GENETIC MARKERS IN RHEUMATOID ARTHRITIS

by

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Abstract

GENETIC MARKERS IN RHEUMATOID ARTHRITIS

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Genetic as well as environmental factors are believed to be of importance in the etiology of rheumatoid arthritis (RA). There are a number of previous studies of genetic markers in RA, but so far no genetic linkage and only a few associations have been found. Of the associations only one (with the HLA antigen DR4) appears to be well documented. In most previous association studies the patients have not been divided according to sex and family history of RA.

In this investigation the HLA antigens A, B and DR and five serum protein systems (Bf, C3, Pi, Hp and Tf) were studied in patients with erosive rheumatoid arthritis (RA), from northern Sweden. Special attention was paid to variations in the strength of associations according to sex and family history of polyarthritis. The following results were found:

1. The frequency of the HLA antigen B27 was significantly increased in the North-Swedish population (16.6%) and among patients with a family history of polyarthritis (42.6%). In agreement with previous investigations a significantly increased frequency of the DR4 antigen was found in the RA patients.

2. In the properdin factor B (Bf) system the S phenotype was found to be significantly increased in male patients and in patients with a family history of polyarthritis, a more severe form of RA and high titres of rheumatoid factor.

3. No significant differences with respect to phenotype or gene frequencies were found in the C3 complement system. Thus, the association between RA and C3 found in previous investigations was not confirmed.

4. A significant increase of rare alpha-1-antitrypsin (Pi) types (MS, MZ, MF and SZ) was found among RA patients. However, the increase concerned mainly Z heterozygotes and was more strongly pronounced among male patients.

5. In the haptoglobin system a significant increase of the Hp2 gene and the Hp2-2 type was found among patients with a family history of polyarthritis, more pronounced among males.

6. A significant increase of the transferrin gene C2 and of the C2 type was found among male RA patients, more pronounced among patients with a family history of polyarthritis.

In 6 out of 8 gene loci studied significant associations were found, which is in agreement with a multifactorial etiology of RA. The results were largely in agreement with the hypothesis that associations would be expected to be stronger in males and in patients with a family history of polyarthritis. A notable finding was the high frequency of first degree relatives (around 40%) with symmetric peripheral polyarthritis of which more than 70% had a diagnosis of RA verified by hospital records.

Key words: Rheumatoid arthritis, family history, genetic marker, HLA B27, HLA DR4, properdin factor B, C3 complement, α1-antitrypsin, haptoglobin, transferrin.

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This thesis is based on the following papers, which will be referred to by their Roman numerals in the text.


V. Rantapää Dahlqvist S, Fröhlander N: Haptoglobin groups and rheumatoid arthritis. Hum Hered, in press.

VI. Rantapää Dahlqvist S, Beckman L: Transferrin C subtypes and rheumatoid arthritis. Hum Hered, in press.
INTRODUCTION

Genetic and environmental factors are believed to be of importance in the etiology of rheumatoid arthritis (RA). RA may, therefore, be heterogeneous and composed of subgroups, which may not always be distinguishable using clinical criteria. In diseases with a multifactorial background, the study of genetic markers, such as cell surface antigens and serum protein polymorphisms, may be useful in the identification of etiological subgroups as well as in the detection of risk factors.

In RA, associations with genetic markers can be expected to be stronger in patients with a family history of RA than in those without. Furthermore, since female sex is a risk factor in RA, associations with genetic markers other than sex would, according to the threshold theory, be expected to be somewhat stronger in males than in females.

There are a number of previous studies of genetic markers in RA, but so far only a few associations have been confirmed and only one, with the HLA factor DR4, appears to be well documented. However, in most previous studies the patients have not been classified according to sex and family history of RA.

The aims of the present investigation were:

1. to study associations between some genetic marker systems and RA in the North-Swedish population
2. to study specifically variations in the strength of associations according to family history of polyarthritis and sex
3. to study the relationship between genetic markers and some clinical variables.

BACKGROUND

Rheumatoid arthritis (RA) is the most prevalent of the inflammatory joint diseases. At onset it is mostly characterized by symmetric peripheral polyarthritis with a progressive course subsequently leading to joint destruction and deformity. In the majority of patients rheumatoid factor (RF) is present in the serum. The prevalence of RA is higher in females, and the male/female ratio varies from about 1:4 among Japanese to about 1:2 in some Caucasian populations (Ohta et al, 1982).

Genetics of RA

The concordance rate of RA in monozygotic twins has been found to be significantly higher than that in dizygotic twins (Lawrence, 1977). The frequency of RA among relatives (family history of RA) has been found to vary considerably between different investigations (Lawrence and Wood, 1968; Lawrence, 1977;
Panayi et al, 1978; Gran et al, 1983a). The variable frequencies may depend on the mode of ascertainment, diagnostic criteria and real differences with respect to familial RA (Lawrence, 1977). Familial aggregation has been found to occur mainly among seropositive cases (Lawrence and Ball, 1958; Lawrence, 1977). Most of the data gathered so far fit a multifactorial mode of inheritance (Lawrence and Wood, 1968; Bennett, 1976; Lawrence, 1977).

The influence of different genetic factors in RA may be elucidated by studies of associations between genetic markers and RA. A genetic marker is a (common) trait with a simple mendelian inheritance e.g. cell surface antigens and genetic variants of serum proteins or red cell enzymes. Associations between genetic markers may have different backgrounds. Either the marker gene may be in linkage disequilibrium with a disease susceptibility gene or the gene product itself may be an etiological factor. Furthermore an association may concern only a particular subgroup of patients, or it may concern severity of the disease but not disease risk per se.

The HLA antigens
The human major histocompatibility complex (MHC) which includes a number of closely linked loci on chromosome 6 (Fig 1), shows an extensive polymorphism (Giles and Capra, 1985). The MHC includes the class I antigens (HLA-A, B and C), the class II (DP, DQ and DR), the class III complement factors (C2, C4A, C4B and Bf) and genes for 21-hydroxylase (21-OH).

The HLA-A, B and C antigens, which are defined by serological methods, are present on all nucleated cells. The antigens are located on a cell surface protein composed of two polypeptide chains: a heavy chain controlled by a gene on chromosome 6, carrying the antigenic determinants and a light chain (β2 microglobeulin) coded by a gene on chromosome 15.

HLA-D region antigens were first studied by the mixed lymphocyte culture (MLC) method (Bach and Hirschhorn, 1964; Bain and Lowenstein, 1964). Serological tests for class II antigens (the DR antigens) were developed later (van Leeuwen et al, 1973). The DR antigens are normally present on B lymphocytes, monocytes, macrophages and Langerhans cells (Giles and Capra, 1985). The class II antigens are located on both of two polypeptide chains (α and β). It is believed that there are at least five different α-chains and seven different β-chains encoded by different loci in the MHC D-region (Giles and Capra, 1985). In the DR locus only the β-chain is polymorphic. There is not always a relationship between Dw and DR specificities, e.g. DR4 is complicated and corresponds to five different Dw specificities (Bodmer, 1984). At least three serological splits have been identified in the DR4 antigen (cf Giles and Capra, 1985) of which one includes Dw4 specificity.

It seems that the primary function of the class I and II antigens involve regulation of immune response. The class I antigens appear to restrict the cytotoxic T cell recognition of foreign antigenic determinants (Doherty and Zinkemagel, 1975) and class II antigens have a restrictive effect on the proliferative responses of

No consistent association has been found between RA and class I antigens. However, in Finland (Isomäki et al, 1975) a significant association has been observed between B27 and RA. Furthermore, in a recent study from northern Norway (Gran et al, 1984a) an increased frequency of B27 in RA (21.5%) was found, which, however, was not statistically significant. It is of interest that in Finland and northern Norway the frequency of B27 is considerably higher than in other Caucasian populations (Tiilikainen et al, 1972; Gran et al, 1984a).

The B27 antigen has been suggested to be associated with severity of RA (Pasternack and Tiilikainen, 1977) and an increased frequency of sacroiliitis in RA (Rantapää Dahlqvist et al, 1984). However, Fallahi et al (1982) did not find any clinical difference between RA patients with and without the B27 antigen.

Patients with a family history of RA showed a significantly stronger DR4 association than patients without a family history in two previous investigations (Panayi et al, 1978; Gran et al, 1983a), but not in a study by Queirós et al (1982). Dw4/DR4 has been reported to be associated with a poorer prognosis of RA (McMichael et al, 1977; Roitt et al, 1978), early onset of RA (Bardin et al, 1982; Gran et al, 1983a; Young et al, 1984), increased frequency of erosive RA (Gran et al, 1984b) and a more progressive (radiologically verified) course (Young et al, 1984). However, most reports have not been able to verify any association between Dw4/DR4 and age at onset (Scherak et al, 1980; Gorodezky et al, 1981; Bardin et al, 1982; Queirós et al, 1982), clinical severity (Stastny, 1980; Gorodezky et al, 1981; Ohta et al, 1982), number and extent of erosions (Scherak et al, 1980; Queirós et al, 1982), presence of subcutaneous nodules (Thomsen et al, 1979; Maeda et al, 1981; Queirós et al, 1982) and drug treatment (Scherak et al, 1980; Maeda et al, 1981; Ohta et al, 1982). Dw4/DR4 has been found to be associated with a positive rheumatoid factor (RF) (Dobloug et al, 1980; Stastny, 1980; Queirós et al, 1982; Ohta et al, 1982) or higher titres of RF (McMichael et al, 1977). In other studies no associations have been found between Dw4/DR4 and the presence or amount of IgM RF (Panayi et al, 1978; Thomsen et al, 1979; Sherak et al, 1980; Stastny, 1980; Maeda et al, 1981).

A significantly decreased frequency of Dw2/DR2 has been reported in RA patients (Panayi et al, 1978; Thomsen et al, 1979), in one study of RF positive patients only (Gran et al, 1983b) and in male patients only (Ohta et al, 1982). DR2 positive patients have been reported to have a milder disease and a significantly lower frequency of subcutaneous nodules (Panayi et al, 1978; Griffin et al, 1984), lower RF titres (Panayi et al, 1978) and less severe radiographic changes (Griffin et al, 1984; Young et al, 1984).

In RA, high titres of RF have been proposed to be associated with DR3 (Panayi et al, 1978) and a significant association has been observed between DR3 and a positive ANA (anti-nuclear antibody) test (Bell and Maddison, 1980; Queirós et al, 1982).

Most studies of associations between HLA antigens and adverse drug reactions in RA have shown contradictory results. There may, however, exist an association between proteinuria after treatment with gold and penicillamine and the DR3 and B8 antigens (Ford, 1984).
The Bf system
Factor B (Bf), a component of the alternative pathway of the complement system, (Fig 2) is controlled by a gene on chromosome 6 located in the HLA complex (see Fig 1) close to the C2 and C4 complement genes (Allen, 1974). In Caucasians the Bf-system has two common genetic variants (F and S) and a number of rare variants (Alper et al, 1972) which can be demonstrated by means of electrophoresis. The electrophoretic patterns of the three common types are shown in Fig 3. The different gene products appear to have equal functional activity (Mauff et al,
1980). The Bf factor is synthesized in the liver but also in macrophages and cells of the synovial tissues (Ruddy et al, 1974). During acute inflammation, the Bf level is increased in serum and synovial fluid (Hedberg, 1964; Ruddy et al, 1974, 1975).

In a previous study of Bf types in RA a significant increase of the S type and a decrease of the FS type was found (Dyer et al, 1984).

The C3 system
The third component (C3) of the complement system (Fig 2) is controlled by a gene on chromosome 19 (Whitehead et al, 1982). The C3 factor is synthesized in liver, macrophages and synovial tissue (Hedberg, 1964; Ruddy et al, 1974, 1975). The C3 system has two common genetic variants (F and S) and a relatively large number of rare variants detectable by electrophoresis (Alper, 1973). Fig 4 shows the electrophoretic patterns of the three common C3 types. A silent (null) allele for C3 is also known, which in the homozygous condition leads to increased susceptibility to infections (cf Tappeiner, 1982). The F variant of C3 has been reported to have an increased affinity for receptors on polymorphonuclear cells (Arvilommi, 1974). The C3 level is increased in serum and synovial fluid during acute inflammation (Hedberg, 1964; Ruddy et al, 1974, 1975).

In previous investigations the frequencies of the C3F type (Farhud et al, 1972) and the C3F gene (Brönnestam, 1973) have been found to be significantly increased in RA.
The Pi system

Alpha-1-antitrypsin ($a_1$-AT) one of the acute phase reactants, is a serum protein with an inhibitory effect on a number of proteases. The gene locus, Pi (protease inhibitor), which is located on chromosome 14 is highly polymorphic with more than 30 codominant alleles (Fagerhol and Cox, 1981). The most common electrophoretic variant was previously called M. By isoelectric focusing according to Frants and Eriksson (1976, 1978) subvariants of M ($M_1$, $M_2$ and $M_3$) have been identified. Fig 5 shows the electrophoretic patterns of some Pi types.

Some $a_1$-AT variants (e.g. Z, S and F) have been found to occur in reduced concentrations in serum. The deficiency alleles are associated with a number of different disorders, in particular with obstructive pulmonary disease (Laurell and Eriksson, 1963; Fagerhol and Cox, 1981). In a number of previous studies the Pi$^Z$ allele has been found to be associated with RA (Cox and Huber, 1976, 1980; Buisseret et al, 1977; Geddes et al, 1977; Arnaud et al, 1979; Breit et al, 1980). There are also studies where no significant association between RA and Pi$^Z$ has been observed (Brackertz and Kueppers, 1977; Collins et al, 1976; Sjöblom and Wollheim, 1977). Thus the association between RA and Z heterozygosity is still under debate. Cox and Huber (1980) claimed that Z heterozygosity was associated mainly with seropositive, erosive RA of the classical type, hence this association would be less likely to show up in less defined patient groups.

There is one previous report of a significant association between RA and the MS phenotype (Breit et al, 1980). However, this has not been confirmed by other investigators.
Alpha-1-antitrypsin is present in synovial fluid at about 75% of the concentration in serum (Brachertz et al, 1975) and increases during inflammation (Pritchard, 1984). It has been hypothesized that deficiency of $\alpha_1$-AT, a major inhibitor of elastase, will increase the destruction of joint cartilage by leucocyte elastase during the inflammatory rheumatoid process (Cox and Huber, 1980).

The haptoglobin system
Haptoglobin (Hp) is a haemoglobin binding serum protein synthesized in the liver. Haptoglobin is an acute phase reactant; plasma concentrations being enhanced during an inflammatory process. The haptoglobin molecule is composed of two $\alpha$- and two $\beta$-chains, and the $\alpha$-chain shows structural similarities with the immunoglobulin light chains (Black and Dixon, 1968). The $\beta$-chains have the binding site for haemoglobin.

A number of genetic variants of the $\alpha$- and $\beta$-chains have been detected by means of electrophoresis (cf Kirk, 1968). The three common types Hp1−1, Hp2−1 and Hp2−2 (see Fig 6) first described by Smithies (1955) and Smithies and Walker (1956) are due to variations in the $\alpha$-chain (Smithies et al, 1966). Furthermore, it has been possible to divide Hp1 into two subtypes: Hp1F and Hp1S. The Hp$^2$ gene
product is a partial duplication (Hp1F and Hp1S) with properties somewhat deviating from those of the Hp1 product.

There is a number of studies of associations between haptoglobin groups and different diseases. Previous studies on association between Hp groups and RA have shown contradictory results. Howard and Ansell (1964), investigating 30 RA patients, observed a significant heterozygote deficiency compared to controls. In two investigations of Swedish RA patients, Nettelbladt and Sundblad (1965, 1967) found a significant decrease of the Hp1 gene. On the other hand, Allison and Blumberg (1958) and Sitton and Dixon (1983) reported no differences between patients and controls with respect to Hp groups.

Whereas the biological functions of haptoglobin are still largely unknown, some properties are of potential significance in RA. Haptoglobin has been recognized as an inhibitor of prostaglandin synthetase and quantitative differences between Hp phenotypes may exist (Jue et al, 1983). Prostaglandins increase the serum levels of haptoglobin possibly by stimulating the synthesis (Shim, 1976), and an existence of a prostaglandin-Hp feed-back mechanism has been proposed.
Haptoglobin has also been shown to inhibit cathepsin B, a lysosomal enzyme (Snellman and Sylvén, 1967), though differences between Hp phenotypes have not been investigated. A feed-back loop involving lysosomal enzymes and acute phase reactants (including Hp) is probable (Koj, 1974).

Haptoglobin possesses antibody-like properties against Streptococcus T4 antigen (Köhler and Prokop, 1978) with phenotype differences. The Hp2-1 and Hp2-2 types act as complete, agglutinating antibodies whilst Hp1-1 acts as an incomplete, blocking antibody (Köhler and Prokop, 1982). A stronger immunological reactivity has been found among Hp2-2 individuals compared to those who are Hp1-1 (Nevo and Sutton, 1968; Baumgarten and Geserick, 1978; Friedel et al, 1979). Petzschmann et al (1980) found Hp2-1 and Hp2-2 to be overrepresented among patients with immune complex nephritis.

The transferrin system
Transferrin is an iron binding serum protein synthesized by the liver. Genetic variations in transferrin were first detected by Smithies (1957, 1958) using starch gel electrophoresis. The most frequent variant was termed C and variants with a faster and slower anodal mobility were termed B and D respectively. The transferrin locus has been assigned to chromosome number 3 (Mc Kusick, 1985).

By isoelectric focusing the common C variant can be separated into several subvariants (Kühnl and Spielmann, 1978, 1979; Constans et al, 1980). In Caucasians the two most common genes are TfC1 and TfC2 (Fig 7).

![Fig. 7. Transferrin C subtypes by isoelectric focusing in polyacrylamide gel. Anode at top.](image-url)
To our knowledge there are no previous studies of associations between transferrin types and RA. However, the transferrin C2 variant has been found to be associated with spontaneous abortion (Beckman et al, 1980), prematurity (Auconi et al, 1982) and phototoxic exzema (Beckman et al, 1985).

Iron ions (McCord and Day, 1978) and to some extent also holotransferrin (Bannister et al, 1982; Motohashi and Mori, 1983) have been found to catalyze the formation of OH· radicals. Holotransferrin has also been suggested to have antioxidative properties (Biemond et al, 1984). There is increasing evidence that oxygen free radicals such as the superoxide (O₂⁻⁻) and hydroxyl (OH·) radicals are involved in the pathogenesis of RA (Greenwald and Moy, 1980; Lunec et al, 1981). Beckman et al (1985) hypothesized that the transferrin C2 variant is more efficient in promoting hydroxyl radical formation and thereby cell damage.

MATERIAL AND METHODS

Patients and controls
Study I was based on 59 patients (not 60 as given in paper I), study II 147 patients, and studies III—VI 200 patients, of which 26 also were included in study II. The diagnostic criteria for RA (ARA criteria) are shown in Table I. Some clinical characteristics of the patients in studies II—VI are summarized in Table II.

All patients had erosive RA and all but three fulfilled the ARA criteria for classical RA (Ropes, 1959). The three remaining patients had definite RA. Erosions were defined as a break in the continuity of the cortex with a diameter ≥ 1 mm (in mtp, mcp and/or pip joints or ulnar styloid process). Rheumatoid factor (RF) titres (Waaler, 1940; Rose et al, 1948) ≥ 1/40 were considered pathological. All patients but five were seropositive. Median RF titre was 1/1280 in the patient materials of studies II—VI. In study II 2 patients out of 147 and in studies III—VI 4 out of 195 had RF titre = 1/40. All patients were examined clinically and their previous disease history was followed by hospital records.

The diagnosis of keratoconjunctivitis sicca (KCS) was made by an ophthalmologist in patients with symptoms of dry eyes. The diagnosis was based on positive Schirmer’s test (≤ 10 mm/5 min considered abnormal) and rose-bengal staining (≤ 4 points considered normal) in both eyes. Sialographic examinations were performed in a few patients (5 in study II, 2 in studies III—VI) with xerostomia. The RA patients with positive tests for KCS and/or sialectasia were regarded as having secondary Sjögren’s syndrome (SS).

The patients in studies II—VI were questioned with respect to occurrence of symmetric peripheral deforming polyarthritis in their first degree relatives. In study II, 54 patients (38%) and studies III—VI 86 patients (43%) reported a positive family history. The disease in the arthritic relatives was evaluated by hospital
Table 1. *American Rheumatism Association Criteria for the Diagnosis of RA*

**CRITERIA**

1. Morning stiffness.
2. Pain on motion or tenderness in at least one joint.
3. Swelling of one joint, representing soft tissue or fluid.
4. Swelling of at least one other joint (soft tissue or fluid) with an interval free of symptoms no longer than 3 months.
5. Symmetrical joint swelling (simultaneous involvement of the same joint, right and left).
6. Subcutaneous nodules over bony prominences, extensor surfaces or near joints.
7. Typical roentgenographic changes which must include demineralization in periarticular bone as an index of inflammation; degenerative changes do not exclude diagnosis of RA.
9. Synovial fluid; a poor mucin clot formation on adding synovial fluid to dilute acetic acid.
10. Synovial histopathology consistent with RA.
   a. Marked villous hypertrophy
   b. Proliferation of synovial cells
   c. Lymphocyte/plasma cell infiltration in subsynovium
   d. Fibrin deposition within or upon microvilli.
11. Characteristic histopathology of rheumatoid nodules biopsied from any site.

To meet criteria 1 to 5, symptoms or signs must be present for at least 6 weeks.

Criteria 2 to 6 must be observed by a physician.

Classic RA – 7 criteria needed.
Definite RA – 5 criteria needed.
Probable RA – 3 criteria needed.
Table II. *Some clinical characteristics of patients in studies I–VI*

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Age at onset years</th>
<th>Disease duration years</th>
<th>Functional class III-IV</th>
<th>Secondary SS %</th>
<th>Maximal ESR ± S.E.</th>
<th>Median RF</th>
<th>Positive ANA %</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>22</td>
<td>40.4 (13–63)</td>
<td>12.5 (1–39)</td>
<td>11</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>16.9</td>
</tr>
<tr>
<td>Study II</td>
<td>48</td>
<td>41.0 (12–81)</td>
<td>15.0 (3–54)</td>
<td>32</td>
<td>15.8</td>
<td>78±2.8</td>
<td>1/1280</td>
<td>15.0</td>
<td>147</td>
</tr>
<tr>
<td>Studies III–VI</td>
<td>100</td>
<td>41.5 (2–70)</td>
<td>14.0 (0.5–47)</td>
<td>49</td>
<td>10.0</td>
<td>74±2.5</td>
<td>1/1280</td>
<td>28.0</td>
<td>200</td>
</tr>
</tbody>
</table>

Figures in parenthesis = range
records (see Table III). About one third of them were known as patients at the Rheumatology Department, Umeå, and more than 70 per cent had a diagnosis of definite or classical RA verified by hospital records, most of them (86% and 90% respectively) from rheumatological clinics. A minor part (3.7–7.0%) of the relatives had arthritic disease, but were not classified as definite or classical RA. In about 20 per cent of the relatives the diagnosis could not be verified.

Table III.  *Clinical diagnosis among first degree relatives of RA patients*

<table>
<thead>
<tr>
<th></th>
<th>Definite or classical RA</th>
<th>Arthritis but not definite or classical RA</th>
<th>Unverified diagnosis</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study II</td>
<td>39</td>
<td>2</td>
<td>13</td>
<td>54</td>
</tr>
<tr>
<td>Studies III–VI</td>
<td>63</td>
<td>6</td>
<td>17</td>
<td>86</td>
</tr>
</tbody>
</table>

Blood donors from the county of Västerbotten served as controls for serum proteins (III–VI) and HLA A and B antigens (I–II). As controls for the DR antigens (II) kidney donors and laboratory staff from central Sweden were used.

**Laboratory techniques**

HLA A and B antigens were determined by the modified microcytotoxicity test (Kissmeyer-Nielsen and Kjerbye, 1967; Mittal et al, 1968) with antisera from northern Sweden.

For DR typing, lymphocyte suspensions were enriched in B cells (Pellegrino et al, 1975) and the cytotoxicity test was a modification of the NIH technique (Ray et al, 1976). Antisera were local or obtained through the Scandiatransplant organisation and the Eighth International Histocompatibility Workshop.

The serum protein systems were studied by the following methods, which are described in more detail in the separate studies:

- C3 by electrophoresis in agarose gels (III).
- Bf by electrophoresis in agarose gels and subsequent immunofixation (III).
- $\alpha_1$-AT and transferrin C subtypes by isoelectric focusing in polyacrylamide gels (IV, VI).
- Haptoglobin by electrophoresis in polyacrylamide gels (V).

**Strategy of analysis and statistical methods**

Association with genetic markers would be expected to be more pronounced in patients with a family history of RA. Female sex is in itself a risk factor in RA,
and one would therefore expect other genetic risk factors to be of relatively greater importance in male patients. Furthermore, some genetic factors may influence disease severity and not disease risk per se. These considerations have served as guidelines in the statistical analysis of the present data. Thus the strategy has been to study marker associations in patients subgrouped according to family history and sex and also associations with clinical variables.

The gene frequencies were calculated by simple gene counting. In the serum protein systems examined phenotypes and genotypes usually coincide, but the existence of rare null alleles cannot be excluded. Thus in a system like C3, the types F and S are assumed to be homozygotes FF and SS respectively even if they theoretically could in some rare instances be F0 and S0 respectively. Null alleles are, however, so rare that they should be of no practical importance in the calculation of gene frequencies.

The agreement between the observed genotype distribution and that expected, assuming a Hardy-Weinberg equilibrium, was calculated using the chi square test. In the general population there is usually a good agreement between observed and expected genotype distributions. However, in patients, with a particular disease, the genotype frequencies are not always in agreement with the Hardy-Weinberg distribution in a marker system showing an association with the disease in question.

The statistical significance was assessed by Student’s t-test, Fisher’s exact test and the Chi square test. In studies of HLA antigens corrected p-values (values corrected for number of tests made) were calculated in order to avoid type I errors. In most cases, however, a priori hypothesis were tested.

The strength of associations was calculated as relative risks, RR (Woolf, 1955) or δ-values (Bengtsson and Thomson, 1981). The δ-value (etiologic fraction) is a more robust measurement especially when the frequency of the marker among patients is high (Svejgaard et al, 1983).

RESULTS

The HLA antigens (I and II)
The frequency of the B27 antigen was found to be significantly increased in the population (blood donors) of northern Sweden compared to southern Sweden and Caucasians in general. In a series of 59 patients from northern Sweden with classical RA, the frequency of the B27 antigen was found to be 29%, which was significantly higher (p < 0.05) than in the controls. It was pointed out that there was a partial genetic relationship between the North-Swedish and Finnish populations, which both showed an increased population frequency of B27 and an association between B27 and RA (Isomäki et al, 1975).
The HLA antigens, A, B and DR were studied in 147 patients with classical, erosive, seropositive RA. These included 54 patients with and 89 without a family history of symmetrical peripheral polyarthritis in first degree relatives. In four patients no reliable information on family history was available. In more than 70% of the arthritic relatives the diagnosis was verified as definite or classical RA.

The frequency of the B27 antigen was significantly (p < 0.001) increased (42.6%) in patients with a family history of polyarthritis compared to patients without a family history of polyarthritis (18.0%) and controls (16.6%).

Compared to the controls (35.1%) the frequency of the DR4 antigen was significantly increased (p < 0.001) in both patients with (67.3%) and without (59.6%) a family history of RA, but there was no significant difference between these two groups of patients.

The strength of association between HLA antigens and RA was calculated as relative risk (RR) and δ-values. The highest RR values, 3.8 and 3.7 for DR4 and B27 respectively, were found among patients with a family history of polyarthritis. The corresponding δ-values were 0.49 and 0.31 respectively.

In RA patients with the B27 phenotype the maximum ESR (mm/h) was significantly (p < 0.01) higher than in B27 negatives. There was no significant antigen association between B27 and DR4 in the patients with or without a family history.

The Bf and C3 systems (III)
In the Bf system there was a significant deviation from the Hardy-Weinberg equilibrium with a deficit of the FS type in patients with a family history of polyarthritis (p < 0.05); this being confined to the males (p < 0.01).

No significant difference was found between patients and controls with respect to the phenotype distribution. However, there was a significant (p < 0.05) decrease of the FS type (and an increase of the S type) among male patients and patients with a family history of polyarthritis. Furthermore, patients with functional classes III and IV (more severe RA) and high titres of the rheumatoid factor (≥ 1/2500) showed a significantly increased S phenotype frequency (p < 0.025).

There was no significant difference between patients and controls with regard to the C3 phenotype and gene frequencies.

The Pi system (IV)
Compared to the controls the frequency of rare types (MS, MZ, MF and SZ) was significantly increased among male patients (p < 0.005) and all patients together (p < 0.025), but not among female patients alone. This increase concerned mainly Z heterozygotes and male patients. The M subtypes showed no association with RA. There were no differences between patients with and without a family history of polyarthritis.
The Hp system (V)
The distribution of haptoglobin types in the patient subgroups were in good agreement with the expected Hardy-Weinberg equilibrium. The Hp2 gene frequency was significantly increased among patients with a family history of polyarthritis compared to those without a family history (p < 0.0005) and to controls (p < 0.005). The phenotype distribution among patients with a family history of polyarthritis differed significantly from patients without a family history (p < 0.005) and controls (p < 0.01). There were no sex differences or associations between haptoglobin groups and functional class or RF titre.

The transferrin system (VI)
In the transferrin system there was no significant departure from the expected Hardy-Weinberg equilibrium. The frequency of the C2 gene was significantly increased among male patients compared to females (p < 0.025) and controls (p < 0.005). The highest frequency of the C2 gene was found in males with a family history of polyarthritis. The frequency of C2 phenotype was significantly increased in all patients (p < 0.025), male patients (p < 0.005) and male patients with a family history (p < 0.005) compared to controls. There was no association between transferrin C types and clinical or laboratory data.

DISCUSSION
Formal genetic studies of RA have indicated a multifactorial inheritance in RA (Lawrence, 1977). In multifactorial diseases the liability (or risk) distribution is a result of the combined effects of many different genetic and/or environmental factors. According to the multifactorial model, individuals whose risk score exceeds a certain border line value (the threshold), are affected. In some disorders there may be sex differences with respect to thresholds. In RA with a higher prevalence in females the threshold can be assumed to be lower for females (Carter, 1969). Among first degree relatives of the afflicted patients the liability curve is shifted to the right compared to that of the population.

Genetic studies of RA are complicated by the heterogeneity existing within the patient group. The diagnosis of RA according to ARA (Ropes, 1959) is based on the fulfilment of a certain number of 11 different criteria, 7 for classical RA, 5 for definite RA and 3 for probable RA. This type of diagnostic definition means implicitly that patients showing different symptoms and criteria may qualify for the same diagnosis. From this follows also that patients who fulfil fewer criteria e.g. probable RA should be more heterogeneous as a group than those with
many criteria e.g. classical RA. Furthermore, all of the 11 criteria are not of the same importance in the diagnosis of RA. Thus the presence of rheumatoid factor and radiographically verified joint erosions are of particular importance and we have, therefore, tried to collect a material where practically all patients have erosive and seropositive RA. This tends to make the patient material homogeneous from a clinical point of view, which is an advantage in studies of differences with respect to sex and family history, but not in studies of severity and other clinical variables.

The association of a genetic marker and a disease may be caused either by linkage disequilibrium between the marker gene and a nearby disease susceptibility gene or by a direct etiological relationship between the gene product and the disease. Previous studies of linkage between HLA genes and RA have not provided any evidence for a deviation from normal segregation (Ström, 1985). The data so far rather indicate a direct etiological effect of the DR4 and B27 antigens. In this work our main interest has been directed towards markers which may be involved in the etiology of RA through mechanisms such as immunological reactions (HLA antigens, complement factors and possibly Hp types) and damage by proteolytic enzymes (α1-AT) and oxygen free radicals (Tf types).

In all marker systems except for the transferrin system statistically significant associations with RA had been found in previous investigations thus providing a basis for a priori hypotheses to be tested in the present work. Also in the transferrin system there was an a priori hypothesis derived from association studies of other disorders.

Since most previous investigations have not included subdivisions of the patient material based on family history and sex, many of the present results are not quite comparable with those from other studies. The strength of the statistical evidence in favour of the associations observed in this study is rather variable.

The association with the TfC2 type was in agreement with the hypothesis, but nevertheless until these results have been confirmed the relationship between TfC2 and RA should be regarded merely as a working hypothesis.

We were not able to verify the previous findings of an increased frequency of the C3F gene (Brönnestam, 1973) and the C3F type (Farhud et al, 1972) in RA. Thus for the moment the evidence in favour of this association is rather weak.

An association between PiZ heterozygosity and RA has been found in 6 previous investigations. In contrast, there are 3 previous studies, where no such association has been observed. Cox and Huber (1980) suggested that Z heterozygosity was associated mainly with seropositive, erosive RA of the classical type. We believe that such an association is likely to exist, even if it is not particularly strong.

The statistical significance of the associations between Bf types and RA is rather weak. However, our results were partly in agreement with those from the
previous study by Dyer et al (1984), and further studies on Bf types in RA are of interest. Such studies should also consider the possibility of a protective effect of FS heterozygosity.

Table IV.  Outcome of studies of association between HLA B27 and RA.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th></th>
<th>Controls</th>
<th></th>
<th>p</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B27 %</td>
<td>No</td>
<td>B27 %</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kueppers et al, 1972</td>
<td>9.6</td>
<td>104</td>
<td>5.9</td>
<td>102</td>
<td>n.s.</td>
<td>C</td>
</tr>
<tr>
<td>Seignalet et al, 1972</td>
<td>14</td>
<td>50</td>
<td>9</td>
<td>300</td>
<td>n.s.</td>
<td>C</td>
</tr>
<tr>
<td>Schlosstein et al, 1973</td>
<td>8</td>
<td>119</td>
<td>8</td>
<td>906</td>
<td>n.s.</td>
<td>D</td>
</tr>
<tr>
<td>Marcolongo and Contu, 1974</td>
<td>4</td>
<td>52</td>
<td>5</td>
<td>398</td>
<td>n.s.</td>
<td>?</td>
</tr>
<tr>
<td>Isomäki et al, 1975</td>
<td>49</td>
<td>45</td>
<td>14</td>
<td>326</td>
<td>0.01 (corr)</td>
<td>C+D+P</td>
</tr>
<tr>
<td>Brautbar et al, 1977</td>
<td>14.3</td>
<td>28</td>
<td>3.0</td>
<td>456</td>
<td>0.012</td>
<td>?</td>
</tr>
<tr>
<td>Matej et al, 1977</td>
<td>16.0</td>
<td>125</td>
<td>13.3</td>
<td>300</td>
<td>n.s.</td>
<td>C</td>
</tr>
<tr>
<td>Morris et al, 1977</td>
<td>19</td>
<td>38</td>
<td>9</td>
<td>700</td>
<td>0.05</td>
<td>C+D+P</td>
</tr>
<tr>
<td>Pasternack and Tiilikainen, 36</td>
<td>70</td>
<td>*</td>
<td>16</td>
<td>120</td>
<td>0.01</td>
<td>?</td>
</tr>
<tr>
<td>Dequeker et al, 1978</td>
<td>19.7</td>
<td>5.6</td>
<td>36</td>
<td>6.6</td>
<td>n.s.</td>
<td>C+D (RF+)</td>
</tr>
<tr>
<td>Ivashova et al, 1978</td>
<td>13</td>
<td>46</td>
<td>10</td>
<td>1200</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Collin et al, 1979</td>
<td>22</td>
<td>27</td>
<td>10</td>
<td>250</td>
<td>n.s.</td>
<td>?</td>
</tr>
<tr>
<td>(cf Roberts &amp; Wentzel, 1981)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isomäki et al, 1979</td>
<td>23</td>
<td>87</td>
<td>14</td>
<td>326</td>
<td>0.05</td>
<td>D</td>
</tr>
<tr>
<td>Thomsen et al, 1979</td>
<td>12.8</td>
<td>47</td>
<td>8.6</td>
<td>1967</td>
<td>n.s.</td>
<td>C+D</td>
</tr>
<tr>
<td>Bjelle et al (I), 1982</td>
<td>29</td>
<td>59</td>
<td>16.6</td>
<td>692</td>
<td>0.05</td>
<td>C</td>
</tr>
<tr>
<td>Gran et al, 1984a</td>
<td>21.5</td>
<td>158</td>
<td>15.9</td>
<td>176</td>
<td>n.s.</td>
<td>C</td>
</tr>
</tbody>
</table>

C = classical
D = definite
P = probable
? = unknown criteria

* 26 RA patients with secondary amyloidosis
44 RA
Previous studies on Hp types in RA have either shown contradictory results or have included patient materials too small to be informative. The importance of our finding of a rather strong association between Hp2-2 and familial RA cannot be fully assessed until confirmed by future studies.

After correction for the number of tests made only the associations with DR4 and with B27 in patients with a family history remained statistically significant. The association with DR4 is now well documented, but most studies of B27 have not shown a significant association with RA. Table IV shows that, out of 16 studies of B27 in RA, there were 6 (including our study I) with a significant difference between patients and controls. In some of the previous studies the number of patients, and thereby the chance of demonstrating a significant difference from the controls, was rather small. Table IV shows that in 14 out of the 16 studies the frequency of B27 was higher among the patients compared to controls, which is significantly different from what would be expected by chance (p < 0.01). Other investigators have not subgrouped their patients according to family history. Our investigation (II) showed a highly significant increase of the B27 antigen among patients with a family history of polyarthritis. It is worth noticing that even if there is a true association between B27 and familial RA, a statistically significant association would be difficult to demonstrate due to the low frequency of familial cases in most patient materials. It is tempting to speculate that the relatively high frequency of B27 among RA patients from Finland, northern Norway and northern Sweden may be due to a high frequency of familial cases.

### Table V. Frequencies of genetic markers (per cent) in patients with and without a family history of polyarthritis and in controls.

<table>
<thead>
<tr>
<th>Genetic markers</th>
<th>Family history</th>
<th>No family history</th>
<th>All patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>B27</td>
<td>42.6***</td>
<td>18.0</td>
<td>27.2**</td>
<td>16.6</td>
</tr>
<tr>
<td>DR4</td>
<td>67.3***</td>
<td>59.6***</td>
<td>63.1***</td>
<td>35.1</td>
</tr>
<tr>
<td>BfS</td>
<td>76.7*</td>
<td>70.2</td>
<td>73.0</td>
<td>64.6</td>
</tr>
<tr>
<td>C3F</td>
<td>4.7</td>
<td>3.5</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Pi deficiency types</td>
<td>8.1</td>
<td>10.5*</td>
<td>9.5*</td>
<td>4.7</td>
</tr>
<tr>
<td>Hp2-2</td>
<td>53.5**</td>
<td>31.6</td>
<td>41.0</td>
<td>38.96</td>
</tr>
<tr>
<td>TfC2</td>
<td>7.1**</td>
<td>3.5</td>
<td>5.1*</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* p < 0.05    ** p < 0.01    *** p < 0.001
The approach to subdivide patients according to family history of polyarthritis has yielded some interesting results (see Table V). The validity of the clinical diagnosis may be questioned in some of the relatives of the RA patients and therefore the term familial polyarthritis has been used to indicate that there may be some doubt about the diagnoses in part of the relatives. However, more than 70% of the relatives had the diagnosis of definite or classical RA verified by hospital records most of them (about 90%) being from rheumatology clinics. Table V shows that in 6 out of 7 marker systems statistically significant associations were found. In 5 of these 6 systems the association was stronger among patients with a family history of polyarthritis.

Sex differences with respect to the strength of the associations were not as pronounced as the differences by family history (Table VI). In five of the marker systems males showed a greater deviation from the controls than females and in two of these systems (Pi and Tf) only the males deviated significantly from the controls.

### Table VI. Frequencies of genetic markers (per cent) in male and female RA patients and in controls.

<table>
<thead>
<tr>
<th>Genetic marker</th>
<th>Males</th>
<th>Females</th>
<th>All patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>B27</td>
<td>24.5</td>
<td>28.6**</td>
<td>27.2**</td>
<td>16.6</td>
</tr>
<tr>
<td>DR4</td>
<td>65.2***</td>
<td>60.0***</td>
<td>63.1***</td>
<td>35.1</td>
</tr>
<tr>
<td>BfS</td>
<td>76.0</td>
<td>70.0</td>
<td>73.0</td>
<td>64.6</td>
</tr>
<tr>
<td>C3F</td>
<td>4.0</td>
<td>4.1</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Pi deficiency types</td>
<td>13.0**</td>
<td>6.0</td>
<td>9.5*</td>
<td>4.7</td>
</tr>
<tr>
<td>Hp2-2</td>
<td>44.0</td>
<td>38.0</td>
<td>41.0</td>
<td>38.96</td>
</tr>
<tr>
<td>TfC2</td>
<td>8.1**</td>
<td>2.0</td>
<td>5.1*</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* p < 0.05  ** p < 0.01  *** p < 0.001

The relative clinical homogeneity of the patient material together with the fact that the patients were mostly studied retrospectively makes the evaluation of a relationship between genetic markers and clinical severity difficult. There were no significant associations between HLA antigens and clinical variables with the exception for an increase of the maximum ESR (mm/h) in patients with the B27 antigen. The BfS type was found to be associated with a more severe form of RA, (functional classes III and IV) and with high titres of RF (> 1/2500). There were no significant associations between clinical data and the other marker systems.
SUMMARY

Genetic as well as environmental factors are believed to be of importance in the etiology of rheumatoid arthritis (RA). There are a number of previous studies of genetic markers in RA, but so far no genetic linkage and only a few associations have been found. Of the associations only one (with the HLA antigen DR4) appears to be well documented. In most previous association studies the patients have not been divided according to sex and family history of RA.

In this investigation the HLA antigens A, B and DR and five serum protein systems (Bf, C3, Pi, Hp and Tf) were studied in patients with erosive RA, from northern Sweden. Special attention was paid to variations in the strength of associations according to sex and family history of polyarthritis. The following results were found:

1. The frequency of the HLA antigen B27 was significantly increased in the North-Swedish population (16.6%) and among patients with a family history of polyarthritis (42.6%). In agreement with previous investigations a significantly increased frequency of the DR4 antigen was found in the RA patients.

2. In the properdin factor B (Bf) system the S phenotype was found to be significantly increased in male patients and in patients with a family history of polyarthritis, a more severe form of RA and high titres of rheumatoid factor.

3. No significant differences with respect to phenotype or gene frequencies were found in the C3 complement system. Thus, the association between RA and C3 found in previous investigations was not confirmed.

4. A significant increase of rare alpha-1-antitrypsin (Pi) types (MS, MZ, MF and SZ) was found among RA patients. However, the increase concerned mainly Z heterozygotes and was more strongly pronounced among male patients.

5. In the haptoglobin system a significant increase of the Hp^2 gene and the Hp2-2 type was found among patients with a family history of polyarthritis, more pronounced among males.

6. A significant increase of the transferrin gene C^2 and of the C2 type was found among male RA patients, more pronounced among patients with a family history of polyarthritis.

In 6 out of 8 gene loci studied significant associations were found, which is in agreement with a multifactorial etiology of RA. The results were largely in agreement with the hypothesis that associations would be expected to be stronger in males and in patients with a family history of polyarthritis. A notable finding was the high frequency of first degree relatives (around 40%) with symmetric peripheral polyarthritis of which more than 70% had a diagnosis of RA verified by hospital records.
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