Tumour necrosis factor-α single nucleotide polymorphisms are not independent of HLA class I in UK Caucasians with adult onset idiopathic inflammatory myopathies

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Objective. To investigate haplotype tagging single nucleotide polymorphisms (SNPs) in the tumour necrosis factor α (TNF-α) gene, in UK Caucasian idiopathic inflammatory myopathy (IIM) patients.
Methods. A cross-sectional, case-control study of four TNF-α SNPs was undertaken, comparing cases of polymyositis (PM) ($n=121$), dermatomyositis (DM) ($n=109$) and myositis overlapping with other connective tissue diseases (CTD-overlap) ($n=73$) with normal subjects ($n=177$). Subgroup analyses were undertaken after stratifying for myositis specific/associated antibodies.
Results. The TNF-308A allele demonstrated a strong association with each myositis disease subgroup *vs* controls [PM, odds ratio (OR) 2.8, 95% confidence interval 1.9–4.3; DM, OR 2.5, 1.6–3.8; CTD-overlap, OR 3.3, 2.1–5.1]. The TNF-308GA/AA genotype frequency was significantly increased *vs* controls (PM, OR 3.7, 2.1–6.3; DM, OR 3.2, 1.8–5.5; CTD-overlap, OR 5.0, 2.6–9.6) suggesting a dominant model. The association was strongest in patients possessing anti-aminoacyl transfer RNA synthetase (anti-synthetase) (OR 5.1, 3.3–8.0) or -PM-Scl (OR 5.0, 2.7-8.9) antibodies. The -1031T allele was also a significant risk factor in DM (OR 2.2, 1.4–3.6), anti-synthetase (OR 2.9, 1.6–5.3) and -PM-Scl (OR 5.6, 1.9–6.4) antibody positive patients. The TNF-308A association was lost after adjusting for HLA-B*08, but remained independent of HLA-DQB1*02 (both are alleles forming part of the common ancestral haplotype). The HLA-B*08/TNF-308A/DRB1*03/DQA1*05/DQB1*02 haplotype was a risk factor in all myositis subgroups *vs* controls (OR 3.0, 1.8–5.3).
Conclusions. TNF-308A and -1031T alleles are significant risk factors in the IIMs. In the IIMs, the TNF-308A allele is part of the common ancestral haplotype, but is not independent of HLA-B*08.

KEY WORDS: Myositis, Polymyositis, Dermatomyositis, Polymorphisms, HLA, TNF-α, Antibodies.

Introduction

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of diseases defined by the presence of acquired muscle inflammation and weakness. The aetiology of the IIMs is unknown, but is thought to be due to a combination of environmental exposures to specific triggers in genetically predisposed individuals [1, 2]. Genetic factors are thought to contribute to the development of PM/DM [3, 4]. Most IIM candidate gene studies to date have been confined to the HLA region and confirm that HLA-DRB1*0301 and DQA1*0501 are risk factors for the development of myositis, especially in patients possessing anti-aminoacyl transfer RNA synthetase (anti-synthetase) antibodies and/or interstitial lung disease (ILD) [5–11]. Other genes within the major histocompatibility complex (MHC) may contribute towards susceptibility in myositis; further disease susceptibility gene searches within this region are challenging because of the strong linkage disequilibrium (LD) between loci and the tendency of certain alleles to be present in combination with each other as preferentially conserved haplotypes.

A possible MHC candidate gene is tumour necrosis factor-α (TNF-α), located within the HLA class III region. At least 12 single nucleotide polymorphisms (SNPs) have been identified within promoter, exonic, intronic and 3′ untranslated regions of the TNF-α gene. Nine of these SNPs are polymorphic within a UK population [12]. A number of TNF-α SNPs have been implicated in various autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), ankylosing spondylitis, Crohn's disease and also in infectious diseases including malaria, leprosy and hepatitis B/C [13]. Associations of the TNF-308A polymorphism have been found in small studies of juvenile and adult onset myositis [14–16]. In individuals of European ancestry, it is known that strong LD exists between the TNF-308A allele and the HLA-DRB1*03-DQA1*05-DQB1*02 common ancestral haplotype (AH), as has been demonstrated in adult onset myositis patients [16]. TNF-α is a pro-inflammatory cytokine with a diverse range of activities [17] and studies have suggested a genetic contribution to TNF-α regulation, whereby possession of the AH or TNF-308A is associated with higher circulating levels of serum TNF-α [18–20].

A number of issues remain unanswered with regards to the TNF gene and its association with the IIMs. It is not known whether TNF-308A is an independent risk factor for the IIMs, or whether the previously reported TNF-308A associations with the IIMs exist due to strong LD within the AH. Furthermore, associations of TNF-α SNPs within defined myositis clinical and serological subgroups have not been sufficiently defined, primarily due to small numbers studied in previous work. In this study, we have utilized TNF-α haplotype-tagging SNPs (the minimum number of SNPs required to be genotyped in order to capture the most frequently occurring haplotypes) previously identified in juvenile oligoarthritis [12]. We investigated the role of the TNF-α gene in a large cohort of IIM patients, to determine whether genetic differences exist between

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different IIM subgroups, according to clinical classification and phenotype.

Methods

Design

This was a cross-sectional, case-control study of TNF-α SNPs, comparing cases of PM, DM and myositis overlapping with another connective tissue disorder (myositis/CTD-overlap) with normal subjects. Subgroup analyses were also undertaken after stratifying by the presence or absence of myositis specific/associated antibodies (MSA/MAAs).

Cases

Between 1999 and 2004, a UK-wide collaboration comprising 56 rheumatologists and four neurologists [the Adult Onset Myositis Immunogenetic Collaboration, (AOMIC)], recruited 303 UK Caucasian patients aged 18 yrs or older from clinical units in 40 teaching and district general hospitals. Patients with PM or DM had probable or definite PM/DM, based on the Bohan and Peter criteria [21, 22]. For myositis/CTD-overlap patients, use of these criteria is problematic as in the UK, myositis is less rigorously diagnosed in the context of another CTD. Thus, 20/73 (27%) myositis/CTD-overlap patients were included if they: (i) met published criteria for their primary CTD [23–27] or mixed connective tissue disease (MCTD) [28]; (ii) possessed at least two of four Bohan and Peter criteria (proximal muscle weakness, elevated muscle enzymes, myopathic EMG changes and diagnostic muscle biopsy) and (iii) possessed at least one MSA/MAA. The remaining 53 myositis/CTD-overlap patients fulfilled criteria for their primary disease/MCTD and for Bohan and Peter probable/definite myositis. A standardized clinical data collection form, detailing demographics and individual clinical details, was used. The collaborating physicians at each study site confirmed/excluded the presence of ILD, by pulmonary function tests and thoracic imaging and cancer-associated myositis (CAM) by relevant investigations. CAM was defined as cancer occurring in patients with definite PM/DM, within 3 yrs of myositis diagnosis (as per modified Bohan and Peter classification [29]). Patients' written consent to participate was obtained according to the Declaration of Helsinki, ethical approval having been gained locally at each participating centre.

Controls

A total of 177 UK Caucasian control subjects were recruited. These were normal subjects recruited from primary population registers as part of previously described epidemiological studies [30, 31]. Collection of data and blood from patients and controls was undertaken under the regulation of the local research ethics committees.

Serological typing

Sera were obtained from patients for determination of MSAs (anti-synthetases: -Jo-1, -PL-7, -PL-12, -EJ, -OJ, -KS; anti-Mi-2, anti-SRP and anti-155/140) and MAAs (anti-PM-Scl, anti-Ku, anti-U1-RNP and U3-RNP), as previously described [9, 32]. The TNF-α genotype and allele frequencies in the controls were comparable with those previously published [12, 16].

Genotyping. DNA was extracted from a peripheral blood sample obtained from both cases and controls using a standard phenol-chloroform method. Genotyping assays were based on the SNaPshot primer extension kit (PE Applied Biosystems, Warrington, UK). ddNTP primer extension methods were designed for polymorphic sites within the TNF-α genomic region. The primer and probe sequences used and Snapshot genotyping assay has been described previously [12].

Four haplotype tagging SNPs previously identified [12] were selected for investigation: three from the TNF-α promoter region (−1031, rs1799964; −863, rs1800630; −308 and rs1800629) and one from intron 1 (+489, rs1800610). Cases were broad-typed for the HLA-B locus, using a commercially available polymerase chain reaction-sequence-specific oligonucleotide probe typing system (Dynal Biotech GmbH, Hamburg, Germany). The HLA class II typing has been described previously [9].

Statistical analyses. Genotyping frequencies for each SNP were tested for Hardy–Weinberg equilibrium (HWE) in each control group. Allelic and genotype frequencies were compared between myositis cases and controls, using Fisher's exact test. Where significant, data were expressed as odds ratios (OR) with exact 95% confidence intervals (CI). A Bonferroni correction was applied to any statistically significant results, by multiplying the derived P-value by the number of tests performed (accounting for four SNPs, three clinical subgroups, 13 antibodies, 26 HLA-B alleles or number of haplotypes where appropriate). Overall HLA-B allele distributions between the myositis subtypes with controls were calculated with exact probabilities calculated using the CLUMP program [33]. Linkage disequilibrium (LD) was calculated using both D' and r^2 values and haplotypes were estimated for selected loci and constructed using the Expectation/Maximisation (EM) algorithm, using HelixTree (version 3.1.2, Golden Helix, Inc., Bozeman, MT, USA). The analyses were also repeated after stratification for myositis serology and presence of CAM. Unless otherwise stated, the statistical package Stata (release 8, Stata Corp., College Station, TX) was used to perform statistical analysis. The study had 80% power to detect the effect of a SNP in each myositis group, with a 17% minor allele frequency conferring an OR of 2.5 at the 5% significance level, assuming a dominant mode of inheritance.

Results

Clinical details

The 303 cases recruited for the study included 121 PM, 109 DM and 73 myositis/CTD-overlap patients. The myositis/CTD-overlap patients had the following primary diagnoses: 48 systemic sclerosis (SSc), nine MCTD, seven Sjögren's syndrome, seven SLE and two RA. There was no significant difference in the mean age at myositis onset between the myositis subgroups (mean 48 years). The overall female percentage was 71% and median duration of disease at data capture was 5 years.

Genotype and allele analysis

Genotype frequencies are summarized in Table 1. TNF-α SNPs showing significant allele associations with various myositis subgroups *vs* controls are summarized in Table 2. A significant increase of the -1031 wild-type TT genotype was noted in the overall myositis group *vs* controls under a recessive mode of inheritance (TT *vs* CC + TC, corrected P [P_{corr}] = 0.008, OR 1.9, 95% CI 1.3–2.9). In DM, the genotype also showed a significant association *vs* controls before multiple comparisons (TT *vs* CC + TC, uncorrected P [P_{uncorr}] = 0.005, OR 2.2, 95% CI 1.2–3.8). The -1031T allele was significantly increased in the DM group, even after adjustment for multiple comparisons (Table 2). Genotype analysis of the -308 SNP revealed a marked increase for AA/GA across all disease subgroups *vs* controls, and a highly significant association under a dominant model. The -308A SNP allele was significantly increased in all disease subgroups *vs* controls. The strongest association was observed within the CTD-overlap group, where the -308A allele was significantly increased in SSc/myositis overlap patients *vs* controls (36% *vs* 15%, P_{corr} < 0.00001, OR 3.3, 1.9–5.5). The -1031T allele association was still present in -308A allele negative cases *vs* controls, before multiple corrections (78% cases *vs* 69% controls,

TABLE 1. Genotype frequencies of TNF-α polymorphisms in myositis patient subgroups and controls

	Controls (%)	PM (%)	DM (%)	CTD-overlap (%)
-1031 (rs1799964)				
Genotype	n = 173	n = 119	n = 109	n = 70
TT	99 (57.2)	84 (70.6)	81 (74.3)	49 (70.0)
TC	62 (35.8)	30 (25.2)	28 (25.7)	21 (30.0)
CC	12 (6.9)	5 (4.2)	0	0
-863 (rs1800630)				
Genotype	n = 168	n = 115	n = 106	n = 70
CC	118 (70.2)	83 (72.2)	82 (77.4)	54 (77.1)
CA	50 (29.8)	31 (27.0)	23 (21.7)	16 (22.9)
AA	0	1 (0.9)	1 (0.9)	0
-308 (rs1800629)[a]				
Genotype	n = 156	n = 117	n = 108	n = 68
GG	112 (71.8)	48 (41.0)	48 (44.5)	23 (33.8)
GA	42 (26.9)	61 (52.1)	55 (50.9)	41 (60.3)
AA	2 (1.3)	8 (6.8)	5 (4.6)	4 (5.9)
+489 (rs1800610)				
Genotype	n = 164	n = 114	n = 106	n = 70
GG	142 (86.6)	97 (85.1)	95 (93.0)	64 (91.4)
GA	22 (13.4)	17 (14.9)	11 (13.0)	6 (8.6)
AA	0	0	0	0

Comparisons are for disease vs controls. PM, polymyositis; DM, dermatomyositis. Probabilities stated are corrected for multiple comparisons.
[a]AA + AG vs GG: PM, $P < 0.00001$, odds ratio 3.7 (95% confidence interval 2.1–6.3); DM, $P = 0.0001$, OR 3.2 (1.8–5.5); CTD-overlap, $P < 0.00001$, OR 5.0 (2.6–9.6).

$P_{uncorr} = 0.03$, OR 1.6, 1.03–2.4). No additional genotype or allele associations were noted when the disease subgroups were combined, and no significant difference in the allele/genotype frequencies was noted when each disease subgroup was compared against the other. No associations with gender were noted for any of the TNF SNPs between cases and controls.

Antibody and clinical associations. A summary of the serological profiles of the AOMIC patients has been previously reported [9]. Stratification of the cases by the presence of anti-synthetase or -PM-Scl antibody status revealed a greater strength of association for both the -308 and -1031 alleles vs controls, compared with the clinical subgroup associations (Table 2). The overall myositis disease association for these SNPs was lost after allowing for the presence of both of these antibodies. Both alleles were also risk factors in patients with ILD, although this association was also lost after accounting for the presence of anti-synthetase antibodies. No further significant associations were noted when the groups were stratified by the presence of other MSA/MAAs including the more DM-specific anti-Mi-2 and 155/140 antibodies, or by CAM. Interaction analysis revealed no influence of age or gender on the SNP associations.

HLA associations. HLA class II associations with the AOMIC IIM cohort have been previously reported [9]. Examining the HLA-B locus, there were highly significant differences in overall allelic distributions between myositis subgroups and controls (Table 3). HLA-B*08 was a risk factor across all myositis disease subgroups vs controls. No other HLA-B allele proved a significant risk or protective factor in the overall group or subgroups. A relative pre-dispositional effect test was performed, to examine whether the effect of other alleles was masked by the relatively increased frequency of HLA-B*08 [34]. The overall exact tests were recalculated without HLA-B*08, after which no further overall differences were detected between the myositis subgroups vs controls.

The TNF-308A SNP is known to be in strong LD with the common ancestral haplotype (AH). Thus, to investigate whether or not the TNF-α SNP associations observed were independent of the AH, multivariate logistic regression models were created with two predictors, TNF-308A and each member of the AH

(HLA-B*08, DRB1*03, DQA1*05, DQB1*02). In the PM and CTD-overlap groups, B*08, DRB1*03, DQA1*05 were each independent predictors after adjustment for the TNF-308A allele, which then lost statistical significance. However, TNF-308A proved an independent predictor after adjustment for DQB1*02 in these subgroups. In contrast, in the DM group, TNF-308A was independent of each AH marker with the exception of B*08. After allowing for the presence of the AH-associated antibodies in the DM group (i.e. the anti-synthetases and anti-PM-Scl), TNF-308A remained independent of DRB1*03 and DRB1*05.

To ascertain which of the tested markers in the AH had the strongest association, a further multivariate logistic regression model was created incorporating all AH markers, including TNF-308A, HLA-B*08, DRB1*03, DQA1*05 and DQB1*02. In the overall myositis group vs controls, after adjusting for the other predictors, HLA-B*08 showed the greatest strength of association (coefficient 1.2 ± 0.5, $P = 0.01$). An interaction analysis was also performed between TNF-308A and each of the tested HLA alleles from the AH. In each instance, no interaction was present, thus excluding any multiplicative effect of the TNF-α marker with the HLA alleles.

LD analysis

All SNPs in the control group were in HWE, except -863, which deviated slightly from HWE ($P = 0.03$). LD pairwise analysis was performed between the TNF-α SNPs using both D′ and r^2 correlation coefficient values (Table 4). The TNF-α SNPs demonstrated significant LD as evidenced by D′ values (D′ > 0.91), with the exception of -308 and +489 (D′ = 0.63). When LD was assessed by the more stringent measure of r^2 (which accounts for differences in allele frequencies), correlation remained strong between TNF-1031 and -863 ($r^2 = 0.5$), but was much weaker between the remaining SNPs ($r^2 < 0.1$), the differing LD values reflecting the variation in allele frequencies between SNPs.

The degree of LD was also estimated with HLA associations in the control group, to determine their relationship within the MHC region. The four TNF-α SNPs were examined against all HLA-B, DRB1, DQA1 and DQB1 broad-type alleles. The TNF-1031, -863 and -308 SNPs showed greatest correlation with the AH alleles ($r^2 > 0.2$), specifically between -308 and B*08 ($r^2 = 0.53$) and between -308 and DRB1*03 ($r^2 = 0.46$). The LD relationship between -308A and the HLA alleles from the AH was strongest for B*08 (D′ = 0.87) followed by DRB1*03 (D′ = 0.71), DQA1*05 (D′ = 0.70) and then DQB1*02 (D′ = 0.62).

Haplotype analysis

Haplotype frequencies for the TNF-α SNPs, HLA-B, DRB1, DQA1 and DQB1 loci were compared between cases and controls (Table 5). In the control population, haplotypes for the TNF-α SNPs existed at a frequency of >6%, and captured 100% of the variation. The TNF-1031T/-863C/-308A/+489G haplotype showed a significant increase in all disease subtypes vs controls. The frequencies were similar to that seen in the -308A univariate allele analysis, suggesting that this haplotype association was attributable to the -308A allele. The -308A allele also formed an extended haplotype with the known HLA myositis associations (HLA-B*08/TNF-308A/DRB1*03/DQA1*05/DQB1*02), and was also a strong risk factor in anti-synthetase (haplotype frequency 39% vs 10%, $P_{corr} < 0.00001$, OR 5.6, 2.9–10.9) and anti-PM-Scl positive patients (haplotype frequency 43% vs 10%, $P_{corr} < 0.00001$, OR 6.6, 2.8–15.1) vs controls. The haplotype frequency in each group was similar regardless of inclusion of the other TNF-α SNPs.

The HLA-B*07/TNF-308G/DRB1*02/DQA1*01/DQB1*06 haplotype was a protective factor for the overall and PM groups

TABLE 2. TNF-α allele associations for myositis disease subtypes and selected antibody subtypes *vs.* controls

	Controls (%)	PM (%)	DM (%)	CTD-overlap (%)	ARS (%)	PM-Scl (%)
-1031 allele	2n = 346	2n = 238	2n = 218	2n = 140	2n = 128	2n = 56
T	260 (75.1)	198 (83.2)	190 (87.2)	119 (85.0)	115 (89.8)	52 (92.9)
C	86 (24.9)	40 (16.8)	28 (12.8)	21 (15.0)	13 (10.2)	4 (7.1)
P_{corr}	–	NS	0.006	NS	0.01	NS
OR (95% CI) for T	–	1.6 (1.1–2.5)	2.2 (1.4–3.6)	1.9 (1.1–3.1)	2.9 (1.6–5.3)	4.3 (1.6-11.3)*
-308 allele	2n = 312	2n = 234	2n = 216	2n = 136	2n = 124	2n = 52
G	266 (85.3)	157 (67.1)	151 (69.9)	87 (64.0)	67 (53.2)	28 (53.9)
A	46 (14.7)	77 (32.9)	65 (30.1)	49 (36.0)	59 (46.8)	24 (46.1)
P_{corr}	–	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
OR (95% CI) for A	–	2.8 (1.9–4.3)	2.5 (1.6–3.8)	3.3 (2.1–5.1)	5.1 (3.3–8.0)	5.0 (2.7–8.9)

ARS, anti-synthetase; OR, odds ratio; CI, confidence interval; P_{corr}, corrected probability; NS, not significant.
*The value for this entry has been corrected.

TABLE 3. Frequency of HLA class I phenotypes in IIM subgroups and controls

HLA	Controls n (%)	Polymyositis n (%)	Dermatomyositis n (%)	CTD-overlap n (%)
B	(n = 158)	(n = 94)	(n = 97)	(n = 60)
07	50 (31.6)	18 (19.1)	18 (18.6)	12 (20.0)
08[a]	36 (22.8)	50 (53.2)	48 (49.5)	39 (65.0)
13	11 (4.4)	3 (3.2)	8 (8.2)	0
14	9 (5.7)	5 (5.3)	8 (8.2)	3 (5.0)
15	24 (15.2)	13 (13.8)	7 (7.2)	7 (11.7)
18	13 (8.2)	5 (5.3)	5 (5.2)	4 (6.7)
27	15 (9.5)	8 (8.5)	6 (6.2)	2 (3.3)
35	21 (13.3)	14 (14.9)	14 (14.4)	9 (15.0)
37	7 (4.4)	5 (5.3)	1 (1.0)	0
38	1 (0.6)	2 (2.1)	1 (1.0)	1 (1.7)
39	6 (3.8)	4 (4.3)	2 (2.1)	2 (3.3)
40	25 (15.8)	13 (13.8)	14 (14.4)	8 (13.3)
41	3 (1.9)	1 (1.1)	1 (1.0)	0
42	0	1 (1.1)	0	0
44	42 (26.6)	16 (17.0)	26 (26.8)	14 (23.3)
45	1 (0.6)	3 (3.2)	2 (2.1)	2 (3.3)
49	2 (1.3)	0	3 (3.1)	3 (5.0)
50	1 (0.6)	1 (1.1)	2 (2.1)	0
51	8 (5.1)	9 (9.6)	4 (4.1)	5 (8.3)
52	0	0	1 (1.0)	0
53	0	0	0	2 (3.3)
55	5 (3.2)	3 (3.2)	8 (8.2)	0
56	2 (1.3)	2 (2.1)	0	1 (1.7)
57	13 (8.2)	3 (3.2)	3 (3.1)	1 (1.7)
58	3 (1.9)	0	1 (1.0)	1 (1.7)
81	0	0	0	0
P		0.003	0.003	0.0001

P = global probability for disease *vs* controls (using genotype data) n (%) = number per percentage of patients with individual phenotypes.
[a]PM *vs* controls, P < 0.00001, OR 3.9 (2.1–6.9); DM *vs* controls, P < 0.00001, OR 3.3 (1.9–5.9); CTD-overlap *vs* controls, P < 0.00001, OR 6.3 (3.1–12.7).

vs controls. Again, the frequency of this haplotype was the same regardless of inclusion of the other TNF-α SNPs (-1031T/-863C/-308G/+489G). TNF-1031C/-863C/-308G/+489G was also protective in overall and DM groups *vs* controls. These other haplotypes may reflect the lower frequency of the -308G allele in cases compared with controls. No significant differences were noted between myositis subgroups.

Discussion

This study used a candidate gene approach to examine haplotype tagging SNPs in the TNF-α region, in order to capture the most frequently found haplotypes. To date, this represents the largest IIM study investigating the TNF-α genomic region. The results confirm that the TNF-308A allele is a risk factor in UK adult onset PM, DM and myositis/CTD-overlap disease. Furthermore, the TNF-308G/A genotype is significantly increased in the disease subtypes *vs* controls, suggesting a dominant mode of inheritance. TNF-308A forms an extended haplotype with HLA alleles forming part of the common AH and is dependent on HLA-B*08, with which it shares strong LD. In contrast,

TNF-308A is independent of HLA-DQB1*02 with which it shares the least LD. In DM, TNF-308A is also independent of the other HLA class II AH alleles, DRB1*03 and DQA1*05. The reason for this is unclear, but it may reflect the different serological profile seen in DM compared with PM and CTD-overlap [9, 35]. No interaction effect is noted between the AH and the TNF alleles which could increase the strength of association. TNF-308A also shows an increased strength of association in patients possessing anti-synthetase and -PM-Scl antibodies (where the AH is a known strong risk factor). For the first time, an association of the -1031T allele has been demonstrated in a myositis disease cohort, especially in patients with DM and anti-PM-Scl antibodies.

The TNF-308A findings are consistent with that seen in the previous smaller myositis studies [14–16] and confirm that risk of disease is spread over a wider area in the MHC than just HLA class I and II alleles. This is reflective of strong LD within the MHC and highlights the difficulty of pinpointing a true disease susceptibility gene for the IIMs within the region. The strongest effect in the AH was at HLA-B*08, a finding confirmed in a combined myositis group tested in a recent US study, although HLA class II alleles conferred a greater strength of association in disease subgroups [10]. Werth *et al.* [15] noted that the frequency of cases with both TNF-308A and HLA-DR3 were not significantly increased between DM cases *vs* controls, although this lack of association may be due to the small patient numbers used in this study. Hassan *et al.* [16] also confirmed that TNF-308A was part of the AH, additionally incorporating two microsatellite markers into the haplotype.

It is known that subjects with the AH have an altered immune response and produce an imbalance of certain cytokines [36]. Certainly, serum TNF-α levels appear to be higher in normal subjects who possess the AH [19]. In a study of the TNF-α region in juvenile DM patients, in comparison with carriers of the TNF-308G allele, -308A carriers synthesized more TNF-α, had a longer disease course and were more likely to have pathological calcification [14]. A recent adult IIM study highlighted that a higher serum TNF:IL-10 ratio was noted in TNF-308A compared with TNF-308G carriers and especially so in females [37]. A tendency to a higher TNF: IL-10 ratio was also noted in anti-Jo-1 and -Ro antibody positive patients, possibly due to the association of these antibodies with the AH. Tying in these findings with the currently observed observation that the TNF-308A allele is in strong LD with the AH, it appears that excess TNF-α production is more likely associated with possession of the AH, rather than the TNF-308A allele alone. It is possible that the AH codes for particular conformational epitopes at the protein level which enhance antigen presentation and the resulting immune and cytokine response [10].

The SNPs tested are haplotype tagging SNPs which means that the true causal SNP may not have been tested directly in the current study. Unlike the previous aforementioned studies, we have not correlated findings with serum data, but it is reasonable

TABLE 4. Pairwise LD between HLA class I, II and TNF-α genes

	B*08	TNF-1031	TNF-863	TNF-308	TNF+489	DRB1*03	DQA1*05	DQB1*02
B08	x	0.04	0.02	0.53	<0.01	0.43	0.27	0.22
TNF-1031	0.97	x	0.97	0.5	0.02	0.02	0.03	0.04
TNF-863	0.97	0.97	x	0.03	0.01	0.02	0.05	0.05
TNF-308	0.87	0.99	0.97	x	0.005	0.46	0.3	0.2
TNF+489	0.53	0.96	0.92	0.63	x	<0.01	<0.01	<0.01
DRB1*03	0.74	0.61	0.97	0.71	<0.01	x	0.62	0.46
DQA1*05	0.75	0.57	0.95	0.7	0.1	0.99	x	0.26
DQB1*02	0.76	0.62	0.97	0.63	0.1	0.93	0.55	x

Figures below diagonal refer to D′ values, figures above the diagonal refer to r^2 values.

TABLE 5. Results of haplotype analysis for HLA class II and TNF-α SNPs

Haplotype				Controls $2n=294$	Overall $2n=550$	PM $2n=212$	DM $2n=208$	CTD-overlap $2n=130$
-1031	-863	-308	+489					
T	C	G	G[a]	55.1	45.2	40.0	51.7	43.5
T	C	A	G[b]	14.6	32.7	34.0	29.3	36.2
C	A	G	G	14.6	12.3	13.7	11.0	12.3
C	C	G	G[c]	9.2	3.1	3.8	2.1	3.4
T	C	G	C	6.5	5.7	7.7	4.5	4.2
				$2n=196$	$2n=470$	$2n=172$	$2n=184$	$2n=114$
HLA-B*08/TNF-308A/DRB1*03/DQA1*05/DQB1*02[d]				10.2	25.7	28.5	19.9	30.7
HLA-B*07/TNF-308G/DRB1*02/DQA1*01/DQB1*06[e]				13.2	4.8	4.6	5.3	4.4

PM, polymyositis; DM, dermatomyositis. All associations are for disease vs controls. Probabilities stated are corrected for the number of haplotypes and subgroups.
[a]Overall, $P=0.03$, OR 0.7 (0.5–0.9); PM, $P=0.02$, OR 0.5 (0.4–0.8).
[b]Overall, $P<0.00001$, OR 2.8 (1.9–4.2); PM, $P<0.00001$, OR 3.0 (1.9–4.7); DM, $P=0.002$, OR 2.4 (1.5–3.9); CTD-overlap, $P<0.00001$, OR 3.3 (2.0–5.5).
[c]Overall, $P=0.001$, OR 0.3 (0.2–0.6), DM, $P=0.009$, OR 0.2 (0.05–0.6).
[d]Overall, $P<0.00001$, OR 3.0 (1.8–5.3); PM, $P<0.00001$, OR 3.5 (1.9–6.5); DM, $P=0.03$, OR 2.2 (1.2–4.2); CTD-overlap, $P<0.00001$, OR 3.9 (2.0–7.6).
[e]Overall, $P=0.0005$, OR 0.3 (0.2–0.6); PM, $P=0.04$, OR 0.3 (0.1–0.8).

to assume parity with findings within other Caucasian myositis populations [14, 16]. The numbers in our study have allowed us to stratify by myositis disease and serological subtype, to examine LD and the relationship between markers within the AH and examine extended haplotypes within the TNF-α and wider MHC region.

To conclude, these findings confirm the association of the TNF-308A allele with IIM susceptibility in a large cohort of UK Caucasians. Significant differences do not exist between the myositis subgroups and the risk appears strongest in anti-synthetase and -PM-Scl patients, i.e. in patients where the AH association is strongest. The TNF-308A association is not independent of HLA-B*08 and other IIM genetic susceptibility markers within the AH, due to the strong LD within the MHC, but does form part of a haplotype with these factors. This study clarifies the role of the TNF-α gene and furthers our understanding of the AH as an important genetic risk factor in the pathogenesis of myositis.

Rheumatology key messages

- TNF-308A is strongly associated with inflammatory myositis.
- The risk appears strongest in anti-synthetase/PM-Scl positive cases
- In the IIMs, TNF-308A is not independent of HLA-B*08.

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