HLA-DRB1*0404 haplotype, although found to be significantly associated with ΔDAS28 in the individual

**Table III.** The linear model with two predictors: baseline DAS28 and CD226.

| Variable | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| (Intercept) | 0.957839 | 0.531244 | 1.803014 | 0.0729 |
| Baseline DAS28 | -0.60283 | 0.084762 | -7.11208 | **2.17 E-11** |
| CD226 (12) | 0.886626 | 0.300816 | 2.947406 | **0.0036** |
| CD226 (22) | 0.680279 | 0.326803 | 2.081615 | **0.0386** |

After correcting for effects of baseline DAS28, no other genetic factor or other factors appear to contribute significantly to the delta DAS28 score; Therefore the two predictors here, baseline DAS28 and CD226 together provide an optimal model to describe the response of this cohort of RA patients.

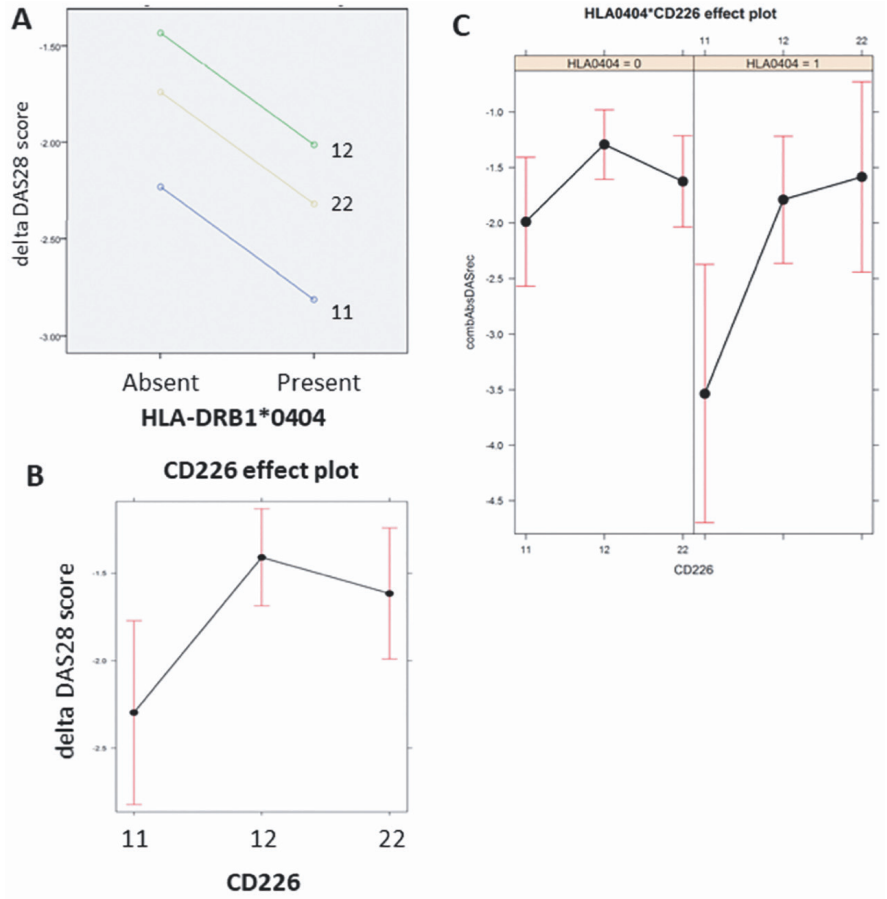


**Fig. 3.** Model of combined effects of HLA-DRB1*0404 allele and CD226 SNP rs763361 on change in DAS28 score after 6 months treatment.
**A**: Non-interaction plot SNP genotypes: 11, wildtype; 12, heterozygous carrier; 22 homozygous carrier of CD226 SNP.
**B**: Effect of CD226 rs763361SNP presence upon change in DAS28 ESR score after 6 months of anti-TNF-α use; 11 is homozygous wildtype TT, 12 is CT and 22 is CC genotype.
**C**: Combined HLA-DRB1*0404 and binary CD226 effect plot. For HLA-DRB1*0404 allele, 0 is absent, 1 is present. For CD226 rs763361 SNP, 11 is homozygous wildtype TT, 12 is CT and 22 is CC genotype.

factor analysis above, was no longer significant after correcting for the effects of baseline DAS28 score.

In summary, the baseline DAS28 score and the CD226 status can be combined in a model to predict anti-TNF-α response in this cohort of RA patients. The results of this model are shown in Table V, and the effects of baseline DAS28 and CD226 genotypes are depicted in Figure 3. As can be seen from this figure, the homologous CD226 genotype 11 conforms to the best response, while patients with either genotype 12 or 22 have significantly worse response than genotype 11.

Given the finding about HLA-DRB1*0404 in the individual factor analysis, we further investigated whether it might still have predictive value in the base model with baseline DAS28 and CD226 as built-in predictors. First, HLA-DRB1*0404 was added to this base model without interaction with CD226. In this simple interaction-free model, HLA-DRB1*0404's effect on ΔDAS28 is just short of statistical significance ($p$=0.051). Secondly, we considered the interaction between CD226 and HLA-DRB1*0404 in the model, and found interesting results. There are significant differences among the 6 genotypes of CD226-HLA-DRB1*0404 combinations; Homozygous absence of the CD226 rs763361 SNP and presence of HLA-DRB1*0404 allele represents the most responsive genotype, which is significantly better than most of other 5 combinations (Fig. 3C).

## Discussion

Rather than grouping all HLA-DRB1 genotypes together as in previous studies (13), this study has for the first time tested individual allele associations with ΔDAS28 across a range of anti-TNF-α treatments. This is also the first study to report a combined predictive model which indicates that patients with presence of HLA-DRB1*0404 and absence of CD226 SNP rs763361 exhibit the largest reductions in DAS28 after anti-TNF-α treatment.

HLA-DRB1*0404 carriers are known to be predisposed to a more severe arthritis phenotype and a higher disease activity (26, 27). In the current study patients with the HLA-DRB1*0404 haplotype did manifest significantly elevated baseline disease activity. Potter *et al*. in a replication study were unable to verify the association of shared epitope genotypes with ΔDAS28 in a study of anti-TNF-α treatment (13). It is therefore of particular interest that the current study, confirmed that the presence HLA-DRB1*0404 was independently associated with a significantly larger drop in disease activity after anti-TNF-α treatment. The association of the *0404 and *0101 alleles with response has been observed previously with etanercept, though intriguingly only when combined with lymphotoxin-α-TNF polymorphisms (28). The influence of TNF-α and TNF receptor gene polymorphisms upon response to anti-TNF-α response

have been extensively studied. The TNF SNPs 489G/A (rs80267959), -308G/A (rs1800629) and -857C/T (rs1799724) are particularly associated with response to etanercept (28-30). Consistent with previous work, a significant association between CHUK (rs11591741) SNP with etanercept response was observed in the subgroup of 70 RA patients who received this anti-TNF-α, though there was no significant association MyD88 SNP (rs7744) (7).

The presence of CD226 SNP rs763361 in the study population was associated with significantly reduced responses to anti-TNF-α treatment. A similar association between rs763361 and anti-TNF-α response in RA has already been reported by Tan *et al*., though as they noted it had only modest strength in a predictive model alone (23). We have therefore attempted in a combined predictive model to control for the possibility that larger changes in ΔDAS28 are not solely due to higher baseline DAS28 in HLA-DRB1*0404 carriers. In the predictive model, only the CD226 SNP contributes to significant drops in disease activity post ant-TNF, once the potentially confounding effects of baseline DAS28 are corrected.

*Biological significance*

It is thought that anti-TNF-α targeting of inflammatory cells with membrane bound TNF enhances antibody dependent cellular cytotoxicity (ADCC) by macrophages and natural killer cells (31). Cell surface activating and inhibitory killer cell immunoglobulin-like receptors regulate natural killer cell functions via HLA class I molecule interaction (32). So it is reasonable to postulate that although the *0404 allele confers higher disease risk and activity, it may also positively modify ADCC mediated apoptosis and clearance by natural killer cells.

CD226 is involved in the effector functions of T helper cells and peripheral T cells exhibit increased CD226 expression in rheumatoid arthritis (33). The rs763361 SNP located in exon 7 of CD226 confers a Gly307Ser change within the cytoplasmic tail of the CD226 receptor. This variant is strongly associated with susceptibility to multiple autoimmune conditions including type 1 diabetes, multiple sclerosis and RA (34). The biological consequence of the variant remains unclear, though it has been hypothesised that downstream effects on phosphorylation at Ser329 may be affected, which is required for cell activation via LFA1 (35).

*Clinical significance*

The ability to correctly predict responders for high cost biologic treatments remains a lofty goal. Previously mentioned studies have reported a number of promising genotypes, but many observe that though associations may be strong or statistically significant, they may be of limited clinical benefit in managing patients. The ability of our model to correctly predict true responders (test sensitivity) is poor (23% HLA0404 and 19% CD226; Suppl. Table S6). However, the ability to distinguish future responders with a positive HLA0404 test and a negative CD226 test was good with positive predictive values of 82% and 83%, respectively. This 'future responder' HLA0404-CD226 combined genotype represents 17% of the study population.

*Other observations*

Although the sample size could have been larger, over 200 RA patients were studied and therefore our study had adequate statistical power. It is also interesting to note that responders (moderate and good) appeared to have higher baseline tender joint counts (TJC) compared to non-responders. It may suggest that the there is a different disease process associated with those that are responding versus those not responding. Although the *0404 allele does not appear to have a significant influence upon baseline TJC, further investigation of the biological pathways associated with TJC factors may be worthy of further study.

## Conclusions

Further association studies of combined haplotypes and other 'omic' and clinical data have the potential to expand the 'predictable' proportion of future responders within a given RA patient population, to a point of clinical usefulness and commercial viability. Even as biosimilar anti-TNF-α treatments have reduced in cost, a pharmacogenetic approach to stratify patient populations could provide a reliable means to rationalise their use toward those most likely to benefit.

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