Association between anti-TNF treatment response and genetic variants within the TLR and NFκB signalling pathways

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ABSTRACT

Objective: To determine whether genetic variation within genes integral to the toll-like receptor (TLR) and NFκB signalling systems, two cardinal regulators of inflammatory and immune responses, contribute towards the observed variation in response to TNF blocking agents in rheumatoid arthritis (RA) patients.

Methods: Pairwise-tagging SNPs spanning 24 candidate genes were selected and genotyped in a large UK cohort of patients receiving anti-TNF therapy for RA. Multivariate regression analyses were performed to test association between individual genotypes, under an additive model, and treatment response at 6-months follow-up, assessed using both the absolute change in DAS28 and the EULAR response criteria. Analyses were performed across subgroups comprising etanercept, infliximab and infliximab/adalimumab treated patients as well as the combined anti-TNF treated cohort. P values < 0.05 were considered statistically significant.

Results: A total of 189 SNPs were successfully genotyped and analysed in 909 patients. Eight SNPs spanning 6 genes demonstrated nominal evidence of association with response (DAS28) across the anti-TNF-treated subgroups, 6 of which were restricted to etanercept-treated patients. Twelve SNPs spanning 9 genes demonstrated nominal evidence of association with treatment response (DAS28 and/or EULAR) across the combined anti-TNF cohort. These included SNPs mapping to *MyD88* (rs7744) and *CHUK* (rs11591741), which were associated under each model applied (Etanercept-treated and combined anti-TNF cohort analysis (DAS28 and EULAR)).

Conclusions: Several SNPs mapping to the TLR and NFκB signalling systems demonstrated association with anti-TNF response as a whole and, in particular, with response to etanercept. Validation of these findings in an independent cohort is now warranted.

INTRODUCTION

The introduction of tumour necrosis factor antagonists (anti-TNF) has improved the treatment and management of rheumatoid arthritis (RA) for many patients. However, these agents are expensive and a substantial proportion of patients do not respond.[1] Even in responders there exists a spectrum of efficacy ranging from complete remission of symptoms to modest improvement. Such variation in treatment response is unlikely to be random, but influenced by genetic, environmental and psychological factors. Identified clinical predictors of response include baseline health assessment questionnaire (HAQ) score, administration of concurrent disease modifying anti-rheumatic drugs (DMARDs), gender and seronegativity.[1-4] Whereas some genetic factors have been proposed to influence treatment response (e.g. TNF, type 2 TNF receptor, Fc-gamma receptors), few have been consistently replicated across study cohorts, indicating a complex relationship with treatment outcome (or simply, a small effect).[5-10] Even in combination these factors have not yet proven to be clinically useful and the identification of additional genetic determinants of treatment response could provide enormous clinical and economic benefit.

The Toll-like receptors (TLR) and NFκB signalling systems are cardinal regulators of inflammatory and immune responses. For instance, TLR sense exogenous and endogenous antigens and downstream pathways (e.g. NFκB, JNK and p38, in particular) signal the production of pro-inflammatory cytokines (e.g. TNF and interleukin 1 (IL1)), chemokines and matrix metalloproteinases (MMPs). In recent years evidence has emerged implicating these signalling systems in the pathogenic, inflammatory and destructive processes characteristic of RA.[11, 12] For instance, increased TLR expression (type 2 and 4, in particular) has been repeatedly described

in synovial tissue from patients with RA and associated with the up-regulation of cytokines and MMPs.[13, 14] In addition, *ex vivo* cultures have linked other components of the TLR signalling pathway (e.g. MyD88 and TIRAP/MAL) to the spontaneous and increased production of TNF, other cytokines and MMPs.[15] Activated NFκB has also been found in RA synovial tissue and may provide a conduit for pro-inflammatory signals initiated by various receptors also implicated in RA (e.g. TLR, IL1β receptor and TNF receptors).[16, 17]

Critically, both systems could contribute towards chronic inflammation in RA via positive feedback loops. Firstly, endogenous markers of tissue injury that are present in RA joints can induce TLR signalling, resulting in further inflammation. Secondly, NFκB is an important activator of TNF-mediated pro-inflammatory signalling but is also stimulated by TNF. Since persistent inflammation defines anti-TNF non-response, these systems provide a logical focus for pharmacogenetic studies of this drug class. Specifically, polymorphisms within the TLR and NFκB pathways that promote more active/pro-inflammatory signals may reduce the efficacy of anti-TNF therapy. We tested this hypothesis in a large UK cohort of patients with RA.

METHODS

Participants

DNA samples from 923 patients receiving anti-TNF therapy for the treatment of RA were available from the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS) (http://www.medicine.manchester.ac.uk/epidemiology/research/arc/genetics/pharmacogenetics/braggss/). This syndicate incorporates collaborations between multiple UK-

wide rheumatology clinics from which eligible patients have been recruited, DNA and serum samples obtained and extensive clinical data, taken at baseline and 6 months follow-up, compiled. The criteria for selection and recruitment of patients have been described in full elsewhere.[4]

Candidate gene and SNP Selection

Twenty four candidate genes were selected for investigation (Table 1). In addition to *TLR 2* and *4*, critical downstream adapter proteins and signalling intermediaries were selected (*TLR1* and 6, *MyD88, Mal*, *IRAK1*, *4* and *M*, *TAB1* and *2*, *TAK1*). *NFKB1* and *2*, and their key regulators were chosen (*IKK1* and *2*, *IkBα*, *IkBβ*, *IkBε*, *SUMO4*, *Rel*, *RelA*, *RelB*), plus one key NFκB target not previously investigated (COX). Marker coverage for each gene included the 10kb upstream and downstream flanking regions. Pairwise tagging SNPs were selected from the CEPH/CEU HapMap dataset (Phase II, release 23a/March08) using Haploview software (minor allele frequency (MAF) >0.05, Hardy-Weinberg p-value>0.05, Min genotype %>90, Max Mendel errors>1, pairwise r^2>0.8).[18, 19] Additional SNPs were included if they were non-synonymous or known to have functional effects (based on information available on NCBI: PubMed, Entrez Gene and dbSNP).

Genotyping

A total of 199 SNPs were selected and genotyped using Sequenom's MassARRAY® iPLEX™ system (Sequenom, Cambridge, UK). Multiplex assays were designed and performed according to the manufacturer's specifications. SNP genotype cluster plots for each assay were manually checked. Quality control (QC) procedures prior to analysis removed any samples with a call rate <80% and any assay with a call rate

<95%. Hardy-Weinberg equilibrium was assessed to identify potential genotyping errors. For SNPs that failed assay design and/or genotyping, alternative tagging SNPs were selected and genotyped, where available.

Statistical analyses

The primary outcome measure was absolute change in the 28 joint count disease activity score (DAS28) between baseline and 6 months follow-up.[20] Multivariate linear regression analyses were performed to investigate association between change in DAS28 and individual SNP genotypes under an additive model. Adjustments were made for independent clinical predictors of anti-TNF response identified in the study cohort, namely baseline DAS28, baseline HAQ score, administration of concurrent DMARDs and gender (Supplementary Table 1). Anti-CCP and RF were also significant predictors of outcome but were not included in the final analysis as data was only available on 75% of patients. Analyses were initially performed according to treatment received (etanercept, infliximab and combined monoclonal antibody group). Adalimumab recipients were not analysed alone due to small numbers. Subsequently, the combined cohort was studied to identify potential class effects, applying the same multivariate model.

In addition, the European League Against Rheumatism (EULAR) response criteria were assessed as a secondary outcome measure (none vs. moderate/good responders) using multivariate logistic regression analyses, applying the same model as described above.[21] This secondary analysis was only performed across the combined anti-TNF drug cohort due to the limited power of the smaller subgroups. All analyses were

performed in Stata (StataCorp, Texas, USA). Power calculations were performed using the Quanto software package.[22, 23]

RESULTS

Genotyping results

A total of 199 SNPs spanning the 24 candidate gene regions were initially genotyped in the 923 patients. These included 93 pairwise tagging SNPs (tagging ~600 polymorphisms) and 95 singleton SNPs selected from the phase II HapMap dataset plus 11 SNPs selected from NCBI (PubMed, Entrez Gene and dbSNP) due to known or potential functional effects (Supplementary Table2). Despite the re-designing and genotyping of alternative assays, 12 SNPs (6%) failed to meet QC thresholds (>95% genotyping success) and were excluded from subsequent analyses (Supplementary Table2). In addition, 14 patients (2%) were removed from analysis due to failure to meet QC thresholds (>80% genotyping success). Consequently, multivariate regression analyses were performed on data generated for 187 SNPs in 909 patients.

For the linear regression analyses across the combined cohort (n=909), there was greater than 90% power to detect a difference ≥0.6 in the absolute change in DAS28 under an additive model, given a minimum MAF of 5%, at the 5% significance level. In the etanercept, infliximab and combined monoclonal antibody subgroups, there was 68%, 64% and 76% power, respectively. Similarly, by comparing EULAR non-responders to moderate/good responders (~20 vs. ~80% of patients, respectively) using logistic regression, the combined anti-TNF group had greater than 80% power to detect an OR ≥2 under the same model.

Characterisation of study cohort and response to anti-TNF therapy

Baseline characteristics for the individual subgroups are presented in Table 2. Forty two percent of patients had received etanercept, 44% infliximab, and 14% adalimumab. Overall, these patients had long-standing, active disease (mean duration 14 years mean, DAS28 of 6.7) with a high degree of disability (mean HAQ score 2.1). At 6 months follow-up, 20% of patients were non-responders, 53% moderate responders and 27% good responders according to the EULAR criteria. The mean change in DAS28 was an improvement of 2.5 points.

Pharmacogenetic predictors of response to individual drugs

Linear regression analyses were first performed according to drug received (Table 3). A total of eight SNPs spanning 6 genes demonstrated evidence of association with the absolute change in DAS28. Interestingly, 6 of these were limited to the etanercept-treated subgroup. Significant differences in response to etanercept were demonstrated with the rs11591741 (*CHUK*), rs7744 (*MyD88*) and rs5743704 (*TLR-2*) polymorphisms. For rs11591741, the linear regression coefficients suggested that, relative to the major homozygotes, heterozygotes and minor homozygotes demonstrated a smaller reduction in DAS28 of 0.267 and 0.534 units on average, respectively. The same effect was demonstrated for rs5743704, with heterozygotes and minor homozygotes demonstrating a smaller reduction in DAS28. However, this could be an artefact of the low MAF (4.8%) calculated across this study cohort. The rs7744 SNP showed the opposite effect, with heterozygotes and minor homozygotes demonstrating a mean greater improvement in DAS28 of 0.390 and 0.780 units compared to major homozygotes, respectively. The remaining 5 SNPs demonstrated nominal evidence of association with treatment response.

Pharmacogenetic predictors of an anti-TNF class effect

Linear regression analyses were subsequently performed across the combined cohort, under the assumption that all three anti-TNF agents act through shared pathways (class effect). Seven SNPs spanning 5 genes demonstrated nominal evidence of association ($p<0.05$) with the absolute change in DAS28 (Table 3). These included SNPs mapping to *CHUK* (rs11591741, rs2230804) and *MyD88* (rs7744) identified in the etanercept-treated subgroup analysis, which demonstrated the same pattern of association but with smaller effect sizes in the combined cohort. Statistically significant evidence for association was lost across the combined cohort for the other 5 polymorphisms suggesting drug specific associations. In contrast, 4 additional SNPs demonstrated association in the combined cohort, which may reflect its greater power. Patients carrying the minor alleles at rs11986055 (*IKBKB*) and rs11541076 (*IRAK-3*) demonstrated a greater improvement in DAS28 compared to major homozygotes. For rs11595324 (*CHUK*) and rs11574851 (*NFκB-2*), patients carrying the minor allele demonstrated a smaller reduction in DAS28.

Finally, logistic regression analyses were performed in the larger combined dataset utilizing the EULAR response criteria as a secondary outcome measure. Of the 7 SNPs associated with DAS28 in the combined anti-TNF dataset, 4 were also associated with EULAR response (Table 4). In particular, rs11591741 (*CHUK*) and rs7744 (*MyD88*) were again associated with this secondary outcome measure. Consistent with the linear regression analysis, for SNPs rs11986055 (*IKBKB*), rs11541076 (*IRAK-3*) and rs7744 (*MyD88*), the odds ratios (OR) indicated that patients carrying the minor allele at these loci had a greater chance of achieving a

moderate/good EULAR response compared to major homozygotes. Carriage of the minor allele of rs11591741 (*CHUK*) was again associated with a lower probability of achieving a moderate/good EULAR response. Additional nominally significant associations were demonstrated between EULAR response and 5 additional SNPs (rs3136645 and rs9403 (*NFKBIB*), rs2206593 (*PTGS2*), rs2289318 (*TLR-2*), rs11096957 (*TLR-10/1/6*)) (Table 4).

DISCUSSION

Analyses were performed to identify common variants spanning 24 candidate genes involved in the NFκB, TLR 2 and 4 signalling pathways that associate with response to anti-TNF treatment in RA patients. Eight SNPs spanning 6 genes (*CHUK, IKBKB, MyD88, NFKBIA, TLR-2, TLR-4*) demonstrated nominally significant associations with absolute change in DAS28 following treatment with individual anti-TNF agents. In addition, 4 of the same genes (*CHUK, IKBKB, MyD88, TLR-2*) plus an additional 5 genes (*IRAK-3, NFκB-2, NFKBIB, PTGS2, TLR-10/1/6*) demonstrated nominal evidence of association with the absolute change in DAS28 and/or EULAR response rates across the combined anti-TNF cohort.

It is important to emphasize that these results arise from exploratory analyses and are reported uncorrected. Indeed, none of the present associations would remain significant following Bonferroni correction. However, such corrections are often considered overly stringent. For instance, although in the current study tagging SNPs were selected as genetic markers, these SNPs demonstrate modest linkage disequilibrium (r^2 = 0.4-0.8) and therefore cannot be considered completely

independent. Nonetheless, and notwithstanding the large size of our cohort, these findings should be considered preliminary, awaiting confirmation in other datasets.

In order to thoroughly explore the data, we chose to analyse and present the results in several ways. Given similarities and differences between agents, pharmacogenetic factors could influence response to individual agents and/or anti-TNF drugs as a group. Hence, we analysed according to both paradigms, acknowledging the reduced power of the smaller drug-specific subgroup analyses. In fact, 8 SNPs demonstrated nominal evidence of association with individual drugs, 6 of which were restricted to the etanercept-treated subgroup. This apparent dichotomy of association according to drug has previously been reported for both genetic and non-genetic factors.[1, 7] Three of these associations (*CHUK* (x2), *MyD88*) remained significant (with diluted effect sizes) in the combined anti-TNF cohort but the others (*IKBKB, NFKBIA, TLR-2, TLR-4*) did not. Although this would be consistent with stronger effects linked to specific drugs, the reduced power and increased risk of both type I and type II errors in smaller subgroup analyses, emphasises the importance of replication in larger datasets. However, to our knowledge BRAGGSS is currently the largest dataset available for such analyses worldwide. Four of the 7 associations demonstrated across the combined anti-TNF cohort were not detected in the individual subgroup analyses, which again may reflect limited power. On a similar line, there is ongoing debate regarding the benefits of switching from one anti-TNF drug to another. Although only a small proportion (6% overall) of our patients had previous exposure to a different anti-TNF agent, exclusion of these patients from the analysis did not change the overall conclusions of the study (data not shown).

There is currently no consensus on the best measure of treatment response in such pharmacogenetic studies. The absolute change in DAS28, which measures response at the level of the individual patient, is, statistically speaking, more powerful due to its continuous scale. In contrast, the EULAR improvement criteria, which measures response at the group level, are more clinically meaningful. We therefore analysed according to both outcomes, although EULAR improvement criteria analyses were limited to the combined cohort because of power limitations. Differences between these two measures may explain why some polymorphisms did not demonstrate associations in both analyses.

Our most robust findings involve associations of *MyD88* and *CHUK* with response in both the etanercept-treated subgroup and combined anti-TNF cohort. Both associated markers were selected as tagging SNPs and, if replicated, these results could present a number of functional possibilities. In *MyD88* the associated SNP rs7744 maps to the 3´UTR and could influence mRNA stability. Alternatively, rs7744 is in strong LD with a SNP, rs156265 (r^2=0.9), which maps to the upstream promoter region and could therefore influence gene expression. In *CHUK*, the associated marker rs11591741 maps to intron 9, is perfectly correlated with an intron 18 SNP (rs11597086) and demonstrates modest LD (r^2>0.5) with 6 other polymorphisms spanning the gene region. Hence, more detailed analyses, including the assessment of functional effects, may be required to identify the true pharmacogenetic association.

With the increasing feasibility of genome wide association studies, there is hope that such designs will aid the identification of markers predictive of anti-TNF treatment efficacy. For instance, although we selected crucial adapter proteins, signalling

intermediaries and regulators involved in the TLR and NFκB pathways, there are a large number of genes implicated in these pathways that we did not investigate, both known and unknown,. However, this does not detract from the power and success of well-designed candidate gene association studies, particularly when drug pathways and mechanisms are often well characterised.[24, 25] MyD88 is the foremost adapter protein essential for TLR intracellular signalling whereas the IκB kinase CHUK (alias: IKK1) is responsible for the phosphorylation and degradation of NFκB inhibitors (IκBs), resulting in NFκB activation and subsequent TNF induction.[26] Many components involved in these pathways, in particular MyD88, have been implicated in the chronic inflammatory and destructive processes characteristic of RA.[13-15] Furthermore, early down regulation of genes within the NFκB signalling system has recently been associated with anti-TNF efficacy.[27] Hence, genetic variation that alters the expression and mediation of these pathways, and consequently TNF activation, may contribute towards anti-TNF treatment response.

In summary, several SNPs mapping to genes involved in the TLR and NFκB signalling pathways demonstrated association with response, particularly to etanercept, but also to anti-TNF drugs as a group. Our data relate to the largest reported cohort of RA patients treated with TNF inhibitors. Nonetheless, as with all pharmacogenetic studies, it is now necessary to validate them in independent cohorts of equal or greater size.

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Table 1. Function and chromosomal locations of candidate genes

Gene names (Aliases)	Functional relevance to study	Chromosomal location	NCBI ID
TLR-1 (TIL) & *TLR-6*[†]	Toll-like receptors 1 and 6	4p14	NCBI-7096 NCBI-10333
TLR-2 (TIL-4)	Toll-like receptor 2	4q32	NCBI-7097
TLR-4 (TOLL)	Toll-like receptor 4	9q33	NCBI-7099
MyD88	Intracellular adaptor molecules	3p22	NCBI-4615
TIRAP (MAL)	for TLRs	11q24	NCBI-114609
IRAK-1 (IRAK)	Mediates TLR signalling	Xq28	NCBI-3654
IRAK-4		12q12	NCBI-51135
IRAK-3 (IRAK-M)	Inhibits TLR signalling	12q14	NCBI-11213
MAP3K7 (TAK1)	Activates NFkB signalling	6q16	NCBI-6885
MAP3K7IP1 (TAB1)		22q13	NCBI-10454
MAP3K7IP2 (TAB2)		6q25	NCBI-23118
CHUK (IKK1, IKBKA)	Phosphorylates NFkB inhibitors	10q24	NCBI-1147
IKBKB (IKK2)	(IkB)	8p11	NCBI-3551
NFkBIA (IkBα)	Inhibitors of NFkB	14q13	NCBI-4792
NFkBIB (IkBβ)		19q13	NCBI-4793
NFkBIE (IkBε)		6p21	NCBI-4794
NFκB-1 (p105, p50)	NFkB p50 subunit & its p105 precursor	4q24	NCBI-4790
NFκB-2 (LYT-10)	NFkB p52 subunit & its p100 precursor	10q24	NCBI-4791
SUMO4 (IDDM5)[‡]	Negative regulator of NFkB	6q25	NCBI-387082
Rel (C-Rel)	Subunits & transcriptional activators of NFkB target genes	2p13	NCBI-5966
RelA (p65, NFκB-3)		11q13	NCBI-5970
RelB (I-Rel)		19q13	NCBI-5971
PTGS2 (COX2)	Major target of NFkB	1q25	NCBI-5743

[†]The *TLR-1/6* region was extended to include *TLR-10*. [‡]The *SUMO4* gene maps within the last intron of *MAP3K7IP2*. Chromosomal locations were taken from the NCBI Build36 assembly.

Table 2. Clinical and demographic characteristics for patient samples treated with each of the 3 anti-TNF drugs

Characteristics	ETA* (n = 386)	INF* (n = 400)	ADA* (n = 123)	Anti-TNF cohort* (n = 909)
Female	77% (298/386)	77% (308/400)	77% (95/123)	77% (701/909)
Age (yrs)	56 (11)	57 (11)	58 (12)	57 (11)
Ever smoked	63% (239/381)	57% (226/397)	57% (68/119)	59% (533/897)
DAS28	6.67 (1)	6.74 (1)	6.53 (1)	6.68 (1)
Duration (yrs)	13.2 (9)	14.4 (10)	13.5 (12)	13.8 (10)
HAQ	2.0 (0.6)	2.2 (0.5)	2.0 (0.5)	2.1 (0.6)
SE positive	80% (194/241)	80% (228/284)	78% (40/51)	80% (462/576)
RF positive	91% (280/308)	87% (242/279)	85% (82/96)	88% (604/683)
Anti-CCP positive	85% (262/308)	81% (227/279)	72% (69/96)	82% (558/683)
Concurrent DMARDs	54% (207/386)	94% (363/386)	60% (67/112)	72% (637/884)
Previous biologic	7% (28/385)	4% (15/397)	11% (14/123)	6% (57/905)
Change in DAS28	-2.5 (1.5)	-2.4 (1.6)	-2.7 (1.6)	-2.5 (1.5)
EULAR†	17%/55%/28% (67/211/108)	22%/54%/24% (90/215/95)	19%/48%/33% (24/59/40)	20%/53%/27% (181/485/243)

*Figures are percentages (numbers) or means (standard deviations). †None/moderate/good responders, ETA=etanercept, INF=infliximab, ADA=adalimumab, Yrs=years, DAS28=28 joint count disease activity score, HAQ=health assessment questionnaire, SE=shared epitope, RF=rheumatoid factor, Anti-CCP=anti-cyclic citrullinated peptide antibody, DMARD=disease modifying anti-rheumatic drugs.

Table 3. Multivariate linear regression results for anti-TNF subgroup and combined analysis

SNP	Gt	Etanercept Freq	Etanercept LR Coef (95% CI), p-value†	Infliximab Freq	Infliximab LR Coef (95% CI), p-value†	Monoclonal Antibodies Freq	Monoclonal Antibodies LR Coef (95% CI), p-value†	Combined Anti-TNF Freq	Combined Anti-TNF LR Coef (95% CI), p-value†
CHUK									
	GG	113	0.267	119	0.108	157	0.143	270	0.194
rs11591741	GC	180	(0.070- 0.464),	185	(-0.108, 0.324),	243	(-0.045, 0.331),	423	(0.058, 0.330),
	CC	81	0.008	84	0.328	109	0.136	190	0.005
	AA	116	-0.198	120	-0.086	155	-0.103	271	-0.14
rs2230804	AG	172	(-0.389, -0.008),	188	(-0.300, 0.129),	247	(-0.289, 0.082),	419	(-0.274, -0.008),
	GG	88	0.041	90	0.432	118	0.275	206	0.037
	TT							835	0.428
rs11595324	TC	-	NS	-	NS	-	NS	63	(0.059, 0.797),
	CC							1	0.023
IKBKB									
	CC	152	0.220	154	-0.046	203	-0.055		
rs10958713	CT	176	(0.014, 0.426),	180	(-0.271, 0.178),	236	(-0.252, 0.143),	-	NS
	TT	53	0.036	58	0.685	73	0.586		
	AA							840	-0.372
rs11986055	AC	-	NS	-	NS	-	NS	58	(-0.722, -0.022),
	CC							3	0.037
IRAK-3									
	TT							614	-0.195
rs11541076	TA	-	NS	-	NS	-	NS	262	(-0.376, -0.014),
	AA							25	0.035
MyD88									
	AA	278	-0.390	286	-0.154	370	-0.102	648	-0.221
rs7744	AG	98	(-0.669, -0.112),	107	(-0.471, 0.162),	137	(-0.365, 0.161),	235	(-0.413, -0.030),
	GG	7	0.006	6	0.338	12	0.444	19	0.024
NFκB-2									
	CC							802	0.398
rs11574851	CT	-	NS	-	NS	-	NS	78	(0.056, 0.741),
	TT							1	.023
NFKBIA									
	AA	346	-0.135	372	0.700	477	0.378		
rs2233407	AT	38	(-0.594, 0.325),	27	(0.144, 1.256),	44	(-0.077, 0.832),	-	NS
	TT	0	0.565	1	0.014	1	0.103		
TLR-2									
	CC	340	0.641	370	-0.418	485	-0.341		
rs5743704	CA	34	(0.181, 1.101),	23	(-1.102, 0.2.67),	30	(-0.936, 0.253),	-	NS
	AA	1	0.006	0	0.231	0	0.260		
	AA	179	-0.245	163	0.032	215	0.039		
rs11935252	AG	166	(-0.459, -0.032),	191	(-0.211, 0.276),	253	(-0.177, 0.255),	-	NS
	GG	38	0.025	38	0.794	46	0.725		
TLR-4									
	AA	286	-0.040	277	-0.310	362	-0.265		
rs7045953	AG	88	(-0.321, 0.240),	107	(-0.605, -0.015),	140	(-0.529, -0.002),	-	NS
	GG	8	0.776	11	0.039	14	0.048		

†Multivariate linear regression analyses were performed under an additive model and adjusted for baseline DAS28, baseline HAQ score, concurrent DMARDs and gender. The major homozygotes were taken as the reference group and coefficient values are per copy of minor "risk" allele. A negative coefficient value indicates a greater reduction in DAS28 compared to major homozygotes, whereas a positive coefficient indicates a smaller improvement. The rs numbers were taken from the dbSNP-b126/NCBI-B36 assembly. SNP=single nucleotide polymorphism, Gt=genotype, Freq=frequency, LR Coef=Linear regression coefficient, CI=Confidence interval. NS-not significant.

Table 4. Multivariate logistic regression results for associated SNPs

SNP	Position	Gt	Freq	EULAR Response, No (%) None	EULAR Response, No (%) Moderate/Good	Logistic Regression OR (95% CI)†	Additive p-value†
CHUK rs11591741	101966491	GG	270	45 (17)	225 (83)	0.774 (0.605, 0.991)	0.042
		GC	423	87 (21)	336 (79)		
		CC	190	45 (24)	145 (76)		
IKBKB rs11986055	42254335	AA	840	171 (20)	669 (80)	3.307 (1.184, 9.239)	0.022
		AC	58	5 (9)	53 (91)		
		CC	3	0 (0)	3 (100)		
IRAK-3 rs11541076	64934595	TT	614	135 (22)	479 (78)	1.467 (1.029, 2.092)	0.034
		TA	262	41 (16)	221 (84)		
		AA	25	3 (12)	22 (88)		
MyD88 rs7744	38159025	AA	648	140 (22)	508 (78)	1.572 (1.075, 2.300)	0.020
		AG	235	38 (16)	197 (84)		
		GG	19	1 (5)	18 (95)		
NFKBIB							
rs3136645	44090489	TT	622	110 (18)	512 (82)	0.647 (0.471, 0.889)	0.007
		TC	259	60 (23)	199 (77)		
		CC	21	9 (43)	12 (57)		
rs9403	44098007	GG	391	63 (16)	328 (84)	0.754 (0.579, 0.982)	0.036
		GC	408	89 (22)	319 (78)		
		CC	94	23 (24)	71 (76)		
PTGS2 rs2206593	184909052	GG	820	154 (19)	666 (81)	0.515 (0.307, 0.862)	0.012
		GA	82	25 (30)	57 (70)		
		AA	1	0 (0)	1 (100)		
TLR-2 rs2289318	154853184	CC	610	111 (18)	499 (82)	0.736 (0.546, 0.993)	0.045
		CG	257	63 (25)	194 (75)		
		GG	34	7 (21)	27 (80)		
TLR-10/1/6 rs11096957	38452886	AA	436	99 (23)	337 (77)	1.324 (1.010, 1.735)	0.042
		AC	362	68 (19)	294 (81)		
		CC	96	13 (14)	83 (86)		

†Multivariate logistic regression analyses adjusted for baseline DAS28, baseline HAQ score, concurrent DMARDs and gender. The major homozygotes were taken as the reference group and odds ratios are per copy of minor "risk" allele. An odds ratio greater than 1 indicates a greater chance of achieving a moderate/good response. The rs numbers and chromosomal positions were taken from the dbSNP-b126/NCBI-B36 assembly. EULAR= European league against rheumatism, SNP=single nucleotide polymorphism, Gt=genotype, Freq=frequency, OR=odds ratio, CI=confidence interval.