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ORIGINAL ARTICLE

Association of a complement receptor 1 gene variant with baseline erythrocyte sedimentation rate levels in patients starting anti-TNF therapy in a UK rheumatoid arthritis cohort: results from the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate cohort

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Eligibility for anti-tumour necrosis factor (TNF) therapy in most European countries is restricted to severe, active rheumatoid arthritis (RA). The DAS28 score is a marker of disease severity and incorporates one of two inflammatory markers, erythrocyte sedimentation rate (ESR) or C-reactive protein. We aimed to determine the relation between genetic variants known to affect ESR and levels of ESR in patients with active RA. DNA samples were genotyped for four single-nucleotide polymorphisms (SNPs) rs7527798 (CR1L), rs6691117 (CR1), rs10903129 (TMEM57) and rs1043879 (C1orf63). The association between SNPs and baseline ESR, baseline DAS28-ESR, and change in DAS28-ESR was evaluated. Baseline ESR was significantly associated with *CR1* rs6691117 genotype ($P = 0.01$). No correlation was identified between baseline DAS28-ESR or change in DAS28-ESR. In conclusion, genetic variation in the gene encoding *CR1* may alter ESR levels but not DAS28-ESR, indicating no adjustment for *CR1* genotype is required in the assessment of patients with severe active RA.

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INTRODUCTION

The development of biologic drugs that block the tumour necrosis factor (TNF) pathway has revolutionized rheumatoid arthritis (RA) treatment and patient prognosis. Anti-TNF drugs reduce joint inflammation, diminish radiological damage and may reduce cardiovascular risk.[1,2] However, due to increased risk of infection, inefficacy in a subset of patients and the economic impact, predictors of treatment response would be a major clinical advance.[3] In the United Kingdom, eligibility for biologics is determined by guidance issued by the National Institute for Health and Clinical Excellence (NICE).[4] Eligibility to commence and maintain treatment with anti-TNF therapy is determined by the 28 joint-count disease activity score (DAS28).[5] The DAS28 is an assessment used to measure the level of disease activity in patients with RA and has been validated in several studies.[6–8] A score of $\geqslant 5.1$ on two separate occasions at least 1 month apart is required before UK patients are eligible for anti-TNF therapy.

DAS28 is a composite index of RA disease activity, which includes number of swollen and tender joints in 28 specified joints, patient global health as measured by a visual analogue scale and one of two inflammatory markers, erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP).[6] CRP is an acute-phase protein that is produced by the liver and is very sensitive to short-term changes in inflammation.[9] ESR is measured by the rate at which red blood cells sediment over 1 h and is reported in mm h^{-1}. In contrast to CRP, raised ESR indicates long-standing, chronic inflammation and is an indirect measure of acute-phase protein levels with a slower response after inflammatory stimulation or resolution.[9] ESR is increased in a variety of conditions including pregnancy, myeloma, anaemia and is further affected by age and gender, but this is not controlled for in the calculation of the DAS28-ESR.[10] A recent genome-wide association study identified single-nucleotide polymorphisms (SNPs) in several genes, which were associated with ESR levels at genome-wide significance thresholds ($P \leqslant 1 \times 10^{-8}$) including *CR1* rs6691117.[11] If this genetic correlation is also observed in RA patients, it could have important clinical implications when assessing eligibility for anti-TNF therapy using the DAS28-ESR. *CR1* encodes complement receptor 1 (CD35), a membrane glycoprotein present on erythrocytes and leucocytes that acts as

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a negative regulator of the complement cascade by increasing clearance of complement opsonized immune complexes, thus preventing immune complex deposition.[12–14]

The aim of the current study was first, to investigate the importance of known genetic variants that affect ESR and determine whether they significantly influence ESR levels in UK patients with active RA, and secondly, to determine whether the genetic variants correlate with treatment response to anti-TNF medication. We aimed to investigate the rs7527798, rs6691117, rs10903129 and rs1043879 SNPs mapping to the *CR1L*, *CR1*, *TMEM57* and *C1orf63* genes, respectively, which have each been associated with ESR levels, to determine their association with baseline ESR, baseline DAS28-ESR and change in DAS28-ESR in patients with RA before and after 6 months therapy with an anti-TNF drug.

MATERIALS AND METHODS

Subjects

DNA samples from patients included in this study were obtained from the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS). Patients eligible for the BRAGGSS cohort were initially identified through the British Society for Rheumatology Biologics Register (BSRBR). The BSRBR is a prospective observational study of patients with rheumatic diseases newly commenced on anti-TNF biologic therapy, who are followed up every 6 months for a period of at least 5 years.[15] One of the fundamental objectives of the BSRBR is to monitor patient progress, as well as the incidence of long- and short-term side effects. The BRAGGSS cohort was developed for the study of genetic predictors of response to anti-TNF biologic therapy. Consultants at contributing centres across the United Kingdom gave permission to identify their patients from the BSRBR; eligible patients were approached by letter and invited to donate blood samples for DNA extraction when they were due for a routine blood test. Samples were posted to the Arthritis Research UK Epidemiology Unit for processing, storage and analyses. All contributing patients provided informed consent, and the study was approved by a multicenter ethics committee (COREC 04/Q1403/37).

Baseline and 6-month DAS28 values were recorded to allow subsequent analysis. Patients were excluded from this study if they had stopped treatment because of adverse events or reasons other than inefficacy, or after any change in their anti-TNF biologic therapy during the follow-up period.

Genotyping

DNA samples were genotyped using the Sequenom MassArray iPLEX system. In each reaction, 10 ng of DNA was used and the protocol was followed according to the manufacturer's instructions (http://www.Sequenom.com). For each marker, negative water controls were included for each experiment, and genotype cluster plots were manually reviewed. In addition, SNPs were assessed for deviation from Hardy–Weinberg equilibrium. For purposes of quality control, a 90% sample threshold and 90% genotyping success threshold were used.

Statistics

Baseline levels of ESR in the cohort studied did not follow a normal distribution and were positively skewed; thus, baseline ESR values were log-transformed before analysis. The association between SNPs and ESR was evaluated with linear regression under an additive effect model. Analyses were repeated adjusting for gender and age at baseline. Linear regression models were also used to analyze DAS28-ESR and change in DAS28-ESR over a 6-month period of treatment with anti-TNF therapy.

These analyses were performed using STATA V.11.2 (http://www.stata.com). Power calculations were performed using Quanto (version 1.2.3) (http://hydra.usc.edu/gxe) under an additive model for a range of marker-allele frequencies.

RESULTS

Clinical response and demographic data were recorded in 2978 patients. In total, 264 stopped their anti-TNF drug for reasons other than inefficacy, 12 had no recorded information regarding a potential change in their therapy and 146 had either an incomplete baseline DAS28 or 6-month follow-up DAS28.

The rs7527798 (*CR1L*), rs6691117 (*CR1*), rs10903129 (*TMEM57*) and rs1043879 (*C1orf63*) SNP markers were genotyped in 1510 DNA samples. The genotyping success rates for rs7527798, rs6691117, rs1043879 and rs10903129 were 96%. Ninety-six individuals were removed due to genotype success $<90\%$.

In total, 1223 samples were successfully genotyped for all four SNPs with recorded baseline DAS28 and 6-month follow-up DAS28. Of the samples with genotype information, ESR serum measurements were available for 1188 samples at baseline (pretreatment) and 1195 samples at 6 months. Table 1 describes the demographic and disease characteristics of the BRAGGSS cohort. Genotype frequencies of all four markers conformed to Hardy–Weinberg equilibrium (Table 2). For 1223 individuals for whom change in DAS28-ESR was available, the study had $>80\%$ power (at the 5% significance threshold) to detect a clinically meaningful difference of 0.6 DAS28-ESR units for an allele frequency of 5%.

As expected, following treatment with anti-TNF therapy, we noted a decrease in serum ESR levels and DAS28-ESR within the BRAGGSS cohort. Age was significantly associated with higher baseline ESR, baseline DAS28 and DAS28 6 months after anti-TNF treatment ($P<0.001, =0.002$ and <0.001, respectively). Female sex was significantly associated with higher baseline ESR, baseline DAS28 and DAS28 6 months after anti-TNF treatment ($P=0.004$, <0.001 and <0.001, respectively).

Two copies of rs6691117 *CR1* GG minor allele were significantly associated with baseline ESR, as shown in Table 2, but not ESR after 6 months of treatment or change of ESR. After correcting for gender and age effects the statistical significance remained ($P=0.01$). Association remained after correcting for anti-CCP serology. The *CR1* rs6691117 GG was not significantly associated with baseline DAS28-ESR, change in DAS28-ESR or DAS28-ESR after 6 months of treatment ($P=0.99$, 0.78, 0.77 respectively). The *CR1L* rs7527798 CC genotype was significantly associated with change in DAS28-ESR following 6 months treatment with anti-TNF therapy ($P=0.05$) but not change in ESR over the same period ($P=0.26$). These data are presented in Supplementary Tables S1–5.

DISCUSSION

Recent reports have identified that genetic variants on chromosome 1 affect ESR levels. This has potentially important consequences for RA patients as eligibility for anti-TNF therapy is determined by DAS28, which may incorporate ESR as a

Table 1. Demographic and disease characteristics

Age[a] (years)	56.75 (10.89)
Disease duration[b] (years)	12 (6–20)
Sex, F: *n* (%)	951 (77.76)
ESR at baseline[b], mm h^{-1}	40 (25–66)
Change in ESR[b], mm h^{-1}	−14 (−3–−30)
DAS28-ESR at baseline[a]	6.67 (0.97)
Change in DAS28-ESR[a]	−2.49 (1.52)
Concurrent methotrexate therapy, *n* (%)	705 (57.65)
Rheumatoid factor positive, *n* (%)	719 (64.31)
Anti-CCP positive, *n* (%)	828 (80.78)
Infliximab, *n* (%)	490 (40.07)
Etanercept, *n* (%)	493 (40.31)
Adalimumab, *n* (%)	240 (19.62)

Abbreviations: DAS28, Disease Activity Score 28; ESR, erythrocyte sedimentation rate; F, female; IQR, interquartile range.
[a]Values are expressed as mean (s.d.).
[b]Values are expressed as median (IQR). All other values are *n* (%). Disease duration was measured in 1180 patients; baseline erythrocyte sedimentation was recorded in 1188 patients; change in ESR was recorded in 1195 patients; and change in DAS28-ESR was available in 1188 patients.

Table 2. Relation of ESR genotypes to baseline ESR levels

SNP	*HWE* (P)	*Genotype*	*Geometric mean baseline ESR (mm h^{-1}) (s.d.)*	n	β-*Coefficient*	P-*value*
CR1L rs7527798	0.30	TT	38.51 (2.00)	598		
		TC	36.11 (2.17)	500		
		CC	36.74 (2.05)	90	−0.042	0.21
CR1 rs6691117	0.46	AA	36.11 (2.13)	750		
		AG	38.67 (2.00)	393		
		GG	48.21 (1.72)	45	0.098	**0.01**
TMEM57 rs10903129	0.43	GG	35.85 (2.19)	364		
		AG	37.71 (2.02)	575		
		AA	38.74 (2.05)	249	0.040	0.18
C1orf63 rs1043879	0.86	AA	36.26 (2.08)	647		
		AG	38.67 (2.07)	461		
		GG	38.79 (2.08)	80	0.049	0.16

Abbreviations: ESR, erythrocyte sedimentation rate; HWE, Hardy–Weinberg expectation; SNP, single-nucleotide polymorphism.
Bold value is a statistically significant result.

biomarker of inflammation. Therefore, as genetic markers affect ESR levels, these markers could influence treatment decisions and could have adverse consequences for those patients who carry the genotypes associated with lower ESR, as they would be less likely to meet eligibility criteria for anti-TNF therapy.

In the current study, two copies of the AA major allele in rs6691117 of the *CR1* gene are significantly associated with lower baseline ESR levels prior to anti-TNF therapy in keeping with the direction of effect reported by Kullo *et al.*[11] ($P = 7 \times 10^{-12}$). Importantly, however, the genotype did not associate with baseline DAS28-ESR. Although we were unable to adjust for all factors that are associated with ESR, in particular haemoglobin levels, we were able to adjust for age and gender that did not qualitatively affect the results. While this is reassuring, the current cohort had severe active RA, and therefore higher ESR compared with the cohort in Kullo *et al.*[11] (46.8 versus 13.1 mm h^{-1} in the discovery cohort).[16] We cannot exclude that the genetic markers affecting ESR levels may still impact DAS28-ESR in patients with less active inflammatory disease than this severe active RA cohort. If the DAS28 thresholds were to be reduced in the future, the genetic markers may influence the eligibility for anti-TNF therapy. Collection of a cohort of patients with early RA would be required to investigate this possibility. Our primary aim was to investigate if genetic markers affected the eligibility to being started on any anti-TNF; we are unable to investigate a class effect between anti-TNF treatments as the reduced sample size would have limited power. CR1 is a potent inhibitor of complement activation and genetic variation within this gene may affect Rouleaux formation and hence ESR. The rs6691117 is a non-synonymous SNP encoding an isoleucine to valine alteration. This may modify the secondary structure of CR1 affecting its ability to clear complement opsonized immune complexes, thereby increasing ESR.

Carriage of the minor allele CC at rs7527798 (*CR1L*) was associated with change in DAS28-ESR, but not baseline ESR or change of ESR. This most likely represents a false-positive result as the rs7527798 polymorphism has not, to our knowledge, been associated with swollen joints, tender joints or patient global health.

Failure to detect an association with baseline or 6-month DAS28-ESR may reflect inadequate power to detect modest effects. However, despite not reaching significance thresholds, the direction of effect for rs7527798, rs6691117, rs10903129 and rs1043879 were in keeping with previously reported directions in a previous association study.[11]

CRP can also be used to calculate DAS28-CRP. A previous study has shown that genetic variants at the *CRP* locus correlate with the level of CRP.[17] However, a recent study has shown that for patients with severe active RA, CRP and DAS28-CRP are not affected by these genetic markers.[18]

CONCLUSIONS

In summary, rs6691117 polymorphism has been shown to influence ESR levels in patients with very active inflammation but not DAS28-ESR. This is reassuring for those patients starting anti-TNF, but further studies with a cohort that includes a wide range of DAS28-ESR levels would be required to ensure rs6691117 does not affect DAS28-ESR and hence eligibility for anti-TNF therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)

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