Research Article

For reprint orders, please contact: reprints@futuremedicine.com

Pharmacogenomics

Previously reported *PDE3A–SLCO1C1* genetic variant does not correlate with anti-TNF response in a large UK rheumatoid arthritis cohort

Aim: A genetic variant has recently reached genome-wide significance for association with TNF-inhibitor response in rheumatoid arthritis patients. Here we undertake a replication study in a UK Caucasian population to test for association with TNF-inhibitor response. **Materials & methods:** The genetic variant, rs3794271, located within the *PDE3A–SLCO1C1* locus was analyzed for correlation with treatment response using both the EULAR classification criteria and absolute change in (Δ) DAS28 scores as outcome measures. **Results:** Genotype data were available from 1750 TNF-inhibitor treated individuals. However, no evidence for association was observed (EULAR: p = 0.91 and ΔDAS28: p = 0.93). Furthermore, no significant associations were observed upon stratification by the anti-TNF received (p > 0.05). **Conclusion:** In the largest replication cohort conducted to date, no evidence for association was observed.

First draft submitted: 30 October 2015; Accepted for publication: 7 March 2016; Published online: 16 May 2016

Keywords: biomarker • rheumatoid arthritis • single nucleotide polymorphism • TNF inhibitors • treatment response

Samantha Louise Smith[1], Darren Plant[2], Xiu Hue Lee[1], Jonathan Massey[1], Kimme Hyrich[3], Ann W Morgan[4], Anthony G Wilson[5], John Isaacs[6] & Anne Barton*[,1,2]

[1]Arthritis Research UK, Centre for Genetics & Genomics, Centre for Musculoskeletal Research, Manchester Academic Health Sciences Centre, The University of Manchester, Manchester, UK

[2]NIHR Manchester Musculoskeletal BRU, Central Manchester Foundation Trust & University of Manchester, Manchester Academic Health Science Centre, Manchester, UK

[3]Arthritis Research UK, Centre for Epidemiology, Centre for Musculoskeletal Research, Manchester Academic Health Sciences Centre, The University of Manchester, Manchester, UK

[4]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds & NIHR Leeds Musculoskeletal Biomedical Research Unit, Leeds Teaching Hospitals NHS Trust, UK

[5]UCD School of Medicine & Medical Science, Conway Institute, University College Dublin, Dublin, Ireland

[6]NIHR Newcastle Biomedical Research Centre, Newcastle upon Tyne NHS Foundation Trust & Newcastle University, Newcastle-upon-Tyne, UK

*Author for correspondence:
Tel.: +44 161 275 1638
Fax: +44 161 275 5043
anne.barton@manchester.ac.uk

Genome-wide association studies (GWAS) have proven highly successful in the identification of rheumatoid arthritis (RA) susceptibility loci; it was therefore hypothesized that this strategy would also prove successful in the identification of genetic variants predictive of treatment response in RA. To date, six small-scale GWAS have been conducted investigating response to TNF-inhibitor biologics [1–6], with little consistency in the findings. However, a SNP at the *PDE3A–SLCO1C1* locus on chromosome 12 (rs3794271) correlating with EULAR response ($p = 3.5 \times 10^{-6}$) in a Danish GWAS (n = 196) [3] has since been replicated in a Spanish RA population (n = 315; $p = 1.74 \times 10^{-5}$) [7]. On meta-analysis of both cohorts, the strength surpassed genome-wide significance thresholds ($p = 3.3 \times 10^{-10}$) [7]. It was estimated that the SNP may account for 10% of the variance observed in treatment response to TNF inhibitors, thereby potentially possessing clinical utility (if used in an algorithm). It is important that further replication be attempted in order to confirm this association in other populations.

The aim of this research was, therefore, to replicate the genome-wide significant genetic association observed at the *PDE3A–SLCO1C1* locus in a larger sample cohort of UK Caucasian RA patients receiving a TNF-inhibitor biologic drug.

Materials & methods

Patient selection

DNA samples from Caucasian patients with RA were selected from BRAGGSS, a prospective longitudinal cohort study, recruiting RA patients across the UK who are about to commence/currently receiving treatment with biologic drugs, described in detail previously [8]. Twenty-eight joint-count disease

Future Medicine part of fsg

activity scores (DAS28) using four variables (the number of tender and swollen joints, erythrocyte sedimentation rate [ESR]/C-reactive protein [CRP] and patient global assessment score) are recorded prior-to and at 3, 6 and 12 months thereafter [9]. The BRAGGSS study was approved by a multicenter ethics committee (COREC 04/Q1403/37).

Definition of treatment response

Response to treatment was assessed using two measures. First, using the EULAR response criteria at 3 or 6 months following treatment, patients were classified into either non-responders (ΔDAS28 score ≤0.6 or 0.6–1.2 and an end score of >5.1), good responders (ΔDAS28 score >1.2 and an end score of ≤3.2) or moderate responders (anything in-between). Second, absolute change in DAS28 scores ($DAS28_{3/6\text{-month}}$ – $DAS28_{baseline}$), a continuous variable, was also used to assess outcome.

Genotyping

Genetic data were extracted from six genome-wide genetic datasets (Affymetrix®, CA, USA; Illumina®, CA, USA). Per platform, sample quality control (QC) involved the removal of ethnic outliers and closely related individuals, while SNP QC involved the removal of SNPs with greater than 5% missing data, SNPs with Hardy–Weinberg p-value of $<5.7 \times 10^{-7}$ and SNPs with minor allele frequencies (MAFs) less than 1%. The remaining SNPs were imputed using the 1000 Genomes reference panel using the IMPUTE2 software. SNPs with high imputation accuracy, in other words, information (INFO) score greater than 0.9 were retained and a second round of QC using the above pipeline was performed. Finally, the SNPs identified from these analyses were extracted.

Statistical analysis

The test for association between the *PDE3A–SLCO1C1* locus and response to TNF-inhibitor treatment was conducted using PLINK version 1.07 [10,11]. Power calculations were performed using Quanto (version 2.4) [12].

The primary test for association was with EULAR response in a logistic regression model, excluding moderate responders. Both univariate and multivariate models were assessed; the multivariate model included baseline DAS28, gender, baseline health assessment questionnaire (HAQ) scores and concurrent disease-modifying anti-rheumatic drug (DMARD) used as covariates. Furthermore, in order to investigate any drug-specific associations, the analyses were repeated following stratification by the TNF-inhibitor drug received.

Finally, SNP dosage data were correlated with absolute change in DAS28 scores (ΔDAS28) in linear regression models (both univariate and multivariate) and the analysis was repeated following stratification by the TNF-inhibitor drug received.

Results

Following QC and the exclusion of patients not receiving TNF-inhibitor biologics, 1750 Caucasian RA patients were available for association analysis. The current study had greater than 95% power to identify the same effect sizes as reported in the previously reported *PDE3A–SLCO1C1* studies [3,7], assuming a minor allele frequency (MAF) of 0.40 for rs3794271 at the 5% significance threshold. The baseline characteristics for the cohort are shown in the table below (Table 1).

The *PDE3A–SLCO1C1* variant, rs3794271, failed to genotype in less than 1% of individuals across the entire cohort; furthermore, the *PDE3A–SLCO1C1* variant was imputed with INFO scores greater than 0.96. The minor G allele of rs3794271, in our study had a MAF of 0.40, which is comparable to the MAF reported by the 1000 Genomes project database for European populations (MAF: 0.36) and those reported by the Danish and Spanish cohorts (MAFs of 0.35 and 0.34, respectively).

For the primary analysis, genotype frequencies for the *PDE3A–SLCO1C1* variant, rs3794271, were compared between EULAR non-responder (n = 359) and good responder (n = 646) patients only. However, no evidence for association was observed (Table 2). Stratifying the analyses by the drug taken showed no significant associations ($p > 0.05$). Specifically, the multivariate p-values for the *PDE3A–SLCO1C1* variant, rs3794271, in the etanercept (n = 323) and infliximab (n = 254) subgroups were 0.52 and 0.74, respectively.

For the secondary analysis of change in DAS28, genotype data from 1750 patients were available for analysis. Following linear regression, no significant associations with treatment response were observed (Table 2). Following stratification by the anti-TNF received, again no significant associations were observed ($p > 0.05$).

Discussion

In this large, well-powered replication cohort from the UK, no evidence for association between the *PDE3A–SLCO1C1* variant, rs3794271 and response to TNF-inhibitor biologics was observed using either the EULAR response criteria or ΔDAS28 as outcome measures. This is in contrast to previous studies where statistically significant associations with EULAR response have been observed (*PDE3A–SLCO1C1*, $p = 3.5 \times 10^{-6}$ and $p = 1.74 \times 10^{-5}$; combined meta-

Table 1. Baseline characteristics of the 1750 Caucasian rheumatoid arthritis patients analyzed in terms of treatment response.

Cohort characteristics	Non-responders	Moderate responders	Good responders
Observations, n (%)	359 (20.5%)	745 (42.6%)	646 (36.9%)
Gender (F), n (%)	292 (81.3%)	587 (78.8%)	473 (73.2%)
Age at baseline (years), me (SD)	56.5 (11.53)	58 (10.96)	55.8 (11.67)
Concurrent DMARDs, n (%)	270 (75.2%)	580 (77.9%)	575 (89%)
Baseline DAS28, me (SD)	6.27 (1.12)	6.59 (0.92)	6.13 (0.88)
End-point DAS28, me (SD)	5.87 (1.17)	4.3 (0.77)	2.31 (0.65)
Change in DAS28, me (SD)	-0.40 (1.02)	-2.29 (0.85)	-3.82 (1.01)
Baseline TJC, med (IQR)	15 (9–21)	16 (11–23)	15 (10–21)
Baseline SJC, med (IQR)	9 (5–14)	10 (6–15)	9.5 (6–14)
Baseline HAQ, med (IQR), n	2.13 (1.75–2.38), 327	2 (1.63–2.38), 693	1.88 (1.25–2.13), 609
Treated with infliximab, n (%)	119 (33.2%)	206 (27.7%)	136 (21.1%)
Smoking status, n (%)†	43 (15.52%)	85 (15.86%)	79 (17.48%)
Treated with adalimumab, n (%)	104 (29%)	188 (25.2%)	255 (39.5%)
Treated with etanercept, n (%)	118 (32.9%)	303 (40.7%)	205 (31.7%)
Treated with golimumab, n (%)	5 (1.4%)	11 (1.5%)	9(1.4%)
Treated with certolizumab, n (%)	13 (3.6%)	37 (5%)	41 (6.3%)

†Smoking status for current smokers (based upon the 1265 patients with smoking data available.
DAS28: Disease activity score in 28-joint; DMARD: Disease modifying anti-rheumatic drug; F: Female; HAQ: Health assessment questionnaire; IQR: Interquartile range; me: Mean; med: Median; SD: Standard deviation; SJC: Swollen joint count in 28-joint; TJC: Tender joint count in 28-joint.

analysis, $p = 3.3 \times 10^{-10}$) [3,7]; the strength surpassing genome-wide significance upon meta-analysis.

The current study of 1750 patients is by far the largest replication study conducted to date, resulting in adequate power (>95%) to detect the same effect sizes as previously reported. For comparison, the Danish and Spanish *PDE3A–SLCO1C1* cohorts comprised 196 and 315 RA patients, respectively, and were further reduced to 135 and 182 patients, respectively, following the exclusion of EULAR moderate responders.

A number of reasons could explain the discrepancy between the current study and the previously reported associations. First, the confounding variables accounted for within the current multivariate analysis [13] were not all accounted for in the previous studies; however, no significant associations were observed within the current univariate and multivariate models suggesting the confounding variables did not explain the differences. Second, the associations observed in the Danish and Spanish cohorts may be population specific. Third, of contrast to the previous reports, treatment response in the current study was assessed at two timepoints. 6 months (n = 1573) and 3 months (n = 177); as compared with the Danish *PDE3A–SLCO1C1* cohort and Spanish cohort where response was assessed at 14 and 12 weeks, respectively [3,7]. However, it has been reported that EULAR moderate and good responders peak following 12 weeks of treatment and remain relatively stable thereafter [14]. Further stratification by the assessment timepoint did not significantly alter the findings ($p > 0.05$; data not shown).

Furthermore, heterogeneity existed within the current study as to how the DAS28 scores were calculated (by using either ESR or CRP within the DAS28

Table 2. Summary statistics of the SNP under investigation for response with anti-TNF treatment.

	EULAR p-value; OR (95% CI)		ΔDAS28 p-value; β-coefficient (95% CI)	
SNP	Univariate model	Multivariate model	Univariate model	Multivariate model
rs3794271 (*PDE3A–SLCO1C1*)	0.91; 1.01 (0.84–1.22)	0.74; 1.04 (0.85–1.27)	0.93; -0.0047 (-0.11–0.10)	0.69; -0.0203 (-0.12–0.081)

Association analysis between anti-TNF response in UK Caucasian RA patients and genotype data for rs3794271 was performed using univariate and multivariate models. The resulting p-values were calculated using additive regression models.
OR: Odds ratio.

calculation). Previous studies have suggested that CRP more accurately reflects inflammation, as ESR can be influenced by age, gender and ethnicity [15,16]. However, it has been demonstrated that use of either inflammatory marker generally results in classifying patients into the same EULAR phenotype with 82.4% agreement and where discrepancies did occur, they were moderate in magnitude [15]. Indeed, where both inflammatory markers were available within the current study an overall agreement of 77.4% in classifying EULAR response was observed (similarly, discrepancies were moderate in magnitude). Further analysis following stratification by the inflammatory marker utilized in the DAS28 calculation did not materially alter the conclusions (data not shown).

Of note, two TNF inhibitors, certolizumab and golimumab, were included in the current study but not in either of the previous reports. However, as they represent a small proportion of the total cohort, 5.2 and 1.43%, respectively, this is unlikely the reason for the lack of replication. Stratification by the anti-TNF received, resulted in non-significant associations for *PDE3A–SLCO1C1* ($p > 0.05$), which is in contrast to the previously reported study by Acosta-Colman *et al.,* which reported that infliximab and etanercept were driving the association with EULAR response [7].

Conclusion

In summary, the largest replication cohort tested to date has found no evidence for association of the *PDE3A–SLCO1C1* locus with TNF-inhibitor response in a UK Caucasian RA population. The lack of association is disappointing given that this locus was the first to exceed genome-wide significance thresholds and has shown consistent evidence for association in two independent European cohorts.

Future perspective

Since the introduction of biological therapies, which have proven highly effective in the treatment of rheumatoid arthritis, the need for biomarkers predictive of treatment response has become even more pressing. However, as of yet, a single biomarker which can accurately predict treatment response has thus far alluded us; with these therapies prescribed on what is essentially a trial and error basis. Such an approach to prescribing biological therapies unsurprisingly therefore, results in a significant proportion of patients not responding satisfactorily to treatment. In the era of stratified medicine, various approaches are being adopted in the search for predictive biomarkers, these include amongst others; genetic, transcriptomic and proteomic studies. However, it is becoming more apparent, that these individualized approaches will not be enough to guide treatment decisions. Rather, systems-based approaches which integrate genetic, transcriptomic, proteomic, clinical and demographic data into predictive algorithms are now being adopted and will most likely be used in clinical settings.

Executive summary

Biological therapies revolutionized the treatment of rheumatoid arthritis, however, currently there is not a biomarker capable of accurately predicting treatment

- Currently biologics are prescribed on a trial and error basis resulting in up to 40% of patients receiving them responding unsatisfactorily.
- Early and effective treatment is key in-order to optimize patient outcome and reduce/limit irreversible joint damage.
- Biologics are costly (costing ~GB£10,000 per patient per year) and are associated with adverse events.
- There is therefore an urgent need to identify biomarkers predictive of response.

Previously, a genetic variant at the *PDE3A–SLCO1C1* locus, rs3794271, had shown replicative evidence in two independent studies for association with treatment response

- The association reached genome-wide significance upon meta-analysis of the Spanish and Danish cohorts.

Replication in a large UK population of rheumatoid arthritis patients found no evidence for association

- Replication was conducted in 1750 UK rheumatoid arthritis patients using both the EULAR classification criteria and absolute changes in DAS28 scores (ΔDAS28) as outcome measures.
- However, we found no evidence to support the use of rs3794271 as a biomarker predictive of response to anti-TNF biologics (EULAR, $p = 0.91$; ΔDAS28, $p = 0.93$).
- Drug-specific effects were also investigated; however, similar observations were observed (i.e., no association as shown by a p-value > 0.05).

Conclusion

- In the largest replication cohort conducted to date, no evidence of association was found to support the use of rs3794271 as a clinically useful predictor of response to anti-TNF biologics in rheumatoid arthritis patients from the UK.

Acknowledgements

We are very grateful to all the patients who consented to participate in this study and to all the nurses who helped collect the samples for this important study.

Financial & competing interests disclosure

SL Smith was funded by an investigator-initiated award to A Barton from Pfizer (award number WS1940162). J Massey is funded by the MRC/Arthritis Research UK stratified medicine award (MATURA, MR/K015346/1). The work is supported by the NIHR's Manchester Musculoskeletal Biomedical Research Unit, Leeds Musculoskeletal Biomedical Research Unit and Newcastle's Biomedical Research Centre. The views expressed in this publication are those of the author(s) and not necessarily of the NHS; the NIHR or the Department of Health. We also thank Arthritis Research for their support (grant number 20385). The authors would also like to acknowledge the assistance given by IT Services and the use of the Computational Shared Facility at The University of Manchester. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained the appropriate institutional review board approval or have followed the principals outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Liu C, Batliwalla F, Li W *et al.* Genome-wide association scan identifies candidate polymorphisms associated with differential response to anti-TNF treatment in rheumatoid arthritis. *Mol. Med.* 14(9–10), 575–81 (2008).

• **This was the first genome-wide association study to investigate biomarkers associated with response to anti-TNF biologics in rheumatoid arthritis.**

2 Plant D, Bowes J, Potter C *et al.* Genome-wide association study of genetic predictors of anti-tumor necrosis factor treatment efficacy in rheumatoid arthritis identifies associations with polymorphisms at seven loci. *Arthritis Rheum.* 63(3), 645–53 (2011).

• **Genome-wide association study conducted using a UK population of rheumatoid arthritis patients about to commence treatment with/currently receiving anti-TNF biologics.**

3 Krintel SB, Palermo G, Johansen JS *et al.* Investigation of single nucleotide polymorphisms and biological pathways associated with response to TNFalpha inhibitors in patients with rheumatoid arthritis. *Pharmacogenet. Genomics* 22(8), 577–589 (2012).

•• **This genome-wide association study was the first to find evidence for association of rs3794271 with response to anti-TNF biologics in a Danish population and was the basis for a replication study conducted by Acosta Colman *et al.***

4 Umicevic MM, Cui J, Vermeulen SH *et al.* Genome-wide association analysis of anti-TNF drug response in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* 72(8), 1375–1381 (2013).

5 Cui J, Stahl EA, Saevarsdottir S *et al.* Genome-wide association study and gene expression analysis identifies CD84 as a predictor of response to etanercept therapy in rheumatoid arthritis. *PLoS Genet.* 9(3), e1003394 (2013).

6 Julià A, Fernandez-Nebro A, Blanco F *et al.* A genome-wide association study identifies a new locus associated with the response to anti-TNF therapy in rheumatoid arthritis. *Pharmacogenomics J.* 16(2), 147–150 (2016).

7 Acosta-Colman I, Palau N, Tornero J *et al.* GWAS replication study confirms the association of *PDE3A–SLCO1C1* with anti-TNF therapy response in rheumatoid arthritis. *Pharmacogenomics* 14(7), 727–734 (2013).

•• **This is the candidate replication study in which rs3794271 successfully replicated in a Spanish cohort of rheumatoid arthritis patients and where upon meta-analysis of both the Danish and Spanish cohorts, the association exceeded genome-wide significance thresholds for association.**

8 Potter C, Hyrich KL, Tracey A *et al.* Association of rheumatoid factor and anti-cyclic citrullinated peptide positivity, but not carriage of shared epitope or PTPN22 susceptibility variants, with anti-tumour necrosis factor response in rheumatoid arthritis. *Ann. Rheum. Dis.* 68(1), 69–74 (2009).

9 Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum.* 38(1), 44–48 (1995).

10 Purcell S, Neale B, Todd-Brown K *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81(3), 559–575 (2007).

11 PLINK (version 1.07) 2007. http://pngu.mgh.harvard.edu/purcell/plink/

12 Quanto (version 1.2.4) 2009. http://hydra.usc.edu/gxe

13 Hyrich KL, Watson KD, Silman AJ, Symmons DP. Predictors of response to anti-TNF-alpha therapy among patients with rheumatoid arthritis: results from the British Society for Rheumatology Biologics Register. *Rheumatology (Oxford)* 45(12), 1558–1565 (2006).

14 van de Putte LB, Atkins C, Malaise M *et al.* Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. *Ann. Rheum. Dis.* 63(5), 508–516 (2004).

15 Wells G, Becker JC, Teng J *et al.* Validation of the 28-joint Disease activity score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann. Rheum. Dis.* 68(6), 954–960 (2009).

16 Jurado RL. Why shouldn't we determine the erythrocyte sedimentation rate? *Clin. Infect. Dis.* 33(4), 548–549 (2001).