α_1 Antitrypsin (PI) phenotypes in two rheumatic diseases: a reappraisal of the association of PI subtypes in rheumatoid arthritis

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SUMMARY α_1 Antitrypsin phenotypes were determined by isoelectric focusing in 225 adult white patients with rheumatoid arthritis (RA), 60 patients with severe rheumatoid arthritis (grade III and IV), 17 sibling pairs—HLA identical but discordant for rheumatoid arthritis, and 122 random patients with Sjögren's syndrome. No significant increase in non-M phenotypes was found in either of the groups of patients with RA, but the association between M subvariants was striking. There was a significant increase in M1M2 phenotype and M2 allotype in both the RA and severe RA groups. This increase in M1M2 was also supported by DR4 positive patients with RA compared with DR4 positive siblings without RA. No apparent association of variant phenotype was found in four subgroups of patients with Sjögren's syndrome.

Key words: genetic association, Sjögren's syndrome.

The functional activity of human α_1 antitrypsin (PI) includes a wide spectrum of protease inhibition in biological fluids. Initially, several genetic variants of this protein were identified by starch gel electrophoresis,¹ but recently the technique of isoelectric focusing has shown further heterogeneity and several subvariants of the common or wild type of phenotype M.² The subvariants of M (M1, M2, and M3) are polymorphic, whereas the other M subvariants (M4 and M5) are probably rare. To date more than 30 variants of the PI system have been established. Most of these are rare, but the alleles PI*S and PI*Z show polymorphic frequencies (>1%) in several human populations. The functional assessment of the PI variants suggests that PI*Null, PI*S, PI*Z, and PI*F alleles are associated with decreased serum concentrations of α_1 antitrypsin. As the proteolytic enzymes seem to be involved in the breakdown of the cartilage in various types of chronic arthritis the low concentrations of α_1 antitrypsin, due to inheritance of low activity alleles, may predispose individuals to rheumatoid arthritis (RA) of a more severe and destructive type.

The available evidence of the association of PI*Z and PI*S alleles with rheumatoid arthritis and their role in the pathogenesis of RA is mutually contradicting. While several investigations have shown a significant increase in MZ and MS phenotypes in patients with RA,^{3–6} a large number of studies do not confirm these findings.^{7–15} A number of previous studies have not analysed the M subvariants. Recent reports of M subvariants in RA have shown an increase of M1 homozygous in Australian patients,¹⁴ whereas a significant increase of M1M2 phenotype has been reported in patients with RA in north west England.¹⁵

The present investigation reports the association of PI subvariants in patients with RA from north east England and provides an up to date reappraisal of the PI status in RA. As there is only one report on the association of PI subtypes in Sjögren's syndrome¹⁶ the present study also includes data on the association of PI phenotypes in various subgroups of Sjögren's syndrome (SS).

Patients and methods

Four groups of patients were included in the study. Group 1: a random collection of 225 adult patients

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fulfilling the criteria of the American Rheumatism Association for the classification of rheumatoid arthritis. All patients were white and from north east England. The details of age range and other characteristics of this patient group have been given previously.¹⁷

Group 2: a separate sample of 60 random patients with severe RA (grade III and IV). The criteria of severity were as given by Steinbrocker *et al.*¹⁸

Group 3: 17 random pairs of siblings, identical for their histocompatibility antigens but discordant for the disease.

Group 4: a total of 145 unrelated patients with SS. The patients were divided into four subgroups: 56 patients with primary SS (P-SS); 46 patients with secondary SS with RA (SS-RA), 15 patients with secondary SS with another recognisable connective tissue disorder other than RA (Sec-SS), and 28 patients with non-systemic sicca syndrome (NS-SS).

The details of clinical features of each subgroup and criteria of classification have already been reported.¹⁹

A random control sample of 316 individuals was derived from blood donors, individuals requested from the general electoral register, and couples referred to the Department of Human Genetics for paternity testing.

PI SUBTYPES

The PI subtyping was performed by thin layer polyacrylamide gel isoelectric focusing using LKB Multiphor apparatus at 10°C. The gel (0.5 mm thick) contained 4.8% acrylamide, 0.15% *N,N'*-methylene-bisacrylamide, 12% glycerol, 6% ampholine pH 4-6 (LKB), and 1.5% of 1% ammonium persulphate. To improve the corridor between the M1 and M2 bands and to identify the intermediate M3 bands various spacer compounds listed in the Sigma catalogue were tried. 3-(*N*-Morpholino)propane sulphonic acid (Sigma) at a concentration of 1.54% w/v in the gel differentiated ideally between M1, M2, and M3 bands (Fig. 1). After polymerisation the gels were prefocused for 20 minutes. Serum samples cleared with 0.1 M dithiothreitol and 0.25 M iodoacetamide were applied to the gels. The isoelectric focusing was carried out at 2000 V for 195 minutes. For staining a 1% solution of Coomassie blue 10B was used.

Results

Tables 1 and 2 compare the number and frequency of PI phenotypes and allotypes in groups 1 (patients with RA) and 2 (patients with severe RA) with those in the control group. In group 1, patients with RA, the heterozygote phenotype M1M2 was signifi-

Fig. 1 Isoelectric focusing of PI phenotypes: 1. M1M2; 2. M1M3; 3. M1M1; 4. M1M1.

cantly increased in frequency, and this increase was at the expense of a significant decrease in the M1M3 heterozygotes. The increase in M2 and the decrease in M3 allotype were significant even after correction for the total number of phenotypes encountered ($p=1.2 \times 10^{-6}$ and 6.4×10^{-10} respectively). In group 2, patients with severe RA, the increase in M1M2 phenotype was significant only before correction ($38.2\% \text{ v } 20.3\%$), but the M2 allotype increase in these patients was highly significant ($p=3.8 \times 10^{-4}$). The trend for a decrease of the M1M3 phenotype was evident here also ($8.6\% \text{ v } 12.3\%$). A decrease in frequency of the M1M1 phenotype was encountered in patients with severe RA, however, which was significant before correction ($36.2\% \text{ v } 49.1\%$; $p=0.048$). There was no significant increase in MZ phenotype or Z allotype in either group 1 or group 2, although in both these patient groups there was a tendency for low S allotypes compared with the control group.

Table 3 gives the distribution of PI phenotypes in

Table 1 *Distribution of PI phenotypes/alleles in patients with RA and controls*

Phenotype/allele	Patients with RA (n=225)		Controls (n=316)		Fisher's p value
	No	%	No	%	
M1M1	98	43.6	155	49.1	0.119
M1M2*	86	38.2	64	20.3	3.5×10^{-6}
M1M3*	4	1.8	39	12.3	1.5×10^{-6}
M2M2	17	7.6	8	2.5	0.006
M3M3	—	—	7	2.2	0.023
M2M3	—	—	4	1.3	0.115
M1S	11	4.9	23	7.3	0.171
M2S	1	0.4	6	1.9	0.137
M3S	—	—	4	1.3	0.115
M1Z	5	2.2	5	1.6	0.406
M2Z	—	—	1	0.3	—
M1F	3	1.3	—	—	0.071
M1	207	92.0	286	90.5	0.328
M2*	104	46.2	83	26.3	1.2×10^{-6}
M3*	4	1.8	54	17.1	6.4×10^{-10}
S	12	5.3	33	10.4	0.023
Z	5	2.2	6	1.9	0.511
F	3	1.3	—	—	0.071
All rare alleles	20	8.9	40	12.7	0.107

*Significant after correction for the total number of phenotypes/alleles encountered.

Table 2 *Distribution of PI phenotypes/alleles in patients with severe RA and controls*

Phenotype/allele	Patients with severe RA (n=58)		Controls (n=316)		Fisher's p value
	No	%	No	%	
M1M1	21	36.2	155	49.1	0.048
M1M2	19	32.8	64	20.3	0.029
M1M3	5	8.6	39	12.3	0.288
M2M2	3	5.2	8	2.5	0.235
M3M3	—	—	7	2.2	0.304
M2M3	5	8.6	4	1.3	—
M1S	2	3.4	23	7.3	0.223
M2S	2	3.4	6	1.9	0.359
M3S	—	—	4	1.3	0.508
M1Z	1	1.7	5	1.6	0.638
M2Z	—	—	1	0.3	—
M1	48	82.8	286	90.5	0.069
M2*	29	50.0	83	26.3	3.8×10^{-4}
M3	10	17.2	54	17.1	0.549
S	4	6.9	33	10.4	0.287
Z	1	1.7	6	1.9	0.703
All rare alleles	5	8.6	40	12.7	0.266

*Significant after the correction for the total number of alleles encountered.

Table 3 *Distribution of PI phenotypes in patients with RA and their HLA identical siblings*

Phenotype	Siblings with RA (n=18)		Siblings without RA (n=16)		Fisher's p value
	No	%	No	%	
M1M1	9	50.0	8	50.0	0.553
M1M2	6	33.2	1	6.3	0.052
M1M3	1	5.6	2	12.5	NS
M1S	1	5.6	0	—	NS
M3S	0	—	1	6.3	NS
M1Var	0	—	2	12.5	NS
M2Var	0	—	1	6.3	NS
M1	0	—	1	6.3	NS
M1Z	1	5.6	0	—	NS

Table 4 Distribution of PI phenotypes in patients with Sjögren's syndrome and controls. Values are the percentage observed frequency

Phenotype	I P-SS* (n=56)	II SS-RA* (n=46)	III Sec-SS* (n=15)	IV NS-SS* (n=28)	Control (n=316)
M1M1	60.7	47.8	73.3	46.4	49.1
M1M2	21.4	19.6	13.3	32.1	20.2
M1M3	8.9	6.5	—	14.3	12.3
M2M2	—	—	—	—	2.5
M3M3	—	—	—	—	2.2
M2M3	—	2.2	6.7	—	1.3
M1S	3.6	15.2	6.7	3.6	7.3
M2S	3.6	—	—	3.6	1.9
M3S	—	2.2	—	—	1.3
M1Z	—	2.2	—	—	1.6
M2Z	—	—	—	—	0.3
M3Z	—	2.2	—	—	—
M1F	1.8	2.2	—	—	—

*P-SS=primary Sjögren's syndrome (SS); SS-RA=secondary SS with rheumatoid arthritis (RA); Sec-SS=secondary SS with a connective tissue disorder other than RA; NS-SS=non-systemic sicca syndrome.

17 sib pairs, HLA identical but discordant for RA. Sixteen of the sib pairs were DR4 positive. In one pair both individuals were affected, but only one had a phenotype M1M2. A comparison of RA positive and RA negative groups showed an increase of M1M2 phenotype in the RA affected group (33.2% v 6.3%). This variation fell just short of significance ($p=0.052$). When only the 16 DR4 positive sibs with RA were compared with the DR4 positive sibs without RA there was a further slight drop in the level of significance ($p=0.062$). No other phenotype showed any significant variation.

Table 4 gives the frequency of PI phenotypes observed in four subgroups of patients with SS and controls. Compared with the control group there was no significant variation in any of the groups of patients with SS, though a slight increase in M1M1 homozygous was evident in P-SS and Sec-SS groups, and of M1S phenotype in the SS-RA group. This lack of association between PI and SS is in agreement with a previous report.¹⁶

Discussion

Several studies to date have investigated a possible association between PI phenotypes and RA. Of these, only five have shown either a marginal or a significant association with Z heterozygote phenotypes (MZ and SZ) and of these, two studies have also shown an increase in the MS phenotype. Geddes and his colleagues found a significant increase of MS and MZ phenotypes in patients with RA complicated by fibrosing alveolitis but not in patients with uncomplicated RA.¹⁰ In the same study patients without RA but with fibrosing alveolitis also showed a significant increase in MZ

phenotype. A more recent report from Pittsburgh, however, failed to confirm this association in their patients with RA and interstitial pulmonary fibrosis.¹² The balance of the available studies is, therefore, in agreement with our present results, i.e., in failing to show an increase in the frequency of the non-M alleles of the PI system in patients with RA and severe RA. We therefore support the view that any increase in the frequency of the Z allele may be related to associated pulmonary manifestations such as fibrosing alveolitis, and that the Z allotype has little or no contribution to the frequency or severity of RA.

On the other hand, the results of the recent studies of PI subvariant in patients with RA have shown an interesting association with M subtypes. Our results show an increase in the M1M2 phenotype in both RA and severe RA groups, and a protective role of the M3 allotype. Our sib pair analysis also reinforces this observation of an increase in the M1M2 phenotype and suggests that this increase is independent of an association with the HLA-DR4 phenotype. Our results are in general agreement with those of Sander and coworkers, who also showed a significant increase in the M1M2 phenotype.¹⁵ Unfortunately, Sander did not differentiate between M2 and M3 phenotypes. This increase in M1M2 phenotypes is not supported by the Pittsburgh survey, though the authors suggest that patients with RA with this phenotype may have an increased risk of developing pulmonary fibrosis.¹² Recent data from Finland, however, do not agree with this latter suggestion.¹³ The above two investigations and our present results do not observe any significant increase in M1 homozygous, as demonstrated by an Australian group.¹⁴

At present it is very difficult to present any convincing explanation of the association of M2 subvariants because the in vitro functional assessment of M subvariants does not show any significant variation in their ability to inhibit elastase or trypsin. As the PI protein has a wide spectrum of inhibition activity a further functional investigation of M subvariants may be warranted. To account for the variation of association in different continental populations in future it will be necessary to consider the population structure of both the control and patient groups. Samples studied from a relatively inbred population may show a homozygous excess as seen in the Australian survey.¹⁴

A genetic predisposition to disease due to an additive effect of alleles present on different parts of human genome has been suggested. For RA, in addition to the association with HLA (DR4), an association with Gm allotypes has been reported.^{20 21} The Gm and PI alleles are linked and situated on chromosome 14. To determine whether the PI alleles are directly involved in the development of RA or are in linkage disequilibrium with another susceptibility locus on chromosome 14 would require a further investigation of chromosome 14 markers in patients with RA with and without pulmonary complications. To resolve the association between RA and M subtypes further analysis with a more refined isoelectric focusing technique using immobilines is necessary.

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