EXTENDED REPORT

Interaction of HLA-DRB1*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study

H Chinoy,[1] S Adimulam,[1] F Marriage,[2] P New,[1] M Vincze,[3] E Zilahi,[3] A Kapitány,[3] A Gyetvai,[3] L Ekholm,[4] P Novota,[5] M Remakova,[5] P Charles,[6] N J McHugh,[7] L Padyukov,[4] L Alfredsson,[8] J Vencovsky,[5] I E Lundberg,[4] K Danko,[3] W E Ollier,[2] R G Cooper[1,2]

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[1]Rheumatic Diseases Centre, Manchester Academic Health Science Centre, The University of Manchester, Salford Royal NHS Foundation Trust, Salford, UK
[2]Centre for Integrated Genomic Medical Research, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK
[3]Division of Clinical Immunology, University of Debrecen, Debrecen, Hungary
[4]Rheumatology Unit, Department of Medicine, Karolinska University Hospital, Solna, Karolinska Institutet, Stockholm, Sweden
[5]Institute of Rheumatology, Charles University, Prague, Czech Republic
[6]Kennedy Institute of Rheumatology, Imperial College, London, UK
[7]Royal National Hospital for Rheumatic Diseases, Bath, UK
[8]Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

Correspondence to
Robert G Cooper, Rheumatic Diseases Centre, Manchester Academic Health Science Centre, The University of Manchester, Salford Royal NHS Foundation Trust, Eccles Old Road, Salford M6 8HD, UK; robert.g.cooper@manchester.ac.uk

ABSTRACT

Objectives HLA-DRB1*03 is strongly associated with anti-Jo-1-positive idiopathic inflammatory myopathies (IIM) and there is now increasing evidence that Jo-1 antigen is preferentially expressed in lung tissue. This study examined whether smoking was associated with the development of anti-Jo-1 antibodies in HLA-DRB1*03-positive IIM.

Methods IIM cases were selected with concurrent information regarding HLA-DRB1 status, smoking history and anti-Jo-1 antibody status. DNA was genotyped at DRB1 using a commercial sequence-specific oligonucleotide kit. Anti-Jo-1 antibody status was established using a line blot assay or immunoprecipitation.

Results 557 Caucasian IIM patients were recruited from Hungary (181), UK (99), Sweden (94) and Czech Republic (183). Smoking frequency was increased in anti-Jo-1-positive IIM cases, and reached statistical significance in Hungarian IIM (45% Jo-1-positive vs 17% Jo-1-negative, OR 3.94, 95% CI 1.53 to 9.89, $p < 0.0001$). A strong association between HLA-DRB1*03 and anti-Jo-1 status was observed across all four cohorts (DRB1*03 frequency: 74% Jo-1-positive vs 35% Jo-1-negative, OR 5.55, 95% CI 3.42 to 9.14, $p < 0.0001$). The frequency of HLA-DRB1*03 was increased in smokers. The frequency of anti-Jo-1 was increased in DRB1*03-positive smokers vs DRB1*03-negative non-smokers (42% vs 8%, OR 7.75, 95% CI 4.21 to 14.28, $p < 0.0001$) and DRB1*03-positive non-smokers (42% vs 31%, $p = 0.08$). In DRB1*03-negative patients, anti-Jo-1 status between smokers and non-smokers was not significantly different. No significant interaction was noted between smoking and DRB1*03 status using anti-Jo-1 as the outcome measure.

Conclusion Smoking appears to be associated with an increased risk of possession of anti-Jo-1 in HLA-DRB1*03-positive IIM cases. The authors hypothesise that an interaction between HLA-DRB1*03 and smoking may prime the development of anti-Jo-1 antibodies.

The idiopathic inflammatory myopathies (IIM) are a group of rare and heterogenous autoimmune diseases characterised by inflammation of skeletal muscle, other organ systems and associated with significant co-morbidities. The aetiopathology of IIM remains poorly understood, but is likely due to interactions between genetic and environmental factors.[1] IIM may be broadly classified according to the traditional clinical subtypes: polymyositis, dermatomyositis, myositis overlapping with another connective tissue disease (CTD), inclusion body myositis and juvenile dermatomyositis.

However, serological status according to circulating myositis-specific antibodies (MSA) or myositis-associated antibodies (MAA) is being increasingly used in the classification of IIM subtypes, and often correlates with well-defined IIM clinical phenotypes. For example, anti-aminoacyl transfer RNA synthetase antibodies are highly specific for IIM and define a clinical subtype labelled the 'anti-synthetase syndrome', characterised by Raynaud's phenomenon, mechanics' hands, arthropathy, interstitial lung disease and myositis.[2] The most frequently found anti-aminoacyl tRNA synthetase antibody is the anti-histidyl tRNA synthetase (Jo-1) antibody. Alleles forming part of the Caucasian MHC 8.1 common ancestral haplotype (HLA-A1-B8-Cw7-DRB1*0301-DQA1*0501-C4A*Q0) occur in strong linkage disequilibrium within Caucasian populations in northern and western Europe, and represent risk factors for a large number of immunopathological diseases.[3] To date, the 8.1 common ancestral haplotype has also been identified as the major risk factor in IIM.[2 4–11] HLA alleles are also associated with the development of MSA/MAA in IIM, and the strong association of anti-aminoacyl tRNA synthetase antibodies and alleles comprising the 8.1 common ancestral haplotype has been confirmed in several IIM studies.[2 8 12–15]

The question arises as to whether and how anti-Jo-1, an antibody against a ubiquitous cytoplasmic antigen, may play a pathogenic role in IIM. In the development of rheumatoid arthritis (RA), an interaction between smoking and alleles forming the HLA shared epitope is thought to prime the development of anti-citrulline-positive antibodies.[16] That Jo-1 is preferentially expressed in lung tissue[17 18] is of potential relevance, bearing in mind that interstitial lung disease may be present in up to 70% of anti-Jo-1-positive patients.[19]

The current study investigated the hypothesis that a gene–environmental interaction between HLA-DRB1*03 and smoking could be of relevance in the development of anti-Jo-1 antibody-positive IIM.

METHODS
Subjects
Five hundred and fifty-seven adult-onset IIM patients, aged 18 years of age or older at disease onset, were recruited from four European countries, UK, Sweden, Hungary and Czech Republic.[2] Patients with polymyositis or dermatomyositis had probable or definite myositis, according to the Bohan and Peter criteria.[20 21] Patients with myositis–CTD overlap either had their primary disease with probable/definite myositis according to Bohan and Peter,[20 21] or had possible myositis but additionally had a confirmed MSA/MAA.[22] The collection of data and blood from patients was undertaken under the regulation of the relevant local research ethics committee, informed consent having been obtained according to the Declaration of Helsinki. Smoking history was ascertained through a retrospective questionnaire including the statement, 'Have you ever smoked as much as one cigarette a day for as long as a year?'.

Anti-Jo-1 autoantibody testing
Serum was obtained from all patients for the determination of MSA including anti-Jo-1 antibodies. Sera from the Czech, Hungarian and Swedish cohorts were tested for antibodies to Jo-1 using a line-immunoassay system (Euroimmun, Lubeck, Germany). Briefly, diluted patient serum was incubated on a rocker with a membrane strip with autoantigens located on nitrocellulose pads. After incubation and washing, bound antibody was detected using a specific anti-IgG antibody linked to an enzyme and visualised using a chromogenic reaction. This was performed according to the manufacturer's instruction using the Euroblot-master automated processor (Euroimmun). Assays were visually interpreted by comparison with known negative and positive control samples. In the case of the UK samples, detection was made by radio-immunoprecipitation, as previously described.[2 23] For anti-Jo-1 testing, the line-immunoassay system and radio-immunoprecipitation are known to correlate well with each other.[24]

Genotyping
DNA was extracted using standard phenol-chloroform or salting-out methods from a peripheral whole blood sample obtained from all cases. As data were readily available for HLA-DRB1 across the four European cohorts, this particular loci was chosen for further investigation. Cases were broad-specificity typed at the HLA-DRB1 loci, using commercially available kits.

Statistical analyses
Individual associations were derived from 2×2 contingency tables using the χ^2 test, or two-tailed Fisher's exact test when individual cells valued five or less. Data were expressed as OR with exact 95% CI. Pointwise p values were not corrected as the association with HLA-DRB1*03 and IIM is well established in the literature.[25 26] The described analyses were also repeated after stratification by smoking and anti-Jo-1 status using multinomial logistic regression. The statistical package Stata (release 9.2) was used to perform statistical analysis.

RESULTS
Clinical subgroups
A total of 557 Caucasian adult-onset IIM patients (women 75%) was recruited from the following countries: Hungary (181), Czech Republic (183), Sweden (94), UK (99) (table 1). The median age of disease onset was 48 years (interquartile range 37–58). No significant difference was noted in the gender or age at onset

Table 1 IIM subgroup frequencies by country

	Polymyositis	Dermatomyositis	Overlap	Total
	n (%)	n (%)	n (%)	n (%)
Hungary	101 (56)	42 (23)*	38 (21)†	181
Czech Republic	82 (45)	95 (52)‡	6 (3)§	183
Sweden	53 (56)	37 (39)	4 (4)	94
UK	40 (40)	39 (39)	20 (20)	99
Total	276 (50)	213 (38)	68 (12)	557

Comparing country with data from other countries combined: *Hungary: dermatomyositis versus polymyositis plus overlap: OR 0.36, 95% CI 0.24 to 0.54, corrected probability p_{corr}<0.0001; †Hungary: overlap versus polymyositis plus dermatomyositis: OR 3.06, 95% CI 1.83 to 5.14, p_{corr}<0.0001; ‡Czech Republic: dermatomyositis versus polymyositis plus overlap: OR 2.34, 95% CI 1.63 to 3.37, p_{corr}<0.0001; §Czech Republic: overlap versus polymyositis plus dermatomyositis: 0.17, 95% CI 0.07 to 0.40, p_{corr}<0.0001.
IIM, idiopathic inflammatory myopathy.

Table 2 Frequency of anti-Jo-1 and smoking status by country

Country	Anti-Jo-1 present n (%)	Smoking ever n (%)
Hungary	29 (16)	39 (22)
Czech Republic	50 (27)	71 (39)
Sweden	14 (15)	51 (54)
UK	22 (22)	55 (56)
Total	115 (21)	216 (39)

Comparison between countries: anti-Jo-1, overall p value 0.026. Smoking status, overall p value <0.0001.

distribution between the cohorts (p=0.065). Fifty per cent of the patients were classified as having polymyositis, 38% dermatomyositis and 12% myositis–CTD overlap disease. A stratification of clinical subgroup status by each participating country is summarised in table 1. An overall difference was present in the distribution of clinical subgroups (p<0.0001). Comparing the Hungarian cohort with other countries combined, the frequency of dermatomyositis was lower and myositis–CTD overlap higher in the Hungarian group. Within the Czech cohort, the dermatomyositis frequency was higher and myositis–CTD overlap frequency lower. Some variation was noted in the frequency of HLA-DRB1*03 between the European cohorts (Hungary, 32.0%; Czech Republic, 43.2%; Sweden, 54.3%; UK, 52.5%; see supplementary table S1, available online only).

Frequency of smoking and anti-Jo-1
The frequency of anti-Jo-1 antibodies was 21% for the overall group (table 2). The highest frequency was noted in the Czech cohort, and the lowest in the Swedish cohort. The frequency of anti-Jo-1 between the countries was broadly similar, although a significant difference was noted in the overall distribution. The overall frequency of 'ever smoking' was 39% and an overall significant difference was noted for the distribution of smoking between the countries. The lowest frequency of ever-smokers was noted in the Hungarian cohort, compared with the highest frequency being seen in the UK cohort. No significant differences in the frequency of smoking were noted across the clinical subgroups.

Relationship between smoking and anti-Jo-1
The frequency of smoking was increased in anti-Jo-1-positive IIM cases across all four cohorts (smoking frequency 50% Jo-1-positive cases vs 36% Jo-1-negative cases, OR 1.83, 95%CI 1.18 to 2.83, p=0.004). This association also reached statistical significance in the case of the Hungarian IIM group (smoking frequency 45% Jo-1-positive cases vs 17% Jo-1-negative cases, OR 3.94, 95% CI 1.53 to 9.89, p=0.0009).

Table 3 Anti-Jo-1 status by smoking and HLA-DRB1*03 status

Smoking status	DRB1*03 status	Anti-Jo-1 positive, n (%)	Anti-Jo-1 negative, n (%)	OR, 95% CI	p Value
Negative	Negative	18 (8)	196 (92)	1.0	
Negative	Positive	39 (31)	88 (69)	4.83, 2.62 to 8.90	<0.0001
Positive	Negative	11 (11)	92 (89)	1.30, 0.59 to 2.87	0.51
Positive	Positive	47 (42)	66 (58)	7.75, 4.21 to 14.28	<0.0001

HLA-DRB1*03-positive cases assigned if they possess one or two copies of DRB1*03.

Table 4 Anti-Jo-1 status by smoking and HLA-DRB1*03 status, and by gender

Smoking status	DRB1*03 status	Anti-Jo-1 positive, n (%)	Anti-Jo-1 negative, n (%)	OR, 95% CI	p Value
Men					
Negative	Negative	4 (10)	35 (90)	1.0	
Negative	Positive	6 (25)	18 (75)	2.92, 0.73 to 11.67	0.13
Positive	Negative	6 (16)	32 (84)	1.64, 0.42 to 6.35	0.47
Positive	Positive	15 (39)	23 (61)	5.70, 1.68 to 19.37	0.005
Women					
Negative	Negative	14 (8)	161 (92)	1.0	
Negative	Positive	33 (32)	70 (68)	5.42, 2.73 to 10.75	<0.0001
Positive	Negative	5 (8)	60 (92)	0.96, 0.33 to 2.78	0.94
Positive	Positive	32 (43)	43 (57)	8.56, 4.20 to 17.5	<0.0001

HLA-DRB1*03-positive cases assigned if they possess one or two copies of DRB1*03.

Relationship between HLA-DRB1*03 and anti-Jo-1

As expected from previous work,[2][15] a strong association between HLA-DRB1*03 and anti-Jo-1 status was observed across all four cohorts combined (DRB1*03 frequency 74% Jo-1-positive cases vs 35% Jo-1-negative cases, OR 5.55, 95% CI 3.42 to 9.14, p=1.3×10^{-14}).

Relationship between HLA-DRB1*03 and smoking

HLA-DRB1*03 was further examined by smoking status. The frequency of HLA-DRB1*03 was increased in smokers, and this association again reached statistical significance in Hungarian IIM (DRB1*03 frequency 59% smokers vs 25% non-smokers, OR 4.39, 95% CI 1.96 to 9.92, p<0.00001).

Investigation of risk of developing anti-Jo-1 antibody conferred by HLA-DRB1*03 and smoking

An analysis was then conducted to examine further the interrelationship between smoking and HLA-DRB1*03, and the risk of the development of anti-Jo-1 antibodies. For the purposes of this analysis, and to maximise statistical power, the four EU cohorts were combined. The HLA-DRB1*03 negative/non-smokers were used as the reference group for these comparisons. The frequency of anti-Jo-1 was significantly increased in both DRB1*03-positive smokers and non-smokers versus DRB1*03-negative non-smokers (table 3). In DRB1*03-negative patients, no significant difference was noted in anti-Jo-1 status between smokers and non-smokers. The frequency of anti-Jo-1 was increased in the DRB1*03-positive group who smoked compared with DRB1*03-positive non-smokers, although this trend did not achieve statistical significance (p=0.08). A similar trend was noted when a separate analysis was made of men and women (table 4). An interaction term was then created between ever-smokers and DRB1*03 status, using anti-Jo-1 as the outcome measure, but no multiplicative effect was observed between smoking and DRB1*03.

DISCUSSION

In four independent European IIM cohorts, the frequency of anti-Jo-1 antibody was approximately 20% in adult-onset disease. Intriguingly, it was observed that the frequency of smoking was increased in anti-Jo-1-positive IIM cases across all four cohorts. The frequency of HLA-DRB1*03 was significantly increased in IIM patients who were smokers in the Hungarian cohort. The frequency of anti-Jo-1 antibody was in fact highest in HLA-DRB1*03-positive smokers compared with the other groups. These findings therefore suggest that smoking may only confer a risk of IIM in HLA-DRB1*03-positive individuals, or alternatively that smoking is a confounding variable for another factor that we did not investigate, for example, interstitial lung disease. Using a statistical interaction term, a relationship was not established between smoking and HLA-DRB1*03, but nevertheless these results do suggest that there is an additional risk of the development of anti-Jo-1 in HLA-DRB1*03-positive cases who are ever-smokers.

The design of the study implies a number of limitations. Environmental triggers may have conferred risk years before IIM onset, therefore it would have been desirable to quantify the number of cigarettes and at what point they were smoked before the onset of IIM accurately. This would have allowed an examination of any cumulative effects of smoking on risk and to assess any temporal relationship of smoking with IIM onset or the commencement of anti-Jo-1 positivity. Examining these issues would, however, only be possible as part of a detailed prospective study. Our study was able to establish that smoking does appear to represent a risk factor for the development of anti-Jo-1 antibodies, but due to a lack of a matched control population, we were not able to establish that smoking was a risk factor per se for IIM, as has been demonstrated in other autoimmune diseases.[16][27] We aim to address this issue as part of a future study. Furthermore, this particular study has not examined other possible MSA/MAA as part of an interaction with smoking. It is possible that in this study, HLA-DRB1*03 may be a marker for a different allele in which linkage disequilibrium is shared. A further issue is the heterogeneity of the HLA class II distribution and the variation of smoking frequency between the European populations, which will have affected the consistency of the results. Overall, a gene–environmental interaction between HLA-DRB1*03 and smoking

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on the multiplicative scale was not proved to be statistically significant, but this may be due to a lack of statistical power. We also estimated the interaction between HLA-DRB1*03 and smoking on the additive scale (data not shown), in which there was a tendency towards an interaction but this was not statistically significant.

Smoking has been shown to induce disease development in other rheumatic diseases. A number of case–control and cohort studies have confirmed the association of smoking with the development of RA. Furthermore, a large case–control study has confirmed a high risk of developing anti-citrullinated antibody-positive RA conferred by a gene–environmental interaction between smoking and HLA-DR shared epitope genes.[16] A further remarkable finding in this study was a high detected proportion of citrulline-positive bronchoalveolar lavage cells in healthy smokers. A further study has shown an association between smoking and systemic lupus erythematosus, and more specifically the occurrence of anti-double-stranded DNA antibodies.[28]

The question arises as to whether smoking may have aetiopathological significance in IIM, for example, by inducing immunomodulatory effects in lung tissues. The Jo-1 antigen is known to be expressed in normal and cancerous lung tissue from non-IIM individuals in higher amounts than observed in other tissues.[17] Granzyme B, a serine protease, is a critical component of the cytotoxic T-cell granule exocytosis pathway,[29] and is thought to mediate muscle cell apoptosis.[30] Most autoantigens targeted in systemic autoimmune diseases, including several aminoacyl tRNA synthetases, are substrates of granzyme B. Jo-1 appears to be uniquely cleaved by granzyme B in position 48 of the N-terminal domain.[31] Furthermore, a unique granzyme B-cleavable form of Jo-1 is found in alveolar epithelium but not in muscle tissue,[18] and the expression of soluble granzyme B in peripheral blood CD8+ T cells appears to be increased in smokers with chronic obstructive pulmonary disease.[32]

In conclusion, the current study has shown that smoking is associated with IIM patients who are either anti-Jo-1 antibody and/or HLA-DRB1*03 positive. There is a suggestion that the risk of the development of anti-Jo-1 antibodies is further increased in IIM patients who are both smokers and possess one or more copies of HLA-DRB1*03. This may have aetiological significance in the development of IIM and may point towards a gene–environmental interaction. Future larger scale work is now required in larger patient cohorts, and in which the effects of other HLA genes and associated antibodies are testable with respect to smoking associations.

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