Investigation of rheumatoid arthritis susceptibility genes identifies association of AFF3 and CD226 variants with response to anti-tumour necrosis factor treatment

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ABSTRACT

Background Anti-tumour necrosis factor (anti-TNF) therapy has proved to be highly successful in treating rheumatoid arthritis (RA), although 30–40% of patients have little or no response. The authors hypothesise that this may be genetically determined. In other complex diseases, susceptibility genes have been shown to influence treatment response. The aim of the current study was to investigate the association of markers within confirmed RA susceptibility loci with the response to anti-TNF treatment.

Methods Eighteen single nucleotide polymorphisms (SNPs) mapping to 11 genetic loci were genotyped in 1012 patients with RA receiving treatment with etanercept, infliximab or adalimumab. Multivariate linear regression analyses were performed using the absolute change in 28 joint count disease activity score (DAS28) between baseline and 6-month follow-up as the outcome variable, adjusting for confounders. p Values <0.05 were considered statistically significant and associated markers were genotyped in an additional 322 samples. Analysis was performed in the combined cohort of 1334 subjects with RA treated with anti-TNF.

Results In the combined analysis, SNPs mapping to AFF3 and CD226 had a statistically significant association with the response to anti-TNF treatment under an additive model. The G allele at rs10865035, mapping to AFF3, was associated with an improved response to anti-TNF treatment (coefficient −0.14 (95% CI −0.25 to −0.03), p = 0.015). At the CD226 SNP rs763361, the C allele conferred reduced response to treatment (coefficient 0.11 (95% CI 0.00 to 0.22), p = 0.048).

Conclusion These results suggest that AFF3 and CD226, two confirmed RA susceptibility genes, have an additional role in influencing the response to anti-TNF treatment.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic potentially disabling disease caused by autoimmune destruction of the synovial joints which affects approximately 1% of the Caucasian population. The introduction of anti-tumour necrosis factor (anti-TNF) biological therapies has dramatically altered the treatment of RA as they show good efficacy in patients resistant to disease-modifying anti-rheumatic drugs (DMARDs) and superior efficacy in the suppression of erosive damage compared with standard DMARDs. However, there remains a significant non-response rate (in the region of 30–40%). The reasons for this remain largely unknown. Furthermore, anti-TNF therapy is associated with expensive annual treatment costs, leading to restrictions in the numbers of patients who may be prescribed these drugs. The identification of predictors of treatment response could potentially reduce the number of non-responding patients, improving the cost-effectiveness of anti-TNF therapies.

Several clinical predictors of response have been determined, including the level of disability at the onset of treatment as measured by the Health Assessment Questionnaire (HAQ) (patients with higher levels of disability at the outset of therapy respond less well); concurrent therapy with DMARDs (co-administration of DMARDs improves response); and the presence of autoantibodies (presence of rheumatoid factor or anticyclic citrullinated peptide antibodies is associated with a poorer response). However, even when these factors were combined, they accounted for less than 20% of the variance in response to anti-TNF agents in one study.

In other complex diseases, polymorphisms in susceptibility genes have been shown to be associated with treatment response. For example, two variants in the established type 2 diabetes (T2D) susceptibility gene TCF7L2 have been shown to influence the response to treatment with sulfonylurea drugs. In the current study we hypothesised that polymorphisms known to have a role in susceptibility to RA may also influence the response to anti-TNF treatment.

We have previously investigated—and found no evidence for—an association of the two major RA susceptibility loci: HLA-DRB1 shared epitope alleles and the PTPN22 R620W polymorphism. However, with the advent of genome-wide association (GWA) studies, there has recently been enormous progress in the identification of RA susceptibility genes. There are now at least 11 additional loci for which association with RA susceptibility has been confirmed in independent data sets, and the aim of the current study was to test the association of these markers with anti-TNF treatment response.
RESULTS

A total of 18 SNPs mapping to the 11 loci investigated were selected for genotyping (table 2). These were polymorphisms with previous evidence for association with RA susceptibility including some proxy SNPs in cases of assay failure, selected using SNAP.19

The initial test cohort comprised 1092 samples; 80 samples were excluded by the <80% QC measure, leaving 1012 samples available for analysis. One SNP (rs13207053) in TNFAIP3 was excluded from analysis due to <80% genotyping success rate, although a perfect proxy for this variant was successfully genotyped (rs13192841).

Two variants mapping to AFF3 and one to the CD226 locus demonstrated statistically significant evidence for association under an additive model (table 3) (AFF3: rs10865035, allele G coefficient −0.16 (95% CI −0.29 to −0.03), p=0.018; rs1160542, allele G coefficient 0.15 (95% CI 0.02 to 0.29), p=0.022; CD226: rs765361, allele C coefficient 0.16 (95% CI 0.03 to 0.29), p=0.016). rs1160542 served as a proxy (r²=0.97) for rs10865035, so these two associations represent a single effect at the combined genotype data from the two cohorts were analyzed. Possible differences in the effect of the associated variants on treatment response between the three anti-TNF drug types were investigated, both by drug type stratification and inclusion of an interaction term in the linear regression model.

In order to increase confidence in the association at these three loci, they were genotyped in an additional 338 anti-TNF agents. These included two regions around the TNFAIP3 locus on chromosome 6q23,7–9 STAT4 on chromosome 1q23,7,10–12 Traf1-C5 on chromosome 9q,7,11,13 a locus encompassing the IL2 and IL21 genes on chromosome 4q27,7,14,15 PRKCB on chromosome 10p,15,7,16 KIF5A on 12q13,7,16 CD40 on 20q13,7,15 CCL21 on 9p13,17 CTLA4 on chromosome 2q, AFF3 also on chromosome 2q and CD226 on 8q22.15,17

Samples

The patient cohort consisted of patients with RA treated with anti-TNF drugs recruited from hospitals across the UK as part of the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS). These patients were originally recruited by the British Society for Rheumatology Biologics Register (BSRBR) and subsequently invited to participate in BRAGGSS, a study of genetic predictors of anti-TNF treatment. Inclusion criteria for enrolment in BRAGGSS were: (1) physician-diagnosed RA; (2) the patient must be registered with the BSRBR, either starting or already receiving treatment with one of the three anti-TNF drugs etanercept, infliximab or adalimumab; and (3) the patient is of Caucasian origin, thus avoiding potential spurious associations arising as a result of population stratification. Patients were excluded from the study if they had missing 28 joint count disease activity score (DAS28) data at either baseline or at follow-up (6 months) or if they had stopped treatment due to adverse events during the follow-up period. The first cohort of BRAGGSS patients used here comprised 1092 patients, while associations were investigated further in an additional 338 patients. Clinical and demographic characteristics for both cohorts are shown in table 1.

Genotyping

Genotyping was performed with 10 ng DNA using the Sequenom MassARRAY iPLEX system according to the manufacturer’s instructions (http://www.sequenom.com/). Duplicate DNA samples were genotyped as part of quality control (QC) assessments.

Analysis of data

Statistical analyses were performed in Stata Version 9.2 (StataCorp, College Station, Texas, USA) and in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/). QC of DNA samples and SNPs was performed by excluding those displaying <80% genotyping success. Multivariate linear regression analysis was used to assess the effect of each SNP genotype on response to treatment, using the continuous variable absolute change in DAS28 between baseline and 6-month follow-up as the outcome measure. Regression analyses were adjusted for confounding variables with a significant effect on anti-TNF treatment response: baseline DAS28, HAQ score, gender and concurrent DMARD therapy. Additive, genotypic, dominant and recessive models of inheritance were tested in Stata. p Values <0.05 were considered statistically significant and no corrections for multiple testing were performed. SNPs reaching statistical significance were genotyped in an additional cohort of patients with RA treated with anti-TNF drugs and the combined genotype data from the two cohorts were analyzed. Possible differences in the effect of the associated variants on treatment response between the three anti-TNF drug types were investigated, both by drug type stratification and inclusion of an interaction term in the linear regression model.

Table 1 Characteristics for first, additional and combined cohorts (1334 samples)

| Characteristics | First cohort (n=1012) | Additional cohort (n=322) | Combined cohort (n=1334) |
|-----------------|---------------------|--------------------------|-------------------------|
| M:F, n [%]      | 229 (22.6):783 (77.4) | 65 (20.2):257 (79.8) | 294 (22.1):1038 (77.9) |
| Mean (SD) age at baseline (years) | 56.5 (11.1) | 56.7 (10.5) | 56.6 (10.9) |
| Mean (SD) disease duration at baseline (years) | 13.9 (8.8) | 12.7 (10.2) | 13.6 (8.9) |
| Mean (SD) HAQ score at baseline | 2.05 (0.56) | 1.9 (0.6) | 2.0 (0.58) |
| Current smoker/ex-smoker/never smoked, n [%] | 171 (16.9):422 (41.7):409 (40.4) | 46 (14.3):135 (41.9):137 (42.6) | 217 (16.3):557 (41.8):544 (40.8) |
| Receiving concurrent DMARD therapy, n [%] | 720 (71.2) | 249 (77.3) | 968 (72.7) |
| Receiving concurrent steroid therapy, n [%] | 414 (40.9) | 127 (39.4) | 541 (40.6) |
| Biologic naïve, n [%] | 949 (93.8) | 298 (92.6) | 1246 (93.5) |
| Mean (SD) baseline DAS28 | 6.69 (0.98) | 6.53 (0.98) | 6.65 (0.98) |
| Mean (SD) change in DAS28 at 6-month follow-up | −2.47 (1.55) | −2.52 (1.40) | −2.48 (1.52) |

DAS28, 28-joint count disease activity score; DMARD, disease-modifying antirheumatic drug; HAQ, Health Assessment Questionnaire.
Table 2 Details of 18 confirmed RA susceptibility gene SNPs selected for genotyping

| Gene  | SNP       | Chr bp | Reason for selection                                      |
|-------|-----------|--------|-----------------------------------------------------------|
| AFF3  | rs10865035| 2 10022166 | Most associated T1D SNP associated with RA15             |
| AFF3  | rs1160542 | 2 10019857 | rs10865035 proxy (r²=0.967)                             |
| STAT4 | rs7574865 | 2 19167287 | Strongest association in US and UK studies16-12          |
| STAT4 | rs101816566| 2 19167814 | rs7574865 proxy (r²=0.951) +49 exon 17 A→G SNP implicated in autoimmunity13 |
| CTLA4 | rs231775  | 2 20444099 | Associated in US population17                          |
| CTLA4 | rs3087243 | 2 204447164| Associated with RA18                                     |
| IL12/IL21 | rs6822844 | 4 123728871| Most associated celiac disease SNP associated with T1D and RA14-15 |
| TNFAIP3 | rs13270033| 6 13800711 | Most strongly associated SNP in RA study9               |
| TNFAIP3 | rs13192841| 6 138008907| rs13270033 proxy (r²=1); second US SNP16                |
| TNFAIP3 | rs5029307 | 6 138236844| Intron 2 SNP                                             |
| CCL21 | rs2812378 | 9 34700260 | Most strongly associated SNP at locus7                   |
| TRAF1 | rs10760130| 9 122741811| Most strongly associated UK SNP11                       |
| TRAF1 | rs2900180 | 9 122746203| Most associated US SNP13                                |
| PRKCG | rs4750316 | 10 6433266 | Most associated SNP at locus14                          |
| KIF5A | rs1678542 | 12 56254982| Most strongly associated SNP at locus15                  |
| CD226 | rs763361  | 18 6568262 | Most associated T1D SNP associated with MS, AITD and RA17 |
| CD40  | rs4810485 | 20 4181354 | Most strongly associated SNP at locus7                   |

A1TID, autoimmune thyroid disease; bp, base pairs; Chr, chromosome; MS, multiple sclerosis; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism; T1D, type 1 diabetes.

DISCUSSION

This investigation of RA susceptibility loci in response to anti-TNF therapy is the largest study of genetic predictors of anti-TNF response performed to date. We have detected nominally significant effects at RA susceptibility variants mapping to the AFF3 and CD226 genes. The identification of an effect on treatment response conferred by polymorphism within a susceptibility gene is not surprising as there are several examples in the literature where complex disease susceptibility genes encode therapeutic targets. For example, in T2D, the established susceptibility gene PPARG encodes a protein which is a target for the thiazolidinedione drugs.20 In RA the drug abatacept, a CTLA4 analogue, was shown to be an effective therapy before the CTLA4 gene was unequivocally demonstrated as associated with susceptibility to RA.7 15 21 There are now examples of polymorphisms within susceptibility genes that influence response to treatment, such as variation in the T2D susceptibility gene TCF7L2 which predicts response to sulfonylureas.8

However, the association of CD226 and AFF3 variants with the anti-TNF response is weak, and the addition of these markers into predictive models including clinical variables has only a modest effect, increasing the R² value from 15.7% to 17.0%. This is in contrast to the large genetic effects seen in studies such as those of response to warfarin therapy. For example, genetic variants in the two genes VKORC1 and CYP2C9 account for about 40% of the variance in warfarin dose.22 In that case the genes were originally targeted as candidates because they were known to lie on the warfarin metabolic pathway, and it may be argued that major genetic effects on the anti-TNF response might be expected to arise from variation within genes implicated in the TNF pathway. However, previous investigations by our group have failed to detect an association between a number of such genes and treatment response.23 One notable exception is the association between the TNF−308 SNP and the response to etanercept, but not infliximab.24

Even with the warfarin story, although major genetic effects have been identified, inclusion of these into models with clinical variables remains only moderately predictive of warfarin dose required or time to stabilise international normalised ratio.25 A subsequent GWA study confirmed an association with VKORC1 and CYP2C9 loci and identified numerous signals which may represent other loci with smaller effects on warfarin requirements. Incorporation of these smaller effects may be required to develop accurate models of prediction.26 Hence, the weak effects detected in the current study may yet prove to be clinically important when combined with other predictors of response to anti-TNF therapy.

In order to detect subtle effects, studies must be adequately powered. One of the most important strengths of this study is the large sample size employed; almost all similar investigations (with notable exceptions25 26 24) have focused on <500 patients with RA. The current sample size provided very high power (>99%) to detect a change in DAS28 of 0.6 units at minor allele frequency ≥0.05. We are therefore confident in excluding modest effects at the variants that did not demonstrate evidence of association in our study.
### Table 3  DAS28 response data by genotype and association p values for 17 successfully genotyped SNPs in 1012 individuals

| SNP   | Gene   | Chr | bp   | Genotype | Count | Mean baseline DAS28 | SD baseline DAS28 | Mean change in DAS28 | SD change in DAS28 | HWE exact p value | Genotypic global p value | Additive model | Dominant model* |
|-------|--------|-----|------|----------|-------|---------------------|-------------------|---------------------|-------------------|-----------------|---------------------------|----------------|-----------------|
|       |        |     |      |          |       |                     |                   |                     |                   |                 |                           |                |                 |
| rs10865035 | AFF3    | 2   | 100202166 | AA       | 240   | 6.72                | 1.02              | -2.28               | 1.57              | 0.089           | 0.051                     | 0.018          | -0.16           |
|        | AG     |     | 532   | 6.69    | 0.96   | -2.51               | 1.51              |                     |                   |                 |                           |                |                 |
|        | GG     |     | 237   | 6.68    | 0.99   | -2.58               | 1.63              |                     |                   |                 |                           |                |                 |
| rs1160542 | AFF3    | 2   | 100198587 | AA       | 247   | 6.70                | 0.97              | -2.59               | 1.61              | 0.050           | 0.066                     | 0.022          | 0.15            |
|        | AG     |     | 530   | 6.69    | 0.96   | -2.49               | 1.51              |                     |                   |                 |                           |                |                 |
|        | GG     |     | 221   | 6.68    | 1.00   | -2.25               | 1.56              |                     |                   |                 |                           |                |                 |
| rs7574865 | STAT4   | 2   | 191672878 | GG       | 564   | 6.73                | 0.99              | -2.57               | 1.55              | 0.400           | 0.051                     | 0.094          | 0.13            |
|        | GT     |     | 389   | 6.65    | 0.95   | -2.33               | 1.58              |                     |                   |                 |                           |                |                 |
|        | TT     |     | 57    | 6.64    | 1.10   | -2.49               | 1.40              |                     |                   |                 |                           |                |                 |
| rs10181856 | STAT4   | 2   | 1916781124 | CC       | 563   | 6.73                | 0.98              | -2.57               | 1.54              | 0.559           | 0.067                     | 0.122          | 0.12            |
|        | CG     |     | 389   | 6.65    | 0.95   | -2.33               | 1.60              |                     |                   |                 |                           |                |                 |
|        | GG     |     | 60    | 6.63    | 1.07   | -2.50               | 1.37              |                     |                   |                 |                           |                |                 |
| rs231775 | CTLA4   | 2   | 20440499 | AA       | 365   | 6.68                | 1.00              | -2.50               | 1.53              | 0.794           | 0.775                     | 0.500          | 0.04            |
|        | AG     |     | 482   | 6.69    | 0.98   | -2.44               | 1.52              |                     |                   |                 |                           |                |                 |
|        | GG     |     | 165   | 6.73    | 0.94   | -2.52               | 1.72              |                     |                   |                 |                           |                |                 |
| rs3087243 | CTLA4   | 2   | 204447164 | GG       | 340   | 6.67                | 0.97              | -2.53               | 1.64              | 0.948           | 0.712                     | 0.570          | 0.04            |
|        | GA     |     | 492   | 6.74    | 0.96   | -2.46               | 1.49              |                     |                   |                 |                           |                |                 |
|        | AA     |     | 178   | 6.61    | 1.05   | -2.41               | 1.57              |                     |                   |                 |                           |                |                 |
| rs6822844 | IL2-IL21 | 4   | 123728871 | GG       | 729   | 6.69                | 0.95              | -2.45               | 1.58              | 0.151           | 0.376                     | 0.317          | -0.09           |
|        | GT     |     | 254   | 6.68    | 1.05   | -2.52               | 1.50              |                     |                   |                 |                           |                |                 |
|        | TT     |     | 31    | 7.00    | 0.84   | -2.70               | 1.33              |                     |                   |                 |                           |                |                 |
| rs13192841 | TNFAIP3 | 6   | 138008907 | GG       | 577   | 6.66                | 0.98              | -2.50               | 1.59              | 0.672           | 0.406                     | 0.469          | 0.05            |
|        | GA     |     | 371   | 6.72    | 0.99   | -2.41               | 1.51              |                     |                   |                 |                           |                |                 |
|        | AA     |     | 64    | 6.77    | 0.92   | -2.60               | 1.48              |                     |                   |                 |                           |                |                 |

**First cohort (n=1012)**
**Table 3 Continued**

| SNP     | Gene    | Chr | bp   | Genotype | Count | Mean baseline DAS28 | SD baseline DAS28 | Mean change in DAS28 | SD change in DAS28 | HWE exact p value | Genotypic global p value | Additive model | Dominant model* |
|---------|---------|-----|------|----------|-------|---------------------|-------------------|----------------------|-------------------|-----------------|-----------------------|----------------|-----------------|
| **First cohort (n=1012)** | | | | | | | | | | | | | |
| rs6920220 | TNFAIP3 | 6   | 138048197 | GG       | 525   | 6.69                | 0.96              | −2.49                | 1.49              | 0.430           | 0.536                 | 0.314          | 0.07            |
|          |         |      |         | GA       | 413   | 6.69                | 1.00              | −2.44                | 1.61              |                |                       |                |                 |
|          |         |      |         | AA       | 71    | 6.68                | 1.02              | −2.43                | 1.68              |                |                       |                |                 |
| rs5029937 | TNFAIP3 | 6   | 138236844 | GG       | 900   | 6.67                | 0.98              | −2.44                | 1.54              | 0.763           | 0.463                 | 0.216          | −0.18           |
|          |         |      |         | GT       | 105   | 6.91                | 0.92              | −2.73                | 1.64              |                |                       |                |                 |
|          |         |      |         | TT       | 2     | 7.46                | 0.35              | −3.43                | 1.01              |                |                       |                |                 |
| rs2812378 | CCL21   | 9   | 34700260 | TT       | 379   | 6.67                | 1.02              | −2.42                | 1.53              | 0.386           | 0.876                 | 0.637          | −0.03           |
|          |         |      |         | TC       | 490   | 6.70                | 0.95              | −2.51                | 1.56              |                |                       |                |                 |
|          |         |      |         | CC       | 140   | 6.74                | 0.96              | −2.48                | 1.62              |                |                       |                |                 |
| rs10760130 | TRAF1   | 9   | 122741811 | AA       | 313   | 6.71                | 0.99              | −2.39                | 1.58              | 0.798           | 0.457                 | 0.981          | 0.00            |
|          |         |      |         | AG       | 492   | 6.68                | 0.94              | −2.54                | 1.53              |                |                       |                |                 |
|          |         |      |         | GG       | 200   | 6.71                | 1.07              | −2.41                | 1.57              |                |                       |                |                 |
| rs2900180 | TRAF1   | 9   | 122746203 | CC       | 425   | 6.73                | 0.98              | −2.47                | 1.61              | 0.077           | 0.730                 | 0.592          | 0.04            |
|          |         |      |         | CT       | 441   | 6.65                | 0.95              | −2.50                | 1.49              |                |                       |                |                 |
|          |         |      |         | TT       | 148   | 6.70                | 1.05              | −2.41                | 1.57              |                |                       |                |                 |
| rs4750316 | PRKCQ   | 10  | 6433266  | GG       | 695   | 6.71                | 0.95              | −2.54                | 1.56              | 0.510           | 0.346                 | 0.387          | 0.07            |
|          |         |      |         | GC       | 283   | 6.67                | 1.01              | −2.33                | 1.50              |                |                       |                |                 |
|          |         |      |         | CC       | 33    | 6.43                | 1.23              | −2.19                | 1.80              |                |                       |                |                 |
| rs1678542 | KIF5A   | 12  | 56254982 | CC       | 419   | 6.72                | 0.98              | −2.50                | 1.55              | 0.448           | 0.724                 | 0.600          | 0.04            |
|          |         |      |         | CG       | 473   | 6.65                | 0.96              | −2.44                | 1.55              |                |                       |                |                 |
|          |         |      |         | GG       | 119   | 6.74                | 1.05              | −2.49                | 1.56              |                |                       |                |                 |
| rs763361  | CD226   | 18  | 65602622 | TT       | 272   | 6.65                | 1.00              | −2.64                | 1.50              | 0.900           | 0.050                 | 0.016          | 0.16            |
|          |         |      |         | TC       | 508   | 6.69                | 0.96              | −2.45                | 1.57              |                |                       |                |                 |
|          |         |      |         | CC       | 232   | 6.75                | 0.98              | −2.32                | 1.57              |                |                       |                |                 |
| rs4810485 | CD40    | 20  | 44181354 | GG       | 607   | 6.68                | 0.95              | −2.50                | 1.54              | 0.048           | 0.398                 | 0.221          | 0.10            |
|          |         |      |         | GT       | 358   | 6.68                | 1.01              | −2.40                | 1.56              |                |                       |                |                 |
|          |         |      |         | TT       | 35    | 6.90                | 0.97              | −2.46                | 1.65              |                |                       |                |                 |

Significant p values (<0.05) are shown in bold type, italic indicates p<0.10.

*Results for the recessive model are not shown; only rs1160542 (AFF3) demonstrated association with treatment response under a recessive model (coef 0.23 (0.01, 0.45), p=0.037).

bp, base pairs; Chr, chromosome; Coef, coefficient for minor allele; DAS, disease activity score; HWE, Hardy–Weinberg equilibrium; SNP, single nucleotide polymorphism.
The associated rs10865035 SNP maps to the 5’ upstream region of AFF3 located on chromosome 2q11. Interestingly, the SNP was associated not only with change in the DAS28 but also with EULAR response criteria; indeed, for the latter analysis, the association remained statistically significant even after applying a stringent Bonferroni correction ($p=0.049$). The gene, also known as FMR2, is preferentially expressed on lymphoid cells and encodes a family of transcription factors that are thought to be implicated in the function of the lymphoid system.27 We speculate that variation in AFF3 may lead to an upregulated inflammatory response by lymphocytes, resulting in more circulating proinflammatory molecules and leading to a reduced response to TNF antagonists; further studies will be required to explore this.

The CD226 gene maps to chromosome 18q22 and encodes a type I membrane protein molecule expressed on the surface of haematopoietic cells which is involved in the triggering of both T and NK cell cytotoxicity. The associated variant (rs765361) is a non-synonymous SNP encoding a glycine to serine substitution in the CD226 gene which maps to chromosome 18q22 and encodes a family of transcription factors that are thought to be involved in the function of the lymphoid system.27 We speculate that variation in AFF3 may lead to an upregulated inflammatory response by lymphocytes, resulting in more circulating proinflammatory molecules and leading to a reduced response to TNF antagonists. The CD226 gene may lead to greater proinflammatory molecule production, thereby explaining why there is a reduced response to anti-TNF drugs.

A limitation of the current investigation is that no correction for multiple testing was applied and, if it was applied, the associations with change in DAS28 would not remain statistically significant (AFF3, $p=0.195$; CD226, $p=0.624$). It is therefore possible that the findings of this study are validated in an independent cohort, such validation was beyond the scope of the current study. Our strategy was to maximise sample size by genotyping all available DNA samples rather than splitting the cohort into test and confirmatory data sets. It is therefore possible that the associations may have arisen due to a type I error. However, our findings are in keeping with those in other complex diseases, in that susceptibility genes may also influence treatment response.5

In summary, we provide evidence for a weak association between SNPs in the AFF3 and CD226 RA susceptibility loci and response to anti-TNF treatment in patients with RA. The percentage of the variance explained by these genetic markers is only 1.3%. It is too early to say whether the response to anti-TNF treatment will be confered through a number of genes, each with a small effect size, or whether genes exist that predict a large percentage of variance to treatment. Candidate gene studies have had limited success, however, in identifying predictors. We hypothesise that the response to treatment is polygenic and that well-powered GWA studies should be able to identify a genetic signature to identify those patients most—or, indeed, least—likely to benefit from these expensive but effective therapies.

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**Competing interests** None.

**Ethics approval** This study was approved by the South Manchester ethics committee (COREC 04/Q1403/37).

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Patient consent** Obtained.

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**Table 4** DAS28 response data by genotype and association $p$ values for three successfully genotyped SNPs in 1334 individuals.

| Gene      | Chr | bp    | Genotype | Global $p$ value | Additive model | Dominant model |
|-----------|-----|-------|----------|------------------|----------------|----------------|
| rs10865035 | 5   | 10202166 | AA       | 0.015            | 0.228          | 0.228          |
| rs7574865  | 2   | 191672878 | GG       | 0.469            | 0.173          | 0.497          |
| rs763361   | 18  | 65682622  | TT       | 0.622            | 0.141          | 0.622          |

Significant $p$ values (<0.05) are shown in bold type, italic indicates $p<0.10$.
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Corrections

The department of one of the authors who co-authored all of the below papers has found that the affiliations were not correct. The correct affiliations for Professor P Emery, for all of the below articles, are: 1Section of Musculoskeletal Disease, Leeds Institute of Molecular Medicine, University of Leeds; 2NIHR Leeds Musculoskeletal Biomedical Research Unit, Leeds Teaching Hospitals Trust, Leeds, UK.