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ASSOCIATION OF HLA-DR4, PROTEASE INHIBITOR PHENOTYPES AND KERATOCONJUNCTIVITIS SICCA WITH PULMONARY ABNORMALITIES IN RHEUMATOID ARTHRITIS

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SUMMARY

One-hundred rheumatoid arthritis (RA) patients were assessed for the association of HLA-DR4, protease inhibitor (Pi) phenotype and keratoconjunctivitis sicca (KCS) with a variety of clinical features, airflow obstruction and bronchial reactivity. Spirometry, lung volume and gas transfer factor measurements were performed to detect airflow obstruction. Bronchial reactivity to inhaled methacholine was assessed by measuring the provocative dose of methacholine causing a 20% fall in FEV1 from the baseline (PD_{20}). Sixty-two patients were HLA-DR4 positive, 87 had Pi MM and 13 MS phenotypes and 37 had positive Schirmer's tear tests. Patients with KCS had a significantly increased history of wheeze (11/37 *vs* 7/63, $P = 0.03$, relative risk (RR) 1.8 [95% CI 1.04, 3.1]), those with HLA-DR4 had a significantly decreased atopy on skin-prick testing [3/62 *vs* 7/38, $P = 0.02$, RR 2.3 [95% CI 1.1, 4.8], Pi phenotypes had no significant effect on respiratory symptoms. Both FEV1 and FVC were significantly higher in the Pi MS group compared to Pi MM group. There was no significant association of HLA-DR4, Pi phenotype and KCS with bronchial reactivity. We conclude that there is no overall significant association of HLA-DR4, Pi phenotype and KCS with airflow obstruction and bronchial reactivity in RA.

KEY WORDS: Rheumatoid arthritis, Airflow obstruction, Bronchial reactivity, HLA-DR4, Protease inhibitor phenotype, Keratoconjunctivitis sicca.

RHEUMATOID arthritis (RA) is associated with several pulmonary manifestations [1] and we have previously shown a significantly increased prevalence of airflow obstruction and bronchial reactivity in unselected patients with RA when compared to controls [2]. The significant increase in airflow obstruction and bronchial reactivity even among life-long non-smoking RA patients suggested that there may be other factors associated with these observed abnormalities.

Three studies have previously found an association between HLA-DR4 and pulmonary abnormalities in RA [3–5]. One reported significant reductions in forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) in HLA-DR4-positive patients compared with HLA-DR4-negative patients [3]. The second study found that 80% of RA patients with airways dysfunction were HLA-DR4 positive, but 66% of their patients with airways dysfunction also had the sicca complex [4]. The third study reported a correlation between HLA-DR4 and the presence of rheumatoid nodules and pulmonary manifestations, but did not specify the nature of pulmonary abnormalities [5].

α_1-Antitrypsin (α_1-AT) or protease inhibitor (Pi) system has over 70 different variants: the most common variants are M, S and Z. The homozygous Pi MM is the commonest variant and is not known to be associated with pulmonary abnormalities. Homozygotes of type Z and heterozygotes of MZ appear to have an increased risk of developing emphysema particularly when they smoke [6]. Deficiencies of α_1-AT have been described with pulmonary abnormalities in RA. Two studies reported a significantly higher frequency of non-M phenotypes in patients with pulmonary fibrosis in RA [7, 8], while another observed an association of Pi MS with rapidly progressive airflow obstruction (three patients) [9]. RA patients with secondary Sjögren's syndrome have been reported to show increased airflow obstruction [3, 4]. However, no previous study has assessed the role of HLA-DR4, Pi phenotype or keratoconjunctivities sicca (KCS) on bronchial reactivity in RA.

The following study addresses the possibility that genetic (inherited) factors may play a part in the expression of airflow obstruction in patients with RA. We have prospectively assessed the role of HLA-DR4, Pi phenotypes and KCS on a variety of clinical features, on airflow obstruction and bronchial reactivity in 100 patients with RA.

PATIENTS AND METHODS

The research project was approved by the Sunderland Health Authority Ethics Committee and written consent was obtained from all subjects. One-hundred consecutive out-patients with RA, as defined by the ARA classification [10], were included in the study.

All the patients visited the hospital twice for study purposes. On the first visit detailed medical, smoking (one pack year = 20 cigarettes daily for 1 yr) and drug histories were obtained. Patients were asked questions based on the Medical Research Council Questionnaire for the respiratory system [11]. Skin-prick tests to house-dust mite, grass pollens, *Aspergillus fumigatus*,

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mixed feathers and any other antigen for which the subject gave a history suggestive of sensitization were done on the anterior aspect of the forearm and compared to the control solution (Bencard). A wheal of 2 mm or more and greater than that of the control was considered positive. Haemoglobin, ESR, autoantibodies and Pi phenotypes were checked and radiographs of hand and chect performed. Rheumatoid factor was determined by latex agglutination test (positive at a dilution of 1:160), HLA-DR4 typing was done by polymerase chain reaction with sequence specific primers [12] and phenotyping of α_1-AT was done by isoelectric focusing [13] using the commercially available PhastGel Dry IEF kit (Pharmacia). Schirmer's tear tests were performed in both eyes in all patients and the response was considered abnormal if there was less than 10 mm wetting over 5 min bilaterally.

Bronchial reactivity to inhaled methacholine was assessed in all the patients as described before [2]. Within one month of the methacholine challenge, detailed pulmonary function tests were performed. They comprised: FEV1 FVC, FEV1/FVC, forced expiratory flow between 25 and 75% of vital capacity (FEF25–75%), forced expiratory flow at 25% (FEF25%), 50% (FEF50%) and 75% (FEF75%) of the vital capacity, residual volume (RV), total lung capacity (TLC) and transfer factor measurement (TLCO).

STATISTICS

The results of FEV1, FVC, FEV1/FVC, FEF25–75%, FEF25%, FEF50%, FEF75%, RV, TLC and TLCO were expressed as a percentage of predicted value for each individual adjusted for age, sex and height [14]. A two-way analysis of variance was used to assess the effect of HLA-DR4, KCS and Pi phenotype on airflow obstruction. The association of HLA-DR4, KCS and Pi phenotype with respiratory symptoms was analysed by logistic regression. A value of $P < 0.05$ was considered to be statistically significant. All analyses were performed using the Minitab statistical package (Minitab Software, Cleocom, Birmingham).

RESULTS

The demographic details and the results of the respiratory symptoms questionnaire are given in Table I. The history of pneumonia given by the patient was not reconfirmed by checking on hospital admission or a previous chest radiograph. Sixty-two patients (62%) were HLA-DR4 positive, 87 had Pi MM (87%) and 13 (13%) had MS phenotypes, while 37 (37%) had KCS on Schirmer's tear testing.

TABLE I
Clinical features of patients with RA

No. of patients	100
Age	58 ± 10 yr (range 22–76)
Sex (female:male)	72:28
Duration of RA	10.5 ± 9 yr (range 1–40)
Wheeze	18
Pleurisy	12
Pneumonia	9
TB	2
Atopy (skin test)	10
Haemoglobin	124 ± 25 g/l [95% CI 119, 129]
ESR	33 ± 25 [95% CI 22, 38]
Erosive arthropathy	57
Abnormal chest radiograph	3
Smoking status	
Non-smokers	36
Current smokers (pack years)	24 [23.5 ± 12, 95% CI 18, 28]
Ex-smokers (pack years)	40 [15 ± 10, 95% CI 12, 19]

Results expressed as mean ± S.D., 95% confidence intervals.

HLA-DR4

There was no significant association of HLA-DR4 status with a history of wheeze, pleurisy or pneumonia (Table II). However, a significantly decreased number of patients in the HLA-DR4 group had atopy on skin-pick testing [3/62 *vs* 7/38, $P = 0.02$, RR = 2.3 (95% CI 1.1, 4.8)]. HLA-DR4 status had no significant effect on spirometry, lung volumes or transfer factor (Table III). There was no significant difference between patients with and those without HLA-DR4 in PD_{20} FEV1 (38/62 *vs* 17/38, $\chi^2 = 2.6$, d.f. 1, $P > 0.05$).

Pi phenotype

There was no significant difference between Pi MM and MS in the history of wheeze, pleurisy, pneumonia and atopy on skin-prick testing (Table II). Patients with Pi MS compared to Pi MM had a significantly higher FEV1 (125 *vs* 99, 95% CI difference −31, −1, $P < 0.05$ and FVC (126 *vs* 109, 95% CI difference −26, 0.0 $P < 0.005$). Table III compares the pulmonary function between Pi MM and MS. There was a significant difference between the Pi MM and MS in the number of patients achieving PD_{20} FEV1 (52/87 *vs* 3/13, $\chi^2 = 6.1$, d.f. 1, $P < 0.025$).

TABLE II
The association of HLA-DR4, KCS and Pi phenotypes with respiratory symptoms

	HLA-DR4		KCS		Pi MS	
	Significance	RR (95% CI)	Significance	RR (95% CI)	Significance	RR (95% CI)
Atopy	0.02	2.3 (1.1, 4.8)	NS	0.8 (0.37, 1.7)	NS	0.7 (0.3, 1.6)
Pleurisy	NS	1.4 (0.7, 2.6)	NS	1.2 (0.66, 2.3)	NS	1.2 (0.4, 3.6)
Pneumonia	NS	1.9 (0.9, 4.2)	NS	1.2 (0.59, 2.6)	NS	0.9 (0.2, 2.7)
Wheeze	NS	1.1 (0.6, 1.9)	0.03	1.8 (1.04, 3.1)	NS	1.7 (0.6, 5.2)

Results are expressed as relative risk (RR) with 95% confidence interval. NS, not significant.

TABLE III
Comparing pulmonary function in the subgroups of HLA-DR4, KCS and Pi phenotype

	HLA-DR4		KCS		Pi phenotype	
	Present (62)	Absent (38)	Present (37)	Absent (63)	MM (87)	MS (13)
	$F_{1,96}$	P	$F_{1,96}$	P	$F_{1,96}$	P
FEV1	1.64	NS	1.12	NS	3.95	0.050
FVC	1.23	NS	0.93	NS	4.84	0.030
FEV1/FVC	0.31	NS	2.04	NS	0.18	NS
FEF25–75%	3.39	NS	0.01	NS	2.55	NS
FEF25%	1.40	NS	1.45	NS	2.79	NS
FEF50%	2.63	NS	0.01	NS	1.23	NS
FEF75%	1.94	NS	0.08	NS	1.21	NS
RV	1.89	NS	0.06	NS	0.08	NS
TLC	3.57	NS	0.96	NS	3.43	NS
DLCO	0.03	NS	0.13	NS	0.17	NS

Pulmonary function between the subgroups of HLA-DR4, KCS and Pi MM:MS are compared by two-way analysis of variance. NS, not significant.

KCS

A significantly increased number of patients in the KCS group complained of wheeze [11/37 *vs* 7/63, $P = 0.03$. RR = 1.8 (95% CI 1.04, 3.1)], but there was no association of KCS with a history of pleurisy, pneumonia or atopy on skin-prick testing (Table II). There was no significant effect of KCS on pulmonary function (Table III) and PD_{20} FEV1 (24/37 *vs* 31/63, $\chi = 2.3$ d.f. 1, $P > 0.5$).

DISCUSSION

We did not observe any significant association of HLA-DR4 status on respiratory symptoms, spirometry, lung volumes, transfer factor and bronchial reactivity. There was a significant decrease in atopy on skin-prick testing in HLA-DR4 patients. We do not know the significance of this, although, atopy is no more common in RA patients than in a control population [15]. We did not assess our patients for further HLA subtyping, but one previous study has found an association of interstitial pulmonary dysfunction with HLA-DQwl allotype in RA [16]

Protease inhibitor (Pi) is the predominant serum anti-protease and protects vulnerable tissue from proteolytic enzymes released by inflammatory cells. Its absence of deficiency can lead to increased destruction of tissues such as the lung leading to emphysema. Whether the effect of mild protease inhibitor deficiency is further potentiated by pre-existing inflammation in the joints and lungs in RA is not known and so far studies have shown conflicting results [7, 9, 17, 18]. Although we observed an increased frequency of MS phenotypes (13%) in our RA patients compared to the reported general UK population (9%) ($P > 0.05$) [19], this had no significant association with respiratory symptoms or bronchial reactivity. Patients in the Pi MS group had significantly higher FEV1, FVC compared to the MM group ($P < 0.05$). This could be explained by the fact that the amount of cigarettes smoked (pack years) was significantly higher in the MM group.

Patients with primary and secondary Sjögren's are known to have an increased frequency of respiratory symptoms [3, 4, 20, 21]. We also observed a significantly increased frequency of wheeze in our patients with KCS. Two previous studies have reported significantly increased bronchial reactivity in patients with primary Sjogren's syndrome [22, 23], but we did not observe a significant association of KCS on bronchial reactivity in our RA population.

In conclusion, we did not find any overall significant association of HLA-DR4 status, KCS and Pi phenotypes on airflow obstruction and bronchial reactivity in RA.

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REFERENCES

1. Kelly CA. Rheumatoid arthritis: classical rheumatoid lung disease; other rheumatoid lung problems. In: Kelly CA, ed. *Baillière's clinical rheumatology. Lung disease in rheumatic disorders*, Vol. 7, No. 1. Baillière Tindall, 1993;30.
2. Hassan WU, Holland CD, Keaney NP, Kelly CA. Bronchial reactivity and airflow obstruction in rheumatoid arthritis. *Ann Rheum Dis* 1994;**53**:411–514.
3. Scott TE, Wise RA, Hochberg MC, Wigley FM, HLA-DR4 and pulmonary dysfunction in rheumatoid arthritis. *Am J Med* 1987;**82**:765–71.
4. Radoux V, Menard HA, Begin R, Decrary F, Koopman WJ. Airways disease in rheumatoid arthritis patients. *Arthritis Rheum* 1987;**30**:249–56.
5. Gladman DD, Anhorn KAB. HLA and disease manifestations in rheumatoid arthritis: a Canadian experience. *J Rheumatol* 1986;**13**:274–6.
6. Hutchinson DCS. The epidemiology of alpha-one antitrypsin deficiency. *Lung* (suppl) 1990;535–42.
7. Geddes DM, Webley M, Brewerton DA, Turton CW, Turner-Warwick M, Murphy AH, Milford A. AAT phenotypes in fibrosing alveolitis and rheumatoid arthritis. *Lancet* 1977;**11**:1049–50.
8. Michalski JP, McComb's CC, Scopelitis E, Biundo JJ, Medgser TA. Alpha-one antitrypsin phenotypes, including M subtypes in pulmonary disease associated with rheumatoid arthritis and systemic sclerosis. *Arthritis Rheum* 1986;**29**:586–91.
9. Mountz JD, Turner RA, Collins RL, Gallup KR, Semble EL. Rheumatoid arthritis and small airways function. *Arthritis Rheum* 1984;**27**:728–36.

10. The Medical Research Council Questionnaire on Respiratory Symptoms 1986. Publication group, Medical Research Council, 20 Park Crescent, London W1N 4AL.
11. Arnett FC, Edworthy EM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatorid arthritis. *Arthritis Rheum* 1988;**31**:315–24.
12. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor–recipient matching in cadaveric transplantation. *Tissue Antigen* 1992;**39**: 225–35.
13. Carpes ML, Posey Y, Brown RK. Separated isoelectric focusing, an improved method of protein analysis and purification. *Anal Biochem* 1977;**77**:166–80.
14. Quanjer PH. Standardized lung function testing. *Bull Eur Physiopathol Respir* 1983;**19**(suppl):39–44.
15. O'Driscoll BRC, Milburn HJ, Kemeny DM, Cochrane GM, Panayi GS. Atopy and rheumatoid arthritis. *Clinical Allergy* 1985;**15**:547–53.
16. Wise RA, Wigley FM, Scott TE, Hochberg MC. HLA-DQw alloantigens and pulmonary dysfunction in rheumatoid arthritis. *Chest* 1988;**94**:609–14.
17. Cow DW, Hubner O. Association of severe rheumatoid arthritis with heterozygosity for alpha-one anti-trypsin deficiency. *Clinical Genetics* 1980;**17**:153–60.
18. Steers G, McMahon MJ, Grennan DM, Hilarby MC. Lack of association of the alpha-one antitrypsin PIZ allele with rheumatoid arthritis or with its extra articular complications. *Disease Markers* 1992;**10**:151–7.
19. Hutchison DCS. The epidemiology of alpha-one antitrypsin deficiency. *Lung* (suppl) 1990;535–42.
20. Kelly C, Gardiner P, Pal B, Griffiths I. Lung function in primary Sjogren's syndrome: a cross sectional and longitudinal study. *Thorax* 1991;**46**:180–3.
21. Gardiner P, Ward C, Allison A, Ashcroft T, Simpson W, Walters H, Kelly C. Pleuropolmonary abnormalities in primary Sjogren's syndrome. *J Rheumatol* 1993;**20**:831–7.
22. La Corte R, Potena A, Bajocchi L, Fabbri L, Trotta F. Increased bronchial responsiveness in primary Sjogren's syndrome. A sign of tracheobronchial involvement. *Clinical Exp Rheum* 1991;**9**:125–30.
23. Gudbjornsson B, Henenstrom H, Stalenheim G, Hallgren R. Bronchial hyperresponsiveness to methacholine in patients with primary Sjogren's syndrome. *Annals Rheum Dis* 1991;**50**:36–40.