Chromosome 14 markers in rheumatoid arthritis

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SUMMARY Phenotype frequencies for variants of the chromosome 14 markers, α_1 antitrypsin (protease inhibitor—Pi), and immunoglobulin heavy chain gene allotypes (Gm and Am) were examined in affected and unaffected members of multicase rheumatoid arthritis (RA) families and compared with published population data. Significantly higher frequencies of phenotypes containing Pi*Z and Pi*S were observed in unrelated index RA cases compared with UK population data. There was also a higher frequency of Pi*Z in family members without RA than in population controls but no such difference for the frequency of Pi*S. No difference in the frequency of PiM1M2 heterozygotes was seen between patients with RA and population controls. An examination of clinical data failed to show any relation between any particular feature of RA and positivity for Pi*Z or Pi*S. No significant differences in frequency of Gm phenotypes were observed between patients with RA and controls. Significant association was found, however, between Pi*Z and Gm phenotypes containing Gm(zax;g). These associations are interpreted as indicating linkage disequilibria between these alleles. No interactions between DR4 and either G1m(z), (a), or (x) allotypes were apparent in patients with RA. A significant association was seen in the index RA cases between DR4 and Pi phenotypes carrying Z or S alleles. Observations from this study provide evidence for the existence of a genetic component for RA susceptibility encoded on chromosome 14. An interactive effect of these genes with DR4 towards susceptibility appears likely.

Key words: Pi, immunoglobulin allotypes, Gm, HLA.

A genetic basis for RA susceptibility is suggested by the known familial tendency to develop this disease and by a higher concordance rate for RA in monozygotic twins than in dizygotic twins.[1] The relatively low figure of 32% concordance for RA development in monozygotic twins gives a clear indication of the role of non-genetic 'environmental' factors contributing to the disease development.

A major component of the genetic susceptibility to RA has been shown to be encoded within the HLA region,[2] with DR4 being associated with RA in many populations.[3] Family studies have now demonstrated linkage of RA susceptibility with HLA.[4]

Additional associations have been described for RA and α_1 antitrypsin (Pi) and IgG allotypes (Gm). It is now known that the genes for Pi and Gm are linked[5] and are located on chromosome 14.[6] The alleles PiZ,[7–10] PiS,[11] and subtypes of PiM[12][13] have been found to be at raised frequency in patients with RA. An association has also been observed with the G1m(x) allotype in DR4 positive patients, suggesting that some form of epistatic interaction between Gm and HLA exists.[14–16]

Associations between RA and chromosome 14 encoded products still have to be well characterised, however, and some studies have failed to show a clear association between RA and either Pi[17–20] or Gm allotypes.[21]

This study therefore had two aims. Firstly, to examine the associations between Pi and Gm with RA and, secondly, to determine the influence of these markers in explaining disease susceptibility in families with RA.

Patients and methods

SUBJECTS

The subjects for this study were drawn from multi-case families with at least two living first degree relatives satisfying American Rheumatism Association (ARA) criteria for classical or definite RA.[22] An explanation of their collection and clinical examination has been reported elsewhere.[4] For this analysis family members were classified into the following groups: (a) index cases, i.e., the first reported RA case for each family (used in testing for associations); (b) affected cases; i.e., family members with classical or definite RA: (c) suspicious cases, i.e., family members in whom there was at least one clinical or laboratory feature suggestive of inflammatory arthritis but who did not satisfy the ARA criteria for definite RA; (d) unaffected cases, i.e., family members who were not affected or suspicious cases.

CONTROL FIGURES TAKEN FROM PUBLISHED DATA

Frequencies for Pi and Gm phenotypes in normal Caucasoid controls have been reported elsewhere.[13 15 23 24]

LABORATORY TECHNIQUES

Pi phenotypes were ascertained for serum samples by isoelectric focusing on commercially available polyacrylamide gel plates (pH range 4·0–5·0; LKB).[25] On completion, gels were stained with Coomassie blue and destained before reading. Pi types were read blind by two independent observers, and samples giving unclear results were rerun.

Immunoglobulin allotypes (Gm, Am, and Km) were identified by haemagglutination inhibition assays of serum samples[26] and included G1m (z,a,x,f), G2m(n), G3m(g,b0,b1,b3,b5,s,t,c3,c5), A2m(1,2), and the $\varkappa$ light chain allotypes Km(1,3). G3m(b0,b1,b3, and b5) usually occur together and are summarised as G2m(b).

HLA typing was performed using procedures described elsewhere.[27 28]

STATISTICAL ANALYSIS

χ^2 Analysis was used to compare phenotype frequencies seen in the unrelated index cases and normal controls. Levels of significance were not corrected for the number of comparisons made.

Similarly, the phenotype frequencies were compared for the index cases and other affected and non-affected individuals within the families. A formal cosegregation analysis was then undertaken

Table 2   Frequency of PiM subtypes in multiple RA case families

PiM subtype	Index cases (n=60)	UK controls* (n=223)
M1M1	55·0 (33)†	73·5 (164)
M1M2	11·7 (7)	12·1 (27)
M1S	18·3 (11)	5·4 (12)
M1Z	8·3 (5)	3·6 (8)
M2M2	5·0 (3)	2·7 (6)
M2S	0·0	1·3 (3)
M2Z	1·7 (1)	0·0

*See ref 13.
†Values are percentage (No).

Table 1   Frequency of Pi phenotypes in multiple RA case families

Phenotype	Index cases alone (n=63)	UK controls* (n=4565)	All affected cases† (n=118)	Suspicious cases‡ (n=89)	Unaffected cases (n=278)
MM	71·4 (45)§	88·5 (4042)	72·0 (85)	76·4 (68)	84·2 (234)
MS	15·9 (10)‖	8·0 (366)	14·4 (17)	13·5 (12)	5·4 (15)
MZ	9·5 (6)¶	1·5 (71)	10·2 (12)	9·0 (8)	9·7 (27)
MF	0·0	0·6 (30)	0·8 (1)	0·0	0·0
M?	1·6 (1)	—	1·7 (2)	0·0	0·4 (1)
SS	0·0	0·4 (16)	0·0	1·1 (1)	0·0
SZ	1·6 (1)	0·2 (10)	0·8 (1)	0·0	0·0
ZZ	0·0	0·04 (2)	0·0	0·0	0·4 (1)

*See text.
†Including index cases.
‡See ref 22.
§Values are percentage (No).
‖Sample v controls 0·05>p>0·01.
¶Sample v controls p<0·001.

of siblings with at least one affected and one non-affected offspring of parents heterozygous for either the S or Z allele. The results were displayed as 2×2 tables.

Results

Table 1 summarises the frequencies of Pi phenotypes in multiple RA case families. When compared with a large panel of UK controls significantly higher frequencies of MZ and MS phenotypes were observed in the unrelated index cases. These increases were also seen in both the overall panel of affected cases and in the suspicious cases. The group of 'suspicious' individuals was differentiated to eliminate any possible patients with RA from the panel of unaffected family members. The frequency of the MS phenotype in the unaffected members (5·4%) was similar to that in the controls (8·0%). In contrast, the frequency of MZ phenotypes remained raised (9·7% *v* 1·5%).

The M1M2 subtypes were available for 60 of the index cases (Table 2). No significant difference in the frequency of M1M2 heterozygotes could be seen between patients with RA and published controls. The reduction in M1 homozygotes in our patients with RA appears to be consistent with the increased frequency of S and Z bearing phenotypes. The Pi status was then compared in the unaffected offspring and those with RA of MS and MZ heterozygous parents in families with at least one offspring of each type. The results showed a slight but non-significant distortion towards affected offspring, who were more likely to inherit the S and Z allele respectively (Tables 3 and 4).

Table 5 summarises the frequencies of Gm phenotypes observed. When compared with published controls no significant difference for any

Table 3 *Offspring of MS heterozygous parents*

Allele	Affected (No)	Unaffected (No)
S	15	12
M	12	20

$\chi^2 = 1.92$, p=NS.

Table 4 *Offspring of MZ heterozygous parents*

Allele	Affected (No)	Unaffected (No)
Z	8	6
M	3	6

$\chi^2 = 1.24$, p=NS.

Table 5 *Gm phenotypes in multiple RA case families*

G1m;G2m;G3m	Index cases (n=62)	Controls* (n=792)	Affected cases† (n=120)	Suspicious cases‡ (n=89)	Unaffected cases (n=271)
f;n;b	40·3 (25)§	43·4 (344)	41·8 (49)	32·6 (29)	37·3 (101)
f;n;bc³c⁵	1·6 (1)	0·5 (4)	0·8 (1)	0·0	0·4 (1)
f;··;b	1·6 (1)	6·3 (59)	2·5 (3)	11·2 (10)	5·5 (15)
zax;··;g	4·8 (3)	4·3 (34)	5·0 (6)	7·8 (7)	6·3 (17)
zaxf;n;g	0·0	0·0	0·0	1·1 (1)	0·4 (1)
zaxf;n;gb	6·5 (4)	9·1 (72)	7·5 (9)	7·8 (7)	5·2 (14)
zaxf;··;gb	4·8 (3)	5·7 (45)	7·5 (9)	4·4 (4)	6·6 (18)
zax;··;gb	3·2 (2)	0·1 (1)	1·7 (2)	0·0	0·0
za;··;g	3·2 (2)	4·5 (36)	5·0 (6)	1·1 (1)	4·1 (11)
zaf;··;gb	14·5 (9)	8·3 (66)	11·7 (14)	10·1 (9)	10·0 (27)
zaf;n;gb	16·1 (10)	15·9 (126)	14·2 (17)	23·6 (21)	21·0 (57)
zaf;··;b	0·0	0·0	0·0	1·1 (1)	0·4 (1)
zaf;··;bst	0·0	‖	0·0	0·0	0·7 (2)
za;··;gb	0·0	0·1 (1)	0·0	1·1 (1)	0·7 (2)
za;··;gb⁰b³b⁵st	0·0	‖	0·8 (1)	0·0	0·4 (1)
za;n;g	1·6 (1)	0·0	0·8 (1)	0·0	0·4 (1)
za;n;gb	0·0	0·0	0·8 (1)	0·0	0·0
zaf;n;bst	1·6 (1)	0·0	0·8 (1)	0·0	0·0
zaf;n;b	0·0	0·5 (4)	0·0	0·0	0·7 (2)

*See ref 29.
†Including index cases.
‡See text.
§Values are percentages (Nos).
‖Rare phenotypes discussed in ref 29.

particular Gm phenotype in patients with RA was seen. The Gm frequencies in all patients with RA and published controls were remarkably similar.

The immunoglobulin $\varkappa$ light chain allotype Km(1) was present in index cases and affected family members at frequencies of 11·4% and 11·3% respectively. A frequency of 17·9% for Km(1) has been reported for unaffected controls.[24] No significant differences were seen between patients with RA and controls for the frequencies of the IgA allotypic markers Am(1) or Am(2).

An interesting observation is that, of the 46 PiZ individuals, 39 had Gm phenotypes containing the G1m(z) and (a) allotypes ($\chi^2=16\cdot6$, p<0·001) and 19 the Gm(zax;g) haplotype ($\chi^2=15\cdot0$, p<0·001). Such associations may reflect linkage disequilibria between these alleles.

An analysis of the relation between DR4 status and Gm allotypes in patients with RA failed to show any increase in the proportion of G1m(z) or G1m(x) allotypes in DR4 positive patients compared with those who were DR4 negative (Table 6). Such an analysis for Pi markers, however, showed a higher proportion of PiZ and PiS containing phenotypes in DR4 positive patients than in those who were DR4 negative (Table 7).

Table 6   *Relations between HLA-DR4 status and Gm markers in RA*

Gm marker	Index cases (n=60)	
	DR4 positive (No)	DR4 negative (No)
G1m(z) positive	21	13
G1m(z) negative	19	7
G1m(x) positive	7	5
G1m(x) negative	33	15

No significant associations.

Table 7   *Relations between HLA-DR4 status and Pi markers in RA*

Pi marker	Index cases (n=61)	
	DR4 positive (No)	DR4 negative (No)
PiS or PiZ positive	16	3
PiS or PiZ negative	24	18

$\chi^2=4\cdot2$, p<0·05.

Discussion

In keeping with some other studies we have observed an increase in the frequency of Pi*Z in patients with RA and, additionally, have shown an increase in the frequency of Pi*S. Other workers have, however, shown no such association. Interestingly, the current study found higher frequencies of PiZ (11·1%) and PiS (17·5%) than a similar but larger series recently published, which found 1·4% and 9·7% respectively, neither of which was significantly increased compared with controls.[20] It is difficult to explain the disparity in these results, but one possible factor is that the index cases in the current study were drawn from multicase families and from sporadic cases in the latter. Thus Pi would appear to 'contribute' towards RA susceptibility. It is known that these particular Pi variants, present at low frequency in the normal population, are associated quantitatively with different levels of serum α_1 antitrypsin. When the concentration of α_1 antitrypsin is expressed as a percentage of that found in the PiMM genotype (i.e., 100%) reduced levels are found for MS (88–80%), MZ (67–59%), SS (58%), SZ (37–36%), and ZZ (7–16%).[23] No quantitative differences have been reported for M1 and M2 subtypes. There is variation between individuals with the same Pi type, however, and some overlap of levels may exist, dependent on the individuals examined.

A reduction in the α_1 antitrypsin level should have the effect of increasing the action of proteolytic enzymes. α_1 Antitrypsin is a major inhibitor of elastase, and it has been suggested that any reduction in its level could lead to increased destruction of joint cartilage by leucocyte elastase released by infiltrating cells during inflammatory rheumatoid processes.[8]

Associations between Pi*Z and a variety of pulmonary disorders have been described,[30 31] including patients with RA and fibrosing alveolitis[19] and individuals with emphysema.[32] These observations, together with the suggestions of increased proteolytic enzyme activity, predict an increase of pulmonary problems or disease severity, or both, in family RA cases with the PiZ variant. Unfortunately the sample size in this study may be too small to address this problem satisfactorily.

The increase of the MS phenotype frequency in patients with RA and its subsequent reduction in unaffected individuals underlines its possible contribution to RA susceptibility. We were unable to show, however, that the observed distortion in the cosegregation of either S or Z with RA in these families is significant. The high frequency of Pi*Z in all groups of family members, including unaffected

members, is unexpected. There have been several reports of a positive transmission bias of PiZ alleles from men,[5] [33] and this may go some way towards explaining this observation. Thus PiZ bearing haplotypes could represent a susceptibility factor contributing towards RA development for certain individuals and through this mechanism of 'loading the dice' may be carried in their family members more frequently than expected by chance.

No particular Gm allotype was raised in frequency for any set of individuals in these families with RA. Associations were seen, however, between certain Pi variants and Gm allotypes and haplotypes. The obvious explanation for this finding is that linkage disequilibrium exist between these alleles. Although it has been shown that Pi and Gm are linked,[6] the recombination fraction between them is estimated to be 0·26,[5] and it would be unusual for linkage disequilibria to be maintained over this distance. An analogy has been drawn between the observed transmission bias of PiZ containing haplotypes and the effects of the T locus in the mouse,[5] which can disturb both segregation ratios and recombination distances between genes.[34] Thus it is not impossible to find certain alleles some distance apart which maintain the phenomenon of linkage disequilibrium.

Previous studies have shown an increase of the G1m(x) allotype in DR4 positive RA patients, implying some level of interaction with the HLA system.[14] [15] This relation could not be observed in our data. A similar situation can be seen, however, whereby significantly more PiS and PiZ containing phenotypes are present in DR4 positive patients than in DR4 negative ones. As linkage disequilibrium appears to exist between Gm(zax;g) and PiZ, a reconciliation between these observations may be that certain chromosome 14 Pi-Gm haplotypes can interact with certain chromosome 6 HLA haplotypes to confer greater RA susceptibility on individuals.

Several hypotheses may explain the contribution of chromosome 14 encoded products to RA susceptibility. One is that Pi may have a direct role in RA pathogenesis and that Gm allotypes, such as G1m(x), may be 'innocent bystanders' carried along through linkage disequilibria. Alternatively, it can be argued that Gm allotypes directly affect the generation of rheumatoid factors.

Another explanation could be that an RA susceptibility gene(s) is in linkage disequilibrium with certain Pi and Gm alleles and could be found more commonly in certain haplotypes. This would not necessarily mutually exclude an additional involvement of Pi and Gm in RA. T cell receptor genes are now known to be encoded also on chromosome

14.[29] [35] It would therefore not be unreasonable to consider this region of chromosome 14 as being a contender for encoding a component of RA susceptibility.

Certain chromosome 14 encoded products may be involved in immunological recognitive processes and after the action of particular pathogenic challenges lead to a potential autoimmune situation whereby the HLA system is implicated in the intensity of the ensuing reaction. Future studies using DNA probes to examine chromosome 14 associations with RA will be of interest.

References

1 Lawrence J S. Rheumatoid arthritis: nature or nurture. *Ann Rheum Dis* 1970; **29:** 357–79.
2 Stastny P. Mixed lymphocyte culture typing cells from patients with rheumatoid arthritis. *Tissue Antigens* 1974; **4:** 571–9.
3 Ollier W, Festenstein H. Rheumatoid arthritis and genetic susceptibility. *Perspectives in Rheumatology* (in press).
4 Ollier W, Silman A, Gosnell N, *et al.* HLA and rheumatoid arthritis: an analysis of multicase families. *Disease Markers* 1986; **4:** 85–98.
5 Gedde-Dahl T, Frants R R, Olaisen B, Eriksson A W, van Loghem E, Lamm L. The Gm-Pi linkage heterogeneity in view of PiM subtypes. *Ann Hum Genet* 1981; **45:** 143–53.
6 Cox D W, Markovic V D, Teshima I E. Genes for immunoglobulin heavy chains and for alpha-1-antitrypsin are localised to specific regions of chromosome 14q. *Nature* 1982; **297:** 428–30.
7 Cox D W, Huber O. Rheumatoid arthritis and alpha-1-antitrypsin. *Lancet* 1976; **i:** 1216–7.
8 Cox D W, Huber O. Association of severe rheumatoid arthritis with heterogeneity for alpha-1-antitrypsin deficiency. *Clin Genet* 1980; **17:** 153–60.
9 Beckman G, Beckman L, Bjelle A, Dahlquist S R. Alpha-1-antitrypsin types and rheumatoid arthritis. *Clin Genet* 1984; **25:** 492–9.
10 Buisseret P D, Pembrey M E, Lessof M H. Alpha-1-antitrypsin phenotypes in rheumatoid arthritis and ankylosing spondylitis. *Lancet* 1977; **ii:** 1358–9.
11 Collins R L, Turner R A, Johnson A M, Whitley N O, McLean R L. Obstructive pulmonary disease in rheumatoid arthritis. *Arthritis Rheum* 1976; **19:** 623–8.
12 Mitchell R J, Fabb S A. Serum proteins, alpha-1-antitrypsin (Pi) and Gc (VDBP) and rheumatoid arthritis. *Disease Markers* 1984; **2:** 461–8.
13 Thomson W, Dyer P A, Sanders P A, Grennan D M. C3, Gm and Pi polymorphisms in rheumatoid arthritis. *Disease Markers* 1986; **4:** 145–50.
14 Propert D N, Kay P, McKluskey J, Zilko P J, Mathews J. Immunoglobulin allotypes in rheumatoid arthritis. In: Dawkins R L, Christiansen F T, Zilko P J, eds. *Immunogenetics in rheumatology. Musculoskeletal disease and D-penicillamine.* Amsterdam: Excerpta Medica, 1982: 127–9.
15 Sanders P A, de Lange G G, Dyer P A, Grennan D M. Gm and Km allotypes in rheumatoid arthritis. *Ann Rheum Dis* 1985; **44:** 529–32.
16 Puttick A, Briggs D, Welsh K, Jacoby R, Williamson E, Jones V. Extended haplotypes in rheumatoid arthritis and preliminary

evidence for an interaction with immunoglobulin genes. *Disease Markers* 1986; **4:** 139–44.

17 Brackertz D, Kueppers F. Alpha-1-antitrypsin phenotypes in rheumatoid arthritis. *Lancet* 1977; **ii:** 934–5.

18 Sjoblom K, Clot J, Serre H. Alpha-1-antitrypsin phenotypes in rheumatoid arthritis. *Lancet* 1977; **ii:** 41–2.

19 Geddes D M, Webley M, Brewerton D A, *et al.* Alpha-1-antitrypsin phenotypes in fibrosing alveolitis and rheumatoid arthritis. *Lancet* 1977; **ii:** 1049–51.

20 Sanders P A, Thomson W, Browne D A, Dyer P A, Grennan D M. α₁ Antitrypsin (PI) allotypes in rheumatoid arthritis. *Ann Rheum Dis* 1986; **45:** 450–3.

21 Gran J T, Gaarder P I, Husby G. IgG heavy chain (Gm) allotypes in rheumatoid arthritis and in healthy individuals seropositive for IgM-rheumatoid factor. *Scand J Rheumatol* 1985; **14:** 144–8.

22 Ropes M W, Bennett G A, Cobb S, Jacox R, Jessar R A. Revision of diagnostic criteria in rheumatoid arthritis. *Bull Rheum Dis* 1958; **9:** 175–6.

23 Cook P J L. The genetics of alpha-1-antitrypsin: a family study in England and Scotland. *Ann Hum Genet* 1975; **38:** 275–87.

24 Fraser G R, Volkers W S, Bernini L F, van Loghem E, Meera-Khan P, Nÿenhuis L E. Gene frequencies in a Dutch population. *Hum Hered* 1974; **24:** 435–48.

25 Welsh S G, McGregor I A, Williams K. Alpha-1-antitrypsin (Pi) phenotypes in a village population from The Gambia, West Africa. *Hum Genet* 1980; **53:** 233–5.

26 Schanfield M S, van Loghem E. Human immunoglobulin allotypes. In: Weir D M, ed. *Handbook of experimental immunology.* Vol 3. Oxford. Blackwell, 1986: 94·1–18.

27 Festenstein H, Adams E, Burke J, Oliver R T D, Sachs J A, Wolf E. The distribution of HLA-A antigens in expatriates from East Bengal living in London. In: Dausset J, Colombani J, eds. *Histocompatibility testing 1972.* Copenhagen: Munksgaard, 1972: 175–8.

28 van Rood J J, van Leeuwen A, Ploem J S. Simultaneous detection of two cell populations by two colour fluorescence and application of the recognition of B-cell determinants. *Nature* 1976; **262:** 795–7.

29 Collins M K L, Goodfellow P N, Spurr N K, *et al.* The human T-cell receptor alpha-chain gene maps to chromosome 14. *Nature* 1985; **314:** 273–4.

30 Fagerhol M K, Cox W D. Pi polymorphism: genetic, biochemical and clinical aspects of alpha-1-antitrypsin. In: Harris H, Hirschorn K, eds. *Advance in human genetics.* New York: Plenum Press, 1981: 1–62.

31 Gadek J E, Crystal R G. Alpha-1-antitrypsin deficiency. In: Stanbury J B, ed. *Metabolic basis of inherited disease.* New York: McGraw-Hill, 1983: 1450–68.

32 Eriksson S. Pulmonary emphysema and alpha-1-antitrypsin deficiency. *Acta Med Scand* 1964; **175:** 197–205.

33 Frants R R, Eriksson A W. PiM subtypes of alpha-1-trypsin in isolate studies. In: Eriksson A W, Forsius H R, Nevanlinna H R, Workman P C, Norio R K, eds. *Population structure and genetic disorders.* London: Academic Press, 1980: 199–210.

34 Bennet D. The T locus of the mouse. *Cell* 1975; **6:** 441–54.

35 Croce C, Isobe M, Palumbo A, *et al.* Gene for alpha-chain of human T-cell receptor: location on chromosome 14 region involved in T-cell neoplasms. *Science* 1985; **227:** 1044–7.