

α_1 Antitrypsin (PI) allotypes in rheumatoid arthritis

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SUMMARY α_1 Antitrypsin (PI) phenotypes were defined in 144 patients with rheumatoid arthritis (RA) and in 223 normal controls. The frequencies of the rare F, S, and Z variants were similar in RA and control groups. No relationships were found between PI allotypes and rheumatoid disease severity or autoantibody titre. The PI M1M2 phenotype was increased in frequency in the RA group, and phenotype frequencies in DR4 positive and negative disease were similar. These results support the suggestion that genes on the 14th chromosome which are linked to PI predispose to RA.

Key words: protease inhibitor, genetic variants, 14th chromosome.

Rheumatoid arthritis is thought to be caused by an interaction between genetic and environmental factors. There is a well established association between RA and HLA-DR4 which is coded for by genes on the sixth chromosome.¹ α_1 Antitrypsin or protease inhibitor (PI) is coded for by genes on the 14th chromosome, which are linked to genes coding for the immunoglobulin heavy chain allotypes (Gm).² There is an association between the G1m(x) allotype and DR4 positive RA,^{3, 4} suggesting that 14th chromosomal genes may interact with genes linked to HLA in the pathogenesis of RA. Previous studies of α_1 antitrypsin phenotypes in RA, however, have given conflicting results. Some have shown an increase in the deficiency allele (PI*Z),⁵⁻⁸ whereas others have shown no such association.⁹⁻¹¹ Most previous studies have not subdivided the functionally normal PI*M allele into subtypes, but one recent study has found an increase in M1 homozygotes in RA.¹² We have re-examined the relationship between α_1 antitrypsin variants and susceptibility to and severity of RA. We have also looked for evidence of an interaction between PI and HLA-DR4.

Patients and methods

Serum samples were obtained from 144 unrelated Caucasoid patients with classical or definite RA¹³ attending the Rheumatology outpatient clinic at Hope Hospital, Salford. One hundred and one

patients were female. The mean age of onset of RA was 47.4 years and 83.1% were seropositive for rheumatoid factor. One hundred and twenty six of the patients had been previously typed for HLA-A, -B, and -DR antigens and 84 for Gm allotypes.⁴

There were 223 Caucasoid controls who were members of hospital staff with no history of RA, or cadaveric kidney donors.

CLINICAL ASSESSMENT

Patients were assessed clinically by one of us (PAS) and case notes studied for previous disease features and laboratory results. Radiographs of hands and feet were available for 121 patients and were graded for erosive changes from 0 (normal) to 40 (most severe) by a modified Larsen score.¹⁴

AUTOANTIBODIES

Sera from patients were tested for rheumatoid factor by the sheep cell agglutination test (SCAT) with RAHA kit (Fujizoki Inc, Tokyo) and for antinuclear factor (ANF) with rat liver substrate and fluorescein conjugated sheep antihuman immunoglobulin. The highest recorded titre was noted for each patient.

PI typing

This was carried out by isoelectric focusing of serum in polyacrylamide gels (LKB Ampholine PAG plates, pH 4-5) according to the manufacturer's instructions. The following PI types were recognised: M, Z, F, and S. We were able to distinguish clearly two subtypes of M, M1 and M2.

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Table 1 PI phenotypes in RA patients and controls

Phenotype	RA patients		Controls		Corrected significance (p _c)
	No	%	No	%	
M1	89	61.8	164	73.5	0.095*
M2	3	2.1	6	2.7	
M1M2	36	25.0	27	12.1	0.01†
M1S	11	7.6	12	5.4	
M2S	1	0.7	3	1.3	
S	2	1.4	1	0.5	
M1Z	1	0.7	8	3.6	
Z	1	0.7	0	0	
M1F	0	0	2	0.9	
Total	144	100	223	100	

* $\chi^2=5.629$, p=0.019, p_c=0.095.† $\chi^2=10.224$, p=0.002, p_c=0.01.

Table 2 PI allotypes in RA patients and controls

Allotype	RA patients		Controls		Corrected significance (p _c)
	No	%	No	%	
M1	137	95.1	213	95.5	
M2	40	27.8	36	16.1	0.04*
S	14	9.7	16	7.2	
Z	2	1.4	8	3.6	
F	0	0	2	0.9	

* $\chi^2=7.213$, p=0.008, p_c=0.04.

STATISTICAL ANALYSIS

The significance of differences in phenotype and allotype frequencies was analysed by a χ^2 test. p Values were corrected for the five allotypes as indicated in the tables (p_c).

Results

The frequencies of PI phenotypes and allotypes in RA and control groups are summarised in Tables 1

and 2. In the RA group the M1M2 phenotype and M2 allotype were increased in frequency and there was a trend for a decrease in the M1 phenotype frequency. There were only two RA patients with the Z allotype.

Clinical, radiological, and laboratory features in RA patients typing for different PI allotypes are summarised in Tables 3, 4, and 5. There were no significant relationships between allotypes and age of onset, sex distribution, and percentage of patients with subcutaneous nodules. Six patients were included who developed proteinuria (≥ 0.5 g/24 h) after gold or D-penicillamine therapy: four were phenotype M1 and two M1S. There were no apparent relationships between x ray scores of joint destruction and PI allotypes. Four RA patients with radiological features of basal pulmonary fibrosis were included and all were PI phenotype M1M2. There were no apparent relationships between rheumatoid factor or ANF titres and PI allotypes. In both RA and control groups PI allotypes were independent of DR and Gm typing. (By contrast the frequency of the G1m(x) allotype was increased in DR4 positive but not DR4 negative RA.⁴)

Discussion

Our results show no increase in frequencies of rare variants of α_1 Antitrypsin (F, S, and Z) in a random group of RA patients seen as hospital outpatients. The S and Z variants are associated with decreased synthesis of α_1 Antitrypsin (60% and 15% of normal respectively), and previous reports of their frequencies in RA have been conflicting.⁵⁻¹² Geddes and coworkers have suggested that the Z variant of α_1 antitrypsin is associated with the pulmonary complications of the disease rather than with RA itself.⁷ Thus differing reported frequencies of rare PI variants in RA may reflect differences in selection of patients with this disease complication. It has also been suggested that the presence of the functionally

Table 3 Clinical features in RA patients typing for different PI allotypes

Allotype	Mean age of onset (years)	Disease onset < 30 years		Male		Nodules		Proteinuria	
		No	%	No	%	No	%	No	%
Overall (n=144)	47.4	15	10.4	43	29.9	39	27.1	6	4.2
M1+ (n=137)	47.2	14	10.2	41	29.9	34	24.8	6	4.4
M2+ (n=40)	47.5	4	10.0	12	30.0	12	30.0	0	0
S+ (n=14)	44.4	1	7.1	4	28.6	4	28.6	2	14.3
Z+ (n=2)	51.5	0	0	1	50	1	50	0	0

Table 4 Radiological features in RA patients typing for different PI allotypes

Allotype	Pulmonary fibrosis		Erosive x ray score		
	No tested	% positive	No tested	% with score <15/40	% with score >25/40
Overall	144	2.8	121	30.6	22.3
M1+	137	2.9	116	30.2	21.6
M2+	40	10.0	32	34.4	28.1
S+	14	0	11	9.1	36.4
Z+	2	0	2	50.0	50.0

Table 5 Laboratory results in RA patients typing for different PI allotypes

Allotype	Rheumatoid factor (SCAT)			Antinuclear factor (ANF)			HLA-DR4		G1m(x)	
	No tested	% with titre $\geq 1/32$	% with titre $\geq 1/1024$	No tested	% with titre $\geq 1/40$	% with titre $\geq 1/1000$	No tested	% positive	No tested	% positive
Overall	142	83.1	29.6	141	48.9	22.0	126	55.6	84	20.2
M1+	135	83.0	28.1	134	49.3	20.9	119	54.6	79	19.0
M2+	39	84.6	23.1	39	51.3	23.1	33	54.5	23	21.7
S+	14	85.7	35.7	14	42.9	21.4	10	50.0	7	14.3
Z+	2	100.0	50.0	2	0	0	2	50.0	1	0

abnormal α_1 antitrypsin allotype Z might influence disease severity,⁵ but we have insufficient numbers of RA patients with this variant to comment on this suggestion.

Our figure for PI*M allotype frequency in normal individuals is similar to that reported in a previous study of an English Caucasian population,¹⁵ and both patient and control group frequencies are in keeping with expected Hardy-Weinberg figures. The Rouen nomenclature meeting in 1978 approved M1, M2, and M3 subtypes of the PI*M variant.¹⁶ With this nomenclature a recent study reported an increase in the PI*M gene frequency in an Australian RA population.¹² In the present study with a standard technique isoelectric focusing in polyacrylamide gel we can clearly distinguish two 'M' subtypes only.¹⁷ Our results of PI M subtype frequencies are not therefore directly comparable with those of workers who have distinguished three M subtypes. We have, however, clearly shown increases in PI 'M1M2' heterozygote and M2 allotype frequencies in patients with RA as compared with those of a local control population. It is likely that this increase represents the effect of a linked gene rather than a direct action of α_1 antitrypsin itself as previous studies have suggested that the M subtypes are functionally equivalent, at least as far as their ability to inhibit elastase and trypsin *in vitro* is concerned.¹⁸

The gene for α_1 antitrypsin is linked to Gm on chromosome 14,² and the present findings are in keeping with previous reports of an association between a Gm allotype and RA^{3,4} and suggest that genes on chromosome 14 linked to both PI and Gm loci increase susceptibility to RA. Unlike the Gm association with RA where the association is with DR4 positive disease only, that between PI and RA appears to be independent of DR status. The reason for this discrepancy is unclear, but further studies of this part of chromosome 14 with more refined techniques to define genetic variants at PI and immunoglobulin heavy chain loci are indicated.

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