Genetics, autoantibodies and clinical features in understanding and predicting rheumatoid arthritis

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Genetics, Autoantibodies and Clinical Features in Understanding and Predicting Rheumatoid Arthritis

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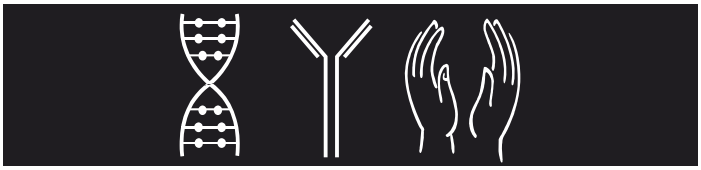

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Chapter 1

GENERAL INTRODUCTION

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a symmetric polyarticular arthritis that most commonly affects the small joints of the hands and the feet, but may involve every synovial joint. Inflammation of the synovial membrane (the joint lining) and destruction of articular structures characterize the disease process that is considered to have an autoimmune nature. Normally, the synovium is relative acellular and has a delicate intimal lining. In RA, the synovium is invaded by inflammatory and immune cells as macrophages, CD4+ T cells and B cells; an increase in macrophage-like synoviocytes and fibroblast-like synoviocytes lead to synovial hyperplasia. Pannus tissue, formed by hypertrophic synovial tissue, subsequently contributes to cartilage destruction by the formation of degrading enzymes and finally the underlying bone of the joint is eroded.

IMPACT OF RHEUMATOID ARTHRITIS

RA is the most common chronic inflammatory arthritis that has a large impact on physical, social and emotional functioning and also creates a large burden of economic costs. RA affects 0.5-1.0% of the adult population in Europe and North America (1,2). In the Netherlands the 'Standaard Diagnose-registratie van Reumatische ziekten' of TNO reports a prevalence of 1.1 per 1,000 men and 2.3 per 1,000 women, standardized for the Dutch population in 2000 (3). From the patient's perspective RA is characterised by pain, stiffness and fatigue (4) and at examination the features are inflammation and destruction of joints. Although the course of RA is highly variable among patients, the physical symptoms often have important functional consequences. The prevalence of work disability ascribed to RA is impressive. Follow-up of patients enrolled in the NOAR cohort (UK) found that in the early years after disease onset about 30% of patients permanently stopped working due to RA, and that patients with RA were 32 times more likely to stop work on health grounds compared to matched controls (5). Albers et al observed in a Dutch cohort of early RA patients work disability in 42% (6). They also stressed the broader impact of RA on daily life as over 40% of patients needed extra rest during day time and 50-60% of patients experienced significant impairment in transport mobility and leisure activities (6). The costs of RA have been studied in detail but estimates vary largely between countries and health systems. The direct costs per patients are estimated at €1,821-11,792 annually and indirect costs at €1,260-37,994 annually (7). The mentioned estimates are means, which mask major inter-individual differences because the distribution of costs is skewed with a small minority of patients accounting for a large proportion of costs (7). In a Dutch study the mean annual direct costs due to RA were estimated to be €5,250 per patient (8).

The prevalence and the huge social consequences of RA underline the importance of thorough understanding of the pathogenesis of RA and the development of effective therapeutic strategies. The last decade it has been recognised that RA needs to be diagnosed early and treated promptly with disease modifying antirheumatic drugs in order to successfully interfere with the disease process and with the progression to joint damage and disability. This new treatment paradigm in combination with new treatment options, have already improved the prospects for patients with RA in general, with improved global disease activity, retardation of joint destruction, prevention of disability and reduction of mortality (9). At present, the ultimate aim of therapeutic interventions in patients with a chronic arthritis is remission. Hopefully, in the future better understanding of the disease process will result in a further shift in treatment strategies. Ideally, the development of RA can be recognised in a very early stage and treatment in this phase is able to hamper progression to the chronic disorder. However, currently, prevention of RA is miles away and the genetic and environment factors that result in RA are far from completely understood.

PATHOPHYSIOLOGY OF AND RISK FACTORS FOR RHEUMATOID ARTHRITIS

Genetic factors

The most important genetic risk factor for RA was documented almost 30 years ago by Stastny by the recognition that HLA-DR4 (HLA-DRB1*04) is associated with RA (10). Later studies showed that also several (other) HLA-DRB1 alleles (*0101, *0102, *0401, *0404, *0405, *0408, *1001 and *1402) were associated with the disease (11-13). The products of these alleles appeared to share an amino acid sequence at position 70-74 in the third hypervariable region of the DRβ1 chain of the HLA-DRB1 molecule (QKRAA, QRRAA or RRRAA). These residues are part of one side of the antigen presenting binding site (Figure 1). The Shared Epitope hypothesis postulates that the shared epitope motif itself is directly involved in the pathogenesis of RA by allowing the presentation of an arthritogenic peptide to T cells (14). Unfortunately, specific arthritogenic peptides that bind to the DR proteins in RA have not been identified. Refinement of the Shared Epitope hypothesis, as well as alternative roles for the shared epitope motif have been proposed (15-17). Although the exact role of the shared epitope in the pathophysiology of RA is still elusive, there is extensive evidence showing an association between the shared epitope encoding alleles and susceptibility to RA as well as severity of RA (18-20). The total genetic contribution to RA has been quantified by the comparison of monozygotic and dizygotic twins and is estimated to be 50-60% (21). The HLA class II molecules are so far the most powerful genetic factor that account for about 30% of the total genetic effect (22).

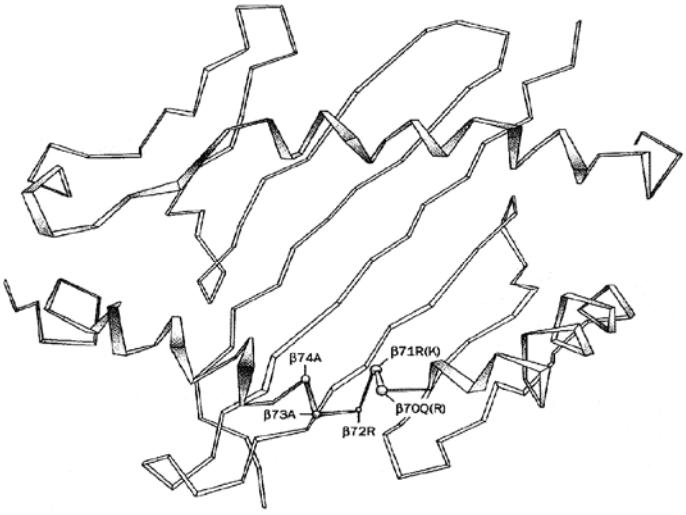

Figure 1. Structure of the HLA-DRB1 molecule. The HLA-DRB1 molecule is composed of an α-chain and a highly polymorphic β-chain. The antigen-presenting binding site is formed by a β sheet (floor) and two helices at both sides. The amino acid residues at position 70-74 are indicated. Based on Brown JH (23).

Autoantibodies

An important reason why RA is considered to be an autoimmune disease is the presence of autoantibodies. The classical autoantibody associated with RA is rheumatoid factor (RF), an autoantibody that is directed at the Fc-part of immunoglobulin G. RF is not unique for RA, and can be found in other autoimmune diseases, infectious diseases and healthy (elderly) persons. The sensitivity of RF varies between 60-70% and the specificity between 50-90% (24).

The latest years, there has been a considerable interest in the observation that the presence of antibodies to citrulline-containing proteins is highly specific for RA. The observation that these antibodies appear early in RA and can be found years before the disease onset (25,26), as well as the finding that citrullinated proteins are expressed in the inflamed joint (27,28), leads to the hypothesis that the anti-cyclic citrullinated peptides (anti-CCP) antibodies are of pathophysiological importance in RA. Citrullination is the posttranslational modification of protein-bound arginine into the non-standard amino-acid citrulline. This process is mediated by the enzym peptidylarginine deiminase and results in a small change in molecular mass and the loss of a positive charge. Although the role of citrullination remains to be determined, it has been proposed that citrullination plays a pivotal role in preparing intracellular proteins for degradation during apoptosis (29,30) and in the regulation of transcription through citrullination of histones (31). Even though citrullination seems to be a nonspecific feature of inflammation, it is not yet clear

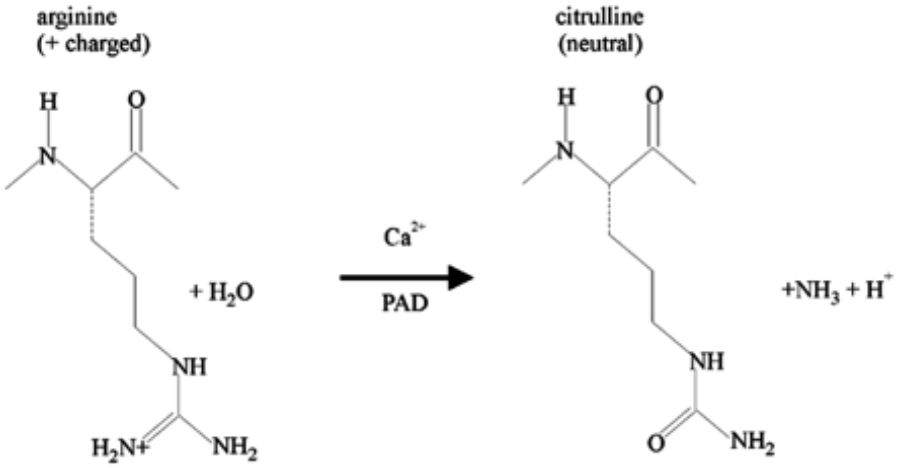

Figure 2. Conversion of arginine to citrulline, mediated by the enzym peptidylarginine deiminase (PAD).

which circumstances lead to breaking tolerance to citrullinated proteins and the development of anti-CCP antibodies. The sensitivity of anti-CCP antibodies for RA is 54-64% and the specificity is about 90-97% (32,33).

UNDIFFERENTIATED ARTHRITIS

In only a minority of the patients that present with recent-onset arthritis to an early arthritis clinic a definite diagnosis can be made directly. Only 22% of the patients that were included in the Leiden Early Arthritis Clinic were diagnosed with RA at the two weeks visit (34) and in a considerable number of patients (about 40%) no diagnosis according to one of the ACR-criteria could be made (34); these patients are identified as undifferentiated arthritis (UA). The disease course of the patients with UA is variable (Figure 3). From several inception cohort studies it is known that about 40-50% of the UA-patients remit spontaneously, whereas one-third develops RA (35-37). The analysis of the clinical evolution of patients with UA is extremely interesting as the analysis of the disease course in combination with genetical and serological factors may allow insight in the factors that are associate with progression towards RA or towards remission. Investigation of UA-patients and the disease course is not only relevant as it may reveal pathophysiological aspects of RA, it also allows the identification of predictive factors for progression to RA. Recognition of independent predictive variables and knowledge on the predictive power of these variables are ingredients to create a prediction model that estimates the chance on RA-development in individual patients with UA. Recent research indicates that

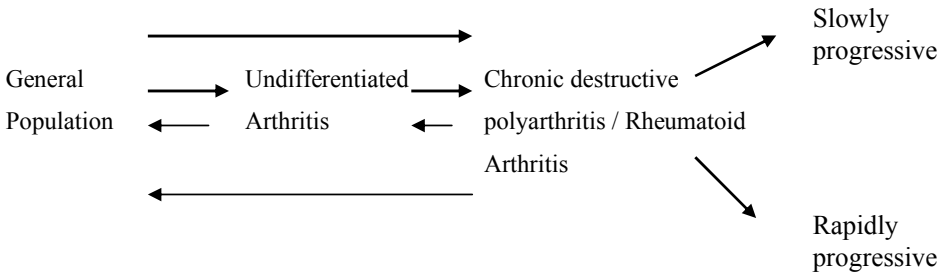

Figure 3. Variable disease course of patients with undifferentiated arthritis.

treatment with methotrexate in very early disease stages as UA is effective in hampering progression to RA (38), providing evidence for the use of disease modifying antirheumatic drugs in UA. Conversely, a considerable amount of UA-patients remit spontaneously and these patients should not be treated with potential toxic drugs. These data underline that at present support for clinicians in treatment decisions in patients with recent-onset UA is urgently needed.

AIM AND OUTLINE OF THE THESIS

The aims of this thesis were mainly three-fold:

1. To investigate the association of several genetic factors with RA.
2. To elucidate the role of the HLA Class II alleles in the development of both anti-CCP antibodies and RA.
3. To identify predictive factors for the development of RA and to develop a prediction model that determines the risk to progress from UA to RA in individual patients.

This thesis is devided in four parts.

In **Part 1** the association between several genetic factors and RA susceptibility and severity is examined.

Although the predisposing effects of the shared epitope encoding HLA-DRB1 alleles are generally accepted, controversy existed regarding the possible protective effects of certain HLA-DRB1 alleles. These alleles contain instead of the shared epitope another common anchor region consisting of the amino acids DERAA. Although some evidence for the protective effect of the presence of DERAA-encoding alleles existed, it was not clear whether the effect of DERAA is really protective or whether the protective effect is attributable to the concomitant absence of predisposing shared epitope alleles. **Chapter 2** investigated the effect of the DERAA-encoding HLA-DRB1 alleles on RA susceptibility and severity and differentiated the protective effect from non-predisposition by comparing subgroups of patients with an equal amount of predisposing alleles.

Tumor necrosis factor (TNF)-alpha is a pro-inflammatory cytokine that plays a significant role in promoting joint inflammation in RA and TNF-alpha blockers are the most effective drugs in the treatment of RA. In 2001 and 2002 two separate groups reported on the association of a Single Nucleotide Polymorphism (SNP) in the TNF-receptor 2 gene in familial RA. Controversy existed on the association of this SNP with RA severity. **Chapter 3** assessed the effect of the *TNFR2* 196 M/R SNP on RA severity by taking advantage of the extremes of the phenotypes that exist in rheumatoid arthritis: the genotype frequencies of the patients that achieved remission and the patients that developed severe destructive disease were compared.

Chapter 4 investigated the association between the C1858T SNP in the protein tyrosine phosphatase non-receptor 22 (PTPN22) gene and RA susceptibility, RA severity and UA. This PTPN22 gene is located at chromosome 1 and encodes for a lymphoid tyrosine phosphatase that mediates the inhibition of T-cell-receptor signaling. The 1858C→T SNP changes the aminoacid at position 620 from arginine (R) to tryptophan (W) and has recently been identified as a risk factor for RA (39). The study described in chapter 4 aimed to replicate the association between this SNP and RA and to extend this finding by studying whether this SNP is also correlated with RA severity and UA.

In **Chapter 5** it is explored whether the receptor for advanced glycation end products (RAGE) G82S polymorphism is an independent risk factor for RA. There are findings that suggest a role for RAGE signaling in the pathogenesis of RA. RAGE seems to be important in its ability to amplify pro-inflammatory immune responses and several RAGE ligands display increased levels in synovial tissue or synovial fluid in patients with RA (40-42). In addition, the G82S SNP has been found to be more prevalent in RA patients compared to controls (43). As this SNP is in strong linkage disequilibrium with the HLA-DR4 allele, chapter 5 examined whether the reported association can be explained through linkage with HLA-DR4.

Chapter 6 reviewed recently identified genetic factors and their contribution to RA.

In **Part 2** associations between the HLA Class II alleles and autoantibodies are described.

Considering the high specificity of anti-CCP antibodies for RA, the finding that the presence of shared epitope encoding HLA-DRB1 alleles correlate with the presence of autoantibodies in RA and the assumption that anti-CCP antibodies are of pathophysiological importance for RA, the nature of the association between HLA and anti-CCP antibodies is further explored. **Chapter 7** compared the shared epitope frequencies of healthy controls and RA patients without and with anti-CCP antibodies in two independent cohorts using two different methods: association and linkage.

In **chapter 8** the association between the non-shared epitope encoding HLA-DRB1 alleles and anti-CCP antibody negative RA is investigated.

The most prominent environmental risk for RA is smoking: smokers have increased levels of RF (44-46) and are more prone to develop RA (46-48). Recently, a gene-environmental interaction between smoking and the shared epitope was described, providing risk for RF-positive but not for RF-negative RA (49). In **chapter 9** it is studied whether a gene-environmental interaction is present for the anti-CCP antibody response. Second, this study investigated whether the interaction between smoking and the shared epitope alleles is unique for RA or is also present in UA.

In chapter 7 it is shown that the shared epitope alleles are only a risk factor for anti-CCP positive RA and not associated with anti-CCP negative RA. As the contribution of the shared epitope containing HLA-alleles to the pathogenesis of RA is not well understood, the findings of chapter 7 led us to evaluate the hypothesis that the shared epitope alleles are mainly a risk factor for anti-CCP antibodies rather than for (anti-CCP positive) RA. To this end, the disease course of patients presenting with UA in combination with the HLA class II alleles and autoantibodies was studied. The results on this analysis, as well as data on the association between the shared epitope alleles and the level of anti-CCP antibodies are described in **chapter 10**.

The results described in chapters 7-10 strongly suggest that the pathogenic mechanisms underlying anti-CCP antibody positive and negative RA are different. These observations inspired subsequent research addressing the question whether anti-CCP-positive RA and anti-CCP-negative RA are different disease entities or have different phenotypical properties. Therefore, in **chapter 11** anti-CCP antibody positive and negative RA patients are compared for several aspects of their phenotype: initial symptoms, signs and acute phase reactants and distribution of joint swelling and severity of radiological joint destruction during the course of the disease.

In **part 3** two different aspects of RA severity are studied.

Chapter 12 investigated *in vitro* characteristics of fibroblast-like synoviocytes (FLS) in relation with the level of joint destruction in patients with RA. FLS are a major constituent of the hyperplastic synovial pannus and *in vitro* studies have shown that the FLS in RA have a transformed behaviour: they express large amount of proteases, express oncogenes and invade normal cartilage. In chapter 12 it is assessed whether the degree of *in vitro* measured invasion is associated with the degree of radiological joint destruction in patients with RA.

Chapter 13 studied the contribution of genetics to RA severity by comparing radiological data of monozygotic and dizygotic twins and unrelated RA-patients. Current research on genetic factors associated with RA is focussed on disease susceptibility. Although the effects of genetic factors on RA severity are of equal interest, so far the contribution of genetics to RA severity is not known.

Part 4 of this thesis deals with the question whether the disease course in arthritis can be predicted. First, in **chapter 14** the current knowledge on risk factors for RA development is reviewed.

The study described in **chapter 15** analysed whether the distribution of arthritic joints at first presentation has a predictive value for the disease course in RA.

Finally, **chapter 16** presents the development of a prediction model for the disease outcome in patients with recent-onset UA and the validation of this model in an independent cohort of UA-patients.

The results of the studies performed in this thesis are summarized and discussed in **chapter 17**.

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Chapter 2

An independent role for protective HLA Class II alleles in rheumatoid arthritis severity and susceptibility

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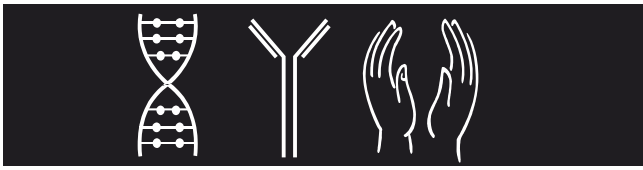

ABSTRACT

Objectives. The most important genetic risk factor for rheumatoid arthritis (RA) is located within the Human Leucocyte Antigen (HLA)-region. HLA-DRB1 alleles encoding for the shared epitope (SE) predispose to RA and to more severe disease. Other HLA-DRB1 alleles harbouring a different epitope, encoded by the amino acids DERAA, have been associated with protection. Due to small cohort sizes, the protective effect on disease severity is still controversial and has never been discerned from non-predisposition (not carrying SE-alleles). This study investigates the effect of the DERAA-encoding alleles on RA severity and susceptibility in a large prospective cohort and differentiates protective effects from non-predisposition by comparing subgroups of patients with an equal amount of predisposition alleles.

Methods. In 440 early RA patients and 423 healthy controls the HLA class II alleles were determined. To study the effect of HLA on disease severity, radiological joint destruction (modified Sharp-van der Heijde method) was determined during 4-years follow-up.

Results. The presence of DERAA-encoding HLA-DRB1-allelles conferred a lower risk to develop RA both in the presence and in the absence of SE-alleles (OR 0.6). In the presence of one SE-allele, the group of patients that carried DERAA had significant less severe radiological destruction at all time points compared to DERAA-negative patient-group with one SE-allele. Additionally, the protective effect of DERAA was detected in the groups of patients that were prone to more severe disease due to the presence of anti-CCP-antibodies or smoking.

Conclusions. DERAA-encoding HLA-DRB1-alleles independently protect against RA and are associated with less severe disease.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex genetic disorder with an estimated heritability of 60% (1). Human Leucocyte Antigens (HLA) Class II molecules are the most powerful recognized genetic factors and contribute to at least 30% of the total genetic effect (2). Extensive evidence exists showing the association between certain frequently occurring HLA-DRB1 alleles (*0101, *0102, *0401, *0404, *0405, *0408, *0410 *1001, *1402) and susceptibility to and severity of RA (3-5). The indicated alleles share a conserved amino acid sequence (QKRAA, QRRAA or RRRAA; also called the shared epitope) at position 70-74 in the third hypervariable region of the DRβ1 chain. These residues are part of an α-helical domain forming one side of the antigen presenting binding site. The Shared Epitope hypothesis postulates that the shared epitope motif itself is directly involved in the pathogenesis of RA by allowing the presentation of a peptide to arthritogenic T cells.

Although the predisposing effects of shared epitope encoding HLA-DRB1 alleles are generally accepted, controversy exists on the existence of protective effects by certain HLA-DRB1 alleles. These alleles contain, instead of the shared epitope, another common anchor-region consisting of the amino acids DERAA. HLA-DRB1 alleles that express this DERAA sequence (DRB1*0103, *0402, *1102, *1103, *1301, *1302, *1304) may protect against RA (6-8). There is some evidence that patients carrying the DERAA sequence have also less erosive disease. However, there are few studies addressing the effect of DERAA on disease severity, and interpretation is hampered either by a retrospective design with variable disease duration (9,10) or by small numbers of patients carrying the DERAA-sequence. Wagner et al. performed a prospective study, but only 7 DERAA-positive patients were followed for 4 years (11). Moreover, it is not clear whether the effect of DERAA encoding HLA-DRB1 alleles is truly protective or is due to the effect of the concomitant absence of predisposing shared epitope encoding HLA-DRB1 alleles.

A number of the initial reports on the protective effects of the DERAA haplotype are based on the Leiden Early Arthritis Clinic (6,12). This cohort started in 1993 and has since then expanded considerably. Presently more than 1800 patients are included. By using this expanded cohort we aim to assess the association of DERAA encoding HLA-DRB1 alleles with 1) RA severity, taking advantage of the fact that at present a substantial number of patients is followed prospectively and 2) susceptibility to RA. This large cohort allows the determination of the possible protective effects of DERAA encoding HLA-DRB1 alleles in the presence of an equal number of shared epitope encoding HLA-DRB1 alleles, thereby permitting differentiation between protection and non-predisposition. Furthermore, the available clinical data allowed to determine whether RA patients exhibiting an extreme of the phenotypic spectrum by achieving clinical remission harbour a different distribution

of HLA-alleles compared to patients with persistent disease. The present data show that HLA-DRB1 alleles encoding the DERAA-sequence are associated with less severe disease at all time points during 4 years follow-up and confer a lower risk to develop RA.

PATIENTS AND METHODS

Study Population

In 1993 an Early Arthritis Clinic was started at the Department of Rheumatology of the Leiden University Medical Center, the only referral center for Rheumatology in a health care region of about 400,000 inhabitants in the western part of the Netherlands (13). General practitioners were encouraged to refer patients directly when arthritis was suspected. Patients referred to the early arthritis clinic could be seen within two weeks and were included in the program when the physician's examination of the patient revealed arthritis and the symptoms had lasted less then two years. For every patient, routine diagnostic laboratory screening was performed. A 44 joint count of swollen joints was performed on entering the study and yearly thereafter. The smoking history was assessed. In this study smokers were patients that smoked (cigarettes, cigars) at inclusion or patients that had smoked previously. The numbers of smoked pack years was not addressed. Non-smokers have never smoked. At present more than 1800 patients are included in the Early Arthritis Clinic, of which approximately 1650 patients have at least one year of follow-up. 440 of these patients fulfilled the diagnosis of RA according to the American College of Rheumatology 1 year after inclusion in the study (376 definite RA and 64 probable RA, according to the ACR criteria of 1987 and 1958 respectively) and had DNA available for genotyping. As it was observed that in the current inception cohort, over 2/3 of probable RA patients developed definite RA in the next year, these probable RA patients were included in the study. A small proportion of the patients involved in the present study (about one third) were also included in previous studies examining the association between HLA-DRB1 alleles and RA using the Leiden Early Arthritis Clinic (6). Informed patient consent was obtained and the study was approved by the local medical ethics committee. A random panel of 423 healthy unrelated Dutch individuals served as control.

HLA genotyping

The HLA class II alleles were determined in all RA patients and controls. The HLA-DRB1 (sub)typing was performed by polymerase chain reaction using specific primers and hybridisation with sequence-specific oligonucleotides. The predisposing shared epitope alleles were DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *0410 *1001, and *1402. The DERAA encoding alleles were HLA-DRB1*0103, *0402, *1102, *1103, *1301, *1302, and *1304. For clarity, this study uses consequently the term DERAA-encoding alleles.

Table 1. HLA-DRB1 genotypes of RA patients and healthy controls

Group	DRB1 genotype	RA patients (N=440)		Controls (N=423)	
		n	%	n	%
A	SE/SE	70	15.9	26	4.9
B	SE/x	187	42.5	124	30.5
C	SE/DERAA	27	6.1	29	6.9
D	x/x	112	25.5	149	35.2
E	x/DERAA	36	8.2	87	20.6
F	DERAA/DERAA	8	1.8	8	1.9

Group B vs. C: OR 0.6, 95%CI 0.3-1.1, p=0.1.
Group D vs. E+F: OR 0.6, 95%CI 0.4-0.97, p=0.03.
Group A+B vs. D: OR 2.3, 95%CI 1.6-3.2, p<0.001.
Patients and controls were categorized according to the HLA-DRB1 genotype.
SE alleles are DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *1001, *1402.
DERAA alleles are DRB1*0103, *0402, *1102, *1103, *1301, *1302, *1304.
x means all other DRB1 alleles.

However this study does not differentiate between the direct effect of these alleles and the effect of other alleles in linkage with the DERAA-encoding HLA-DRB1 alleles; the observed effects might therefore also be the result of a haplotype containing the DERAA-encoding alleles. For the analysis a division in six groups according to the HLA-DRB1 alleles was made: homozygous for shared epitope (SE/SE, group A), one shared epitope allele (SE/x, group B), one shared epitope and one DERAA allele (SE/DERAA, group C), no shared epitope or DERAA alleles (x/x, group D), one DERAA allele (x/DERAA, group E) and two copies of a DERAA encoding allele (DERAA/DERAA, group F), see Table 1.

Radiographic progression

Radiographs of hands and feet were made at baseline, at one year and yearly thereafter. Radiographs were scored using the modified Sharp-Van der Heijde method (14). The rheumatologist that scored the radiographs was blinded to the clinical data and unaware of the study question. At inclusion radiographs were scored of 324 patients, 305 patients had radiographs at 1 year follow-up, 259 patients at 2 year follow-up, 216 patients at 3 year follow-up and 197 patients at 4 years follow-up. The fact that at the moment of analysis not all patients had achieved 4 years follow-up is inherent to the design of an inception cohort.

Extremes of the phenotypes: clinical remission

Comparing the extreme phenotypes of a disease can elucidate the presence or absence of an association between an allele and disease severity (15,16). For this study patients that developed clinical remission, the best clinical course possible, were selected. Patients in remission had no signs of arthritis in the absence of disease-modifying drugs and were therefore discharged from the outpatient clinic. Patients were only discharged after they

had been at least one year in remission without the use of disease-modifying drugs. Eighty patients achieved clinical remission; all had fulfilled the ACR criteria for RA (62 patients for definite RA and 18 patients for probable RA, according to the ACR criteria of 1987 and 1958 respectively).

Statistical analysis

To differentiate the protective effects from the effects due to non-predisposition, analysis was performed using subgroups of patients with an equal amount of predisposing shared epitope alleles. To determine the effect of the DERAA-encoding alleles in the presence of one shared epitope allele the subgroups SE/DERAA and SE/x were compared (group B vs. C, see table 1); to assess the effect of the DERAA-encoding alleles in the absence of shared epitope alleles the subgroups X/DERAA and DERAA/DERAA were compared with x/x (group E+F vs. D, see table 1). An alternative method to identify the causative HLA-factor truly responsible for the association is described by Svejgaard and Ryder (17). This method uses a two-by-four table that is subsequently analysed using various two-by-two tables involving stratification of each of the two factors against each other. The association of DERAA with RA susceptibility was analysed and presented according to both methods. For the analysis of the severity data, subgroups with an equal amount of predisposing shared epitope alleles were compared. Odds ratio's (OR) with 95% confidence intervals (95% CI) were calculated using the method of Woolf Haldane; p values were calculated using the chi square test. Differences in means between groups were analysed with the Mann Whitney test or t-test when appropriate. In all tests, p values less than 0.05 were considered significant.

RESULTS

Susceptibility

To study the effect of the presence of DERAA on the susceptibility to RA, patients and controls were divided in 6 groups according to their HLA-DRB1 status (Table 1). In total 71 RA patients (16%) and 124 controls (29%) carried DERAA encoding HLA-DRB1 alleles. (OR 0.5, 95%CI 0.3-0.7, $p<0.0001$) First, the effect of DERAA in the absence of shared epitope allele was assessed by comparing group D with E+F. DERAA positive persons had a significantly lower risk to develop RA (OR 0.6, 95%CI 0.4-0.97, $p=0.03$). Comparing group B with group C revealed that in the presence of one shared epitope allele the DERAA-encoding alleles reduce the risk to develop RA, although the observed effect was not statistically significant (OR 0.6, 95%CI 0.3-1.1, $p=0.1$).

Additionally the same data were analysed according to the Svejgaard approach (see Table 2). The presence of DERAA conferred a significant lower risk to develop RA both

Table 2. HLA-DRB1 genotypes of RA patients and healthy controls, analysed according to the approach of Svejgaard and Ryder (17)

Shared epitope encoding DRB1	DERAA encoding HLA-DRB1	Number of patients (N=440)	Number of controls (N=423)
+	+	27	29
+	–	257	150
–	+	44	95
–	–	112	149

Comparison	Entries of 2x 2 table				
	a	b	c	d	
SE + vs. –	284	156	179	244	OR 2.5 95%CI 1.9-3.3, p<0.0001
DERAA + vs. –	71	369	124	299	OR 0.5 95%CI 0.3-0.7 p<0.0001
SE in DERAA +	27	44	29	95	OR 2.0 95%CI 1.0-4.0, p=0.03
SE in DERAA –	257	112	150	149	OR 2.3 95%CI 1.6-3.2, p<0.0001
DERAA in SE +	27	257	29	150	OR 0.5 95%CI 0.3-0.99 p=0.03
DERAA in SE –	44	112	95	149	OR 0.6 95%CI 0.4-0.97, p=0.03

in shared epitope negative and shared epitope positive patients (OR 0.6, 95%CI 0.4-0.97, p=0.03 and OR 0.5, 95%CI 0.3-0.99, p=0.03 respectively).

As anti-CCP antibodies are highly associated with RA, we wished to analyse whether the presence of DERAA was correlated with the anti-CCP status of patients. Therefore the effect of DERAA on the risk to develop RA was assessed in anti-CCP positive and anti-CCP negative RA patients separately. The presence of DERAA conferred a lower risk to develop both anti-CCP positive RA (OR 0.3, 95%CI 0.1-0.4) and anti-CCP negative RA (OR 0.7, 95%CI 0.5-1.0).

The effect on disease susceptibility of shared epitope alleles in the absence of DERAA was assessed by comparing group A+B versus D (Table 1) and similarly according the Svejgaard approach (Table 2). Shared epitope positive persons had an odds of 2.3 to develop RA compared to shared epitope negative patients (95%CI 1.6-3.2, p< 0.001).

As the HLA-DRB1 alleles are in linkage disequilibrium with certain HLA-DQ alleles (DQ3 and DQ5, see ref 22), the above-described analysis was also performed using HLA-DRB1-DQ genotypes. Similar results on predisposition to RA were found using HLA-DRB1-DQ genotypes instead of using HLA-DRB1 alleles solely (Table 3).

Table 3. HLA-DRB1 and -DQ genotypes of RA patients and healthy controls

Group	DQ genotype	DRB1 genotype	RA patients (N=440)		Controls (N=423)	
			n	%	n	%
1 a	3/3	SE/SE	31	7.0	9	0.9
b		SE/x	10	2.2	2	1.7
c		x/x	2	0.5	2	0.5
2 a	3/5	SE/SE	29	6.6	11	2.6
b		SE/x	5	1.1	2	0.5
3 a	5/5	SE/SE	9	2.0	6	1.4
b		SE/x	1	0.2	0	0
4 a	3/x	SE/SE	1	0.2	0	0
b		SE/x	100	22.7	58	13.7
c		x/x	16	3.6	16	3.8
5 a	5/x	SE/x	71	16.1	62	14.7
b		x/x	1	0.2	0	0
6 a	3/x	SE/DERAA	14	3.2	12	2.8
b		x/DERAA	3	0.7	7	1.7
7 a	5/x	SE/DERAA	13	3.0	17	4.0
b		x/DERAA	1	0.2	0	0
8	x/x	x/x	93	21.4	131	31.0
9	x/x	x/DERAA	32	7.3	80	18.9
10	x/x	DERAA/ DERAA	8	1.8	8	1.9
Predisposition alleles + (gr1-5)			276		168#	
Predisposition alleles – (gr8)			93		131#	
Protection alleles – (gr 4b+5a)			171		120*	
Protection alleles + (gr 6a+7a)			27		29*	
Protection alleles – (gr 8)			93		131°	
Protection alleles + (gr9+10)			40		88 °	

Patients were categorized according to the presence or absence of DQ3 or DQ5 heterodimers and subdivided for DRB1 alleles. SE alleles are DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *1001, *1402. DQ3 means DQB1*0301, *0302, *0303, *0304, *0401, or *0402 in combination with DQA1*03. DQ5 means DQB1*0501 in combination with DQA1*0101 or *01040. DERAA alleles are DRB1*0103, *0402, *1102, *1103, *1301, *1302, *1304. x means all other DQ or DRB1 alleles.
OR 2.3 95%CI 1.7-3.3, p<0.001.
* OR 0.7, 95%CI 0.4-1.2, p=0.14
° OR 0.6, 95%CI 0.4-1.0, p=0.05

All the above-mentioned results did not change when the 64 patients with probable RA were excluded and the 376 patients with definite RA were analysed (comparison of group B with C OR 0.6, 95%CI 0.3-1.1, p=0.06; comparison of group D with E+F OR 0.6, 95%CI 0.4-1.0, p=0.04; comparison of groups A+B with D OR 2.1, 95%CI 1.1-4.0, p=0.01).

In conclusion, these data show that carriership of DERAA-encoding HLA-DRB1 alleles protects to develop RA in individuals with a shared epitope allele as well as in individuals without shared epitope alleles.

Severity

To asses the influence of the presence of DERAA encoding HLA-DRB1 alleles on radiological joint destruction, Sharp-van der Heijde scores during 4 years of follow-up were compared in subgroups of patients with an equal of amount of shared epitope encoding HLA-DRB1 alleles, thereby excluding a possible confounding effect due to a difference in predisposing alleles. Although the rate of joint destruction in the whole group of shared epitope negative patients was very low, the effect of carrying one or two DERAA alleles in the absence of shared epitope alleles was determined by comparing the radiological scores of group E+F versus D. The mean (± SEM) Sharp-van der Heijde scores at inclusion and 1, 3 and 4 years of follow up were respectively 2.9 ± 0.6, 8.1 ± 1.8, 12.4 ± 2.4, and 15.1 ± 3.6 in the patients not carrying a protection allele (group D) and 5.0 ± 2.2, 7.8 ± 3.4, 8.6 ± 3.7, and 15.2 ± 7.3 in the patients with one or two protection alleles (group E+F) ($p = 0.4$, 0.9, 0.3 and 0.9 respectively). Thus, the presence of DERAA-encoding alleles in patients with absence of shared epitope does not result in significantly lower radiological scores. As anti-CCP antibodies are associated with more severe disease (18), we assessed the influence of DERAA on disease severity in anti-CCP positive and negative patients separately. This analysis revealed that in shared epitope negative anti-CCP positive RA patients, the presence of DERAA associates with significantly less severe disease at all points in time except inclusion (see Figure 1). In shared epitope negative, anti-CCP negative patients the rate of joint destruction was too low to observe differences between DERAA positive and negative patients.

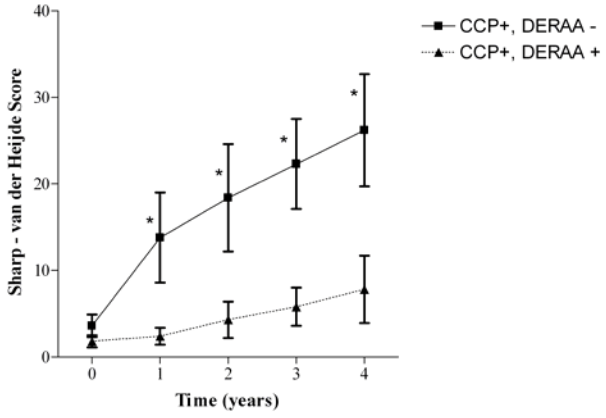

Figure 1. Sharp-van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of shared epitope negative anti-CCP positive RA patients in the presence and absence of DERAA-encoding alleles. * $P < 0.05$.

Because patients not carrying shared epitope alleles have a less destructive disease compared to shared epitope positive patients (comparison of groups D versus A+B, Figure 2), we subsequently assessed the effect of DERAA on disease severity in shared epitope positive patients, as in this group of patients with more severe disease the window to read out an eventual protective effect is larger. Moreover, by analysing the subgroups of patients with an equal amount of shared epitope alleles a possible confounding effect due to differences in predisposing alleles was excluded. Comparing the Sharp-van der Heijde scores of group B with group C (see Table 1 for the division in groups) showed significant lower Sharp-van der Heijde scores at all time points during 4 years follow-up in the DERAA positive group (Figure 3, $p<0.001$ at inclusion, 1 and 2 years follow-up, $p<0.01$ at 3 years and $p<0.05$ at 4 years follow-up). Thus, DERAA-encoding alleles protect against severe disease in the presence of one shared epitope allele.

Considering the association between anti-CCP antibodies and RA severity (18), we wished to assess whether the observed protective influence of DERAA is dependent on the presence or absence of anti-CCP antibodies. Therefore, the effect of DERAA in the presence of one shared epitope allele was analysed in anti-CCP positive and negative patients separately. The protective effect of DERAA remained in both anti-CCP positive and negative RA patients (Figure 4). Not only anti-CCP antibodies are known to associate with RA severity, also the environmental factor smoking is reported to correlate with more severe disease (19). To further confirm the protective effects of the DERAA-encoding alleles we analysed the effects of DERAA in patients that were prone to more severe disease due to smoking. Therefore, the effect of DERAA in the presence of one shared epitope allele was assessed for smokers and non-smokers separately. Non-smoking patients that were DERAA-positive showed a trend for lower radiological scores ($p=0.06$ and 0.07 at 1 and 4

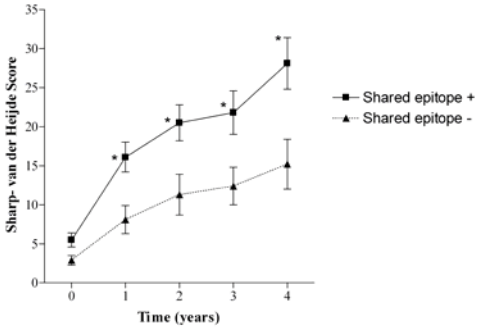

Figure 2. Sharp- van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of RA patients with or without shared epitope alleles in the absence of DERAA –encoding alleles. * P<0.05.

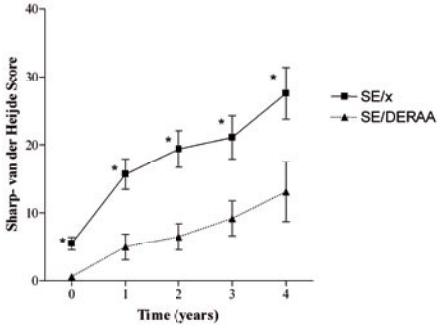

Figure 3. Sharp- van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of RA patients with and without DERAA-encoding HLA-DRB1 alleles in the presence of one shared epitope allele. * P<0.05.

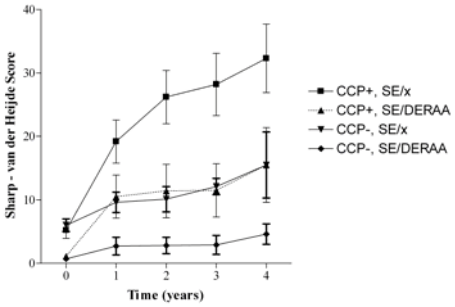

Figure 4. Sharp-van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of RA patients with and without DERAA-encoding alleles in the presence of one shared epitope encoding allele, for anti-CCP positive and anti-CCP negative patients separately.

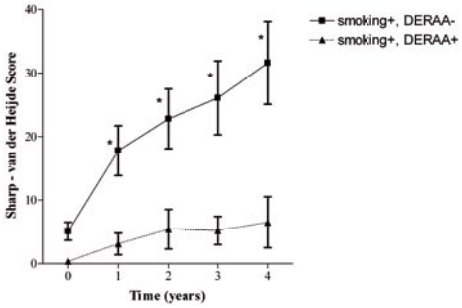

Figure 5. Sharp-van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of shared epitope positive smoking RA patients in the presence or absence of DERAA -encoding alleles. * P<0.05.

year follow-up respectively). In smokers the presence of DERAA correlated with significant lower Sharp-Van der Heijde scores at all time points except inclusion ($p<0.05$, Figure 5). As smoking might correlate with anti-CCP antibodies (non published data S. Linn -Rasker) a Mantel-Haenszel analysis revealed that a trend to a protective effect of DERAA in both anti-CCP positive and negative smoking RA patients was present (data not shown).

In conclusion, 1) DERAA-encoding HLA-DRB1 alleles are associated with less severe joint destruction in patients that also carry a HLA-DRB1 encoding shared epitope allele, 2) this protective effect remains after correction for anti-CCP antibodies and 3) DERAA-encoding alleles also exhibit a protective effect in severe disease that is associated with smoking.

Extremes of the phenotypes: clinical remission

To assess a possible association between HLA and clinical remission, we identified 80 patients that obtained clinical remission without the use of disease modifying drugs. Clinical remission was achieved after a mean follow-up of 3.9 years (SD 2.5 years). The patients in the remission group were in 62% of cases female, had a mean age of 57.7 ± 15.4 years (mean ± SD) and were in 12% anti-CCP antibody positive. The 360 patients that did have persistent RA were in 66% of cases female, had a mean age of 55.4 ± 16.4 years and were in 57% anti-CCP antibody positive. There was no different distribution of DERAA-encoding HLA-DRB1 alleles in patients that obtained remission compared to patients with persistent RA. Overall, 18% of patients that obtained remission carried DERAA alleles, versus 16% of the RA patients with persistent disease. Likewise, when the distribution of DERAA in the presence or absence of shared epitope alleles was evaluated, no differences were found in the remission or persistent RA group. In addition, the distribution of shared epitope encoding HLA-DRB1 alleles in the absence of DERAA alleles was studied in the

remission and persistent RA group. Fifty-five percent of patients that achieved clinical remission carried shared epitope alleles, compared to 73% of the patients with persistent RA. This indicates that shared epitope alleles occurred significantly less frequent in the patients that achieved clinical remission (OR 0.5, 95%CI 0.3-0.8, p=0.003). In conclusion, RA patients that achieve clinical remission have significantly less frequent shared epitope alleles but do not carry more DERAA-encoding HLA-DRB1 alleles.

DISCUSSION

This study investigates the associations between HLA Class II alleles and RA and describes the protective effects of DERAA-encoding HLA-DRB1 alleles on RA severity and susceptibility. The question whether the effect of DERAA is truly protective or only the result of the absence of predisposing shared epitope-encoding HLA-DRB1 alleles has been surrounded with some controversy. In the current study the comparison of subgroups allowed to differentiate the effects of protection and non-predisposition. This study shows that the DERAA-encoding HLA-DRB1 alleles independently reduce the risk to develop RA.

More importantly however, our study shows in a large prospective cohort that DERAA encoding alleles are associated with less severe radiological destruction in patients that were predisposed to severe RA by the presence of shared-epitope alleles at all time point during 4 years of follow-up. The protective effect of DERAA remained after stratification for anti-CCP antibodies. Stratification for smoking, another risk factor for severe disease, showed that DERAA particularly protects in patients that are also predisposed to more severe disease by smoking. All together, these data indicate that the protective influence of DERAA can be detected in patients that are prone to severe disease, by either the presence of shared epitope alleles, anti-CCP antibodies or smoking. In patients with a low rate of joint destruction such as shared epitope negative and anti-CCP negative RA patients, the current data set is not sufficiently powered to answer the question whether a protective effect of DERAA is present in these patients. Intriguingly, the differences in Sharp-van der Heijde scores between DERAA-positive and negative patients (in presence of a shared epitope allele) are as large as the differences in Sharp-van der Heijde scores between shared epitope positive and negative patients (see Figure 2 and 3). Thus, the protective effect of DERAA-encoding alleles on radiological joint destruction seems to be of a similar magnitude as the predisposing effect of shared epitope alleles.

The chance to achieve clinical remission is lower for patients carrying a predisposition allele, but is not higher in patients carrying protection alleles. Although we cannot explain these observations at this moment, these findings suggest that the disease-pro-

moting mechanisms that are associated with shared epitope alleles are distinct from the mechanisms involved in tempering disease-progression. In this respect, it is tempting to speculate that the protective pathways associated with the expression of DERAA-encoding HLA-alleles are able to dampen the effector pathways underlying bone and cartilage breakdown, but that they do not affect the principal pathway that drives chronicity.

Although the number of patients with 4 years follow-up in the current study is higher compared to previous studies on the protective effect of DERAA on RA severity, the present study lacked sufficient power to address the question of a dose effect of DERAA. This is due to the finding that homozygosity for DERAA in RA-patients is rare (2% of the RA patients in this cohort). Of these 8 patients, 5 patients had at the moment of analysis a follow-up of 2 years and only 2 had a follow-up of 4 years. Remarkably, the total Sharp-van der Heijde score of these patients was 1.0 ± 1.0 at inclusion, 1.6 ± 1.0 and 0 ± 0 at 2 and 4 years follow-up (mean ± SEM), indicating that RA patients with two copies of DERAA seems to have a non-destructive disease course. As the radiological scores of the patients that are homozygous for DERAA are lower than the patients heterozygous for DERAA, a gene-dose effect is possible. However, the number of homozygous patients is too low for definite conclusions.

Although so far not much data are available on the association between protective HLA Class II alleles and RA severity, well-designed studies are available on the association between protective HLA alleles and disease susceptibility (8,20,21). However, the definition of protective alleles differs in these studies. De Vries et al. considered alleles with amino acid D at position 70 as protecting. In this way more alleles than those encoding for D^{70}ERAA were classified as protective (e.g HLA-DRB1 *07, *1201, *1501) (20). Reviron et al. concluded on a different hypothesis (electric charge of the HLA pocket) that alleles with a neutral or negative charge in their P4 pocket protect to develop RA. These alleles contain not only the DERAA-encoding HLA-DRB1 alleles but also other HLA- alleles, among which HLA-DRB1*08 (21). Our results confirm and extend these observations by focussing on the DERAA-encoding HLA-alleles and by analysing the effects of these alleles on disease severity. The observed effects of the presence of DERAA might be the direct result of the DERAA-encoding alleles or might be the result of HLA-haplotypes that contain the DERAA-encoding HLA-DRB1 alleles.

The known predisposing effect of the shared epitope alleles on RA susceptibility and severity is confirmed in this study. Previously, it has been hypothesised by our group that predisposition to RA is not only controlled by shared epitope alleles but is also conferred by HLA-DQ alleles (22). Support for a role of HLA-DQ came from studies on collagen induced arthritis in HLA-DQ transgenic mice (23). The so-called RA-protection hypothesis

further implied that DERAA is only protective in the presence of certain DQ3 or DQ5 heterodimers (22). The data of the current study were analysed both using HLA-DRB1 genotypes and using HLA-DR-DQ genotypes, revealing similar results. The predisposing HLA-DQ and -DRB1 alleles are strongly associated in our population; therefore differentiation of the individual effects of HLA-DR and HLA-DQ was not feasible. As the present study reveals that DERAA protects against RA not only in patients with predisposing HLA-DR alleles or HLA-DR-DQ genotypes but also confers a lower risk to develop RA in patients without these predisposing genotypes, the previously published RA-protection hypothesis should be amended.

It has been demonstrated that peptides carrying the DERAA motif are naturally processed by human APC and it has been suggested that the protective effect of DERAA is mediated by a specific protective T cell response (24). Although our results clearly show that the presence of a predisposing haplotype is not required to observe the protective effect associated with DERAA, it is conceivable that the DERAA-sequence itself is presented towards T-cells with protective activities. Interestingly alleles carrying the DERAA sequence, particularly DRB1*13 alleles, not only protect from (severe) RA but have also been associated with a milder disease outcome in other diseases, such as a reduced progression to active chronic hepatitis C and B (25,26), a lower incidence of cervical carcinoma (27) and a reduced rejection of renal transplants (28). These findings are intriguing and point to the importance to elucidate the biological pathways underlying these associations, as they might unveil new insights on immune regulation in relation to the HLA-system.

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Chapter 3

No association between Tumor Necrosis Factor Receptor Type 2 gene polymorphism and rheumatoid arthritis severity: a comparison of the extremes of phenotypes

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ABSTRACT

Objectives. To assess the association between the tumor necrosis factor receptor 2 (*TNFR2)* 196 M/R single nucleotide polymorphism and rheumatoid arthritis (RA) severity by taking advantage of the extremes of phenotypes that exist in arthritis.

Methods. Out of the Leiden Early Arthritis Cohort (1700 patients), we selected patients that initially had the diagnosis of definite or probable RA according to ACR criteria and developed complete remission (71 patients) or had the worst progression to destructive disease (72 patients). A group of 135 healthy controls was included. In these groups the *TNFR2* genotype was determined.

Results. The extremes of phenotypes did not differ significantly in genotype distribution. No difference in genotype distribution between rheumatoid arthritis patients and healthy controls was observed.

Conclusions. Our study demonstrates that even by comparing the extremes of phenotypes no association between the *TNFR2* genotype and disease severity can be detected in Caucasian patients with sporadic RA.

INTRODUCTION

Recent reports have suggested an association between the tumor necrosis factor receptor 2 (*TNFR2)* 196 M/R single nucleotide polymorphism (SNP) and susceptibility to rheumatoid arthritis (RA), restricted to familial RA (1,2). Two recent studies have reported conflicting results concerning the involvement of *TNFR2* 196 M/R SNP as a genetic factor of RA severity (3,4). Whereas Glossop et al. reports no association with this SNP and radiological and functional RA severity (3), Constantin et al. recently found a worse Health Assessment Questionnaire score in patients carrying the *TNFR2* 196R allele (4). However, in both studies the distribution of severity of RA in the relatively small number of patients is limited. An association between *TNFR2* 196M/R and severity would emerge best comparing patients with the best and the worst course, e.g. comparing the extreme phenotypes. Therefore, out of a cohort of 1700 patients we selected RA patients who developed complete remission and those patients of this cohort that had the worst progression to destructive disease. In these two unique groups of patients the *TNFR2* genotype was determined.

PATIENTS AND METHODS

The Leiden Early Arthritis Cohort consists of all patients that were referred to the Department of Rheumatology of the Leiden University Medical Center with recent onset arthritis. From 1993 till 2003 over 1700 patients have been included and followed. Follow-up included Health Assessment Questionnaire, Disease Activity Score, and radiographs of hands and feet at 0, 6 and 12 months and yearly thereafter (5). To detect the patients with the best course we selected those patients that at the enter of the cohort had 1) a symmetric arthritis of the small joints, 2) morning stiffness of at least one hour and 3) the diagnosis of definite or probable RA according to ACR criteria who 4) achieved long-term and complete remission (no signs of arthritis in the absence of disease modifying drugs) and were therefore discharged. Patients were not discharged when less than one year in remission without the use of disease modifying drugs. In total 78 patients fulfilled the criteria; of 71 patients DNA was available for *TNFR2* genotyping. To select the patients with the most progressive disease, the RA patients with the highest rate of joint destruction as measured by the Sharp/van der Heijde method at radiographs of hands and feet after one year of follow-up were selected. 79 Patients had a total score > 15; of 72 patients was DNA available. All patients included reported to have Caucasian grandparents. A group of 135 healthy controls was included; all these persons were Caucasian and had no family history of RA. Informed patient consent was obtained and the study was approved by the local Medical Ethical Committee under code P237-94.

Genotyping of *TNFR2* 196 M/R polymorphism was performed blinded, by PCR-restriction fragment polymorphism (N1a III) as previously described (6). Two samples of 71 and 72 patients provides a power of 82% to detect differences with 95% confidence and an odds ratio of 3 based on a 196R allele frequency of 22%. The X^2-test was used to compare the genotype distribution between the two groups of RA patients. All allele and genotype frequencies were in Hardy Weinberg equilibrium.

RESULTS

The frequencies of the 196M and 196R alleles among the 143 RA patients were 78% and 22% respectively. Table 1 depicts patients' characteristics and the genotype distributions of both groups of RA patients and of the healthy controls. In the remission group, 49 patients (69%) had fulfilled the ACR-criteria for definite RA, and 22 patients (31%) the ACR criteria for probable RA. The extremes of phenotypes did not differ significantly in genotype distribution, as well as determined in the total group of patients as when assessed in the first half of patients and replicated in the second half. In addition, there was no significant difference in Health Assessment Questionnaire and Disease Activity Score when the 196R and 196M alleles were compared (data not shown). No difference in genotype distribution between rheumatoid arthritis patients and healthy controls was

Table 1. Characteristics of and *TNFR2* 196 M/R gene polymorphism in rheumatoid arthritis patients with complete remission and severe progression.

	Complete Remission (No 71)	Severe Progression (No 72)
Age (mean SD)	57.6 ± 16.9	55.6 ± 16.8
Male/Female, No (%)	30 (42.3) / 41 (57.7)	25 (34.7) / 47 (65.3)
RF positive, No (%)	7 (9.9)	30 (41.7)
Total Sharp/van der Heijde score at inclusion	3.1 ± 7.2	11.0 ± 15.9
Total erosion score	0.8 ± 2.7	4.7 ± 9.2
Total joint space narrowing score	2.3 ± 5.4	6.1 ± 8.2
Total Sharp/van der Heijde score after 1 year follow-up	5.3 ± 8.3	36.1 ± 24.5
Total erosion score	1.9 ± 2.7	21.3 ± 17.0
Total joint space narrowing score	3.4 ± 6.5	14.7 ± 11.0
TNFR2 196 M/R genotype		
MM No (%)	43 (60.5)	50 (69.4)
MR No (%)	23 (32.4)	19 (26.4)
RR No (%)	5 (7.0)	3 (4.2)
Shared Epitope positive No, (%)	30 (42)	57 (79)

The *TNFR2* 196 M/R genotype distributions in the 135 healthy controls was: MM 81 (60.0%), MR 44 (32.6%), RR 10 (7.4%).

observed as well (Table 1). Patients in the severe progression group were more frequent shared epitope positive than patients in the remission group (odds ratio 5.2, 95% confidence interval 2.3-11.7) (Table 1).

DISCUSSION

In this study no association between the *TNFR2* 196 M/R gene polymorphism and severity in Caucasian patients with sporadic RA was observed. By comparing the genotypes of the patients with the worst and the best course out of a cohort of more than 1700 patients an association between *TNFR2* and severity, if present, very likely would have been detected. With the sample sizes used we have a power of 82% to detect differences with an odds ratio of 3. In general the numbers used in a power calculation are based on the difference between a 'normal' population of rheumatoid arthritis patient that contains many phenotypes and healthy controls that contain some phenocopies. In this circumstance a power of 80% to detect differences with an odds ratio of 2 is usually accepted. In the present study the power was enhanced by selection of extremes of the phenotypes. This method is supposed to enlarge the difference and can make a study more powerful. Therefore in this study we accept the magnitude of the odds ratio to be at least 3. The odds ratio for the association between HLA- Class II alleles and RA severity is about 3 in inception cohorts (7). In our design we observed an odds ratio for shared epitope positivity and RA severity of 5, indicating the power of the present approach.

The allele and genotype distributions in this Dutch study are similar to those in French (2,4), British (3), Italian (8) and Swedish (9) studies, in which allele frequencies of 75-79% are reported for the 196M allele and allele frequencies of 20-25% for the 196R allele. The fact that in the present study the gene distribution of patients and healthy controls were comparable confirms previous findings of an absent association between *TNFR2* and susceptibility to sporadic RA (1,2).

The *TNFR2* gene is located on chromosome 1p36 and consists of 10 exons and 9 introns. A SNP at codon 196 in exon 6 (ATG → AGG) results in a nonconservative amino acid substitution: methionine (M) → arginine (R). Little is known on the functionality of this amino acid substitution. In Japanese patients with systemic lupus erythematosus (SLE) it is demonstrated that the 196 *TNFR2* SNP has no influence on receptor binding of TNF-α or on receptor shedding, but that the 196R allele more effectively transduces signals for IL-6 production than does 196 M allele (10). However also in these Japanese SLE patients the 196R allele was not associated with disease severity (10).

In summary, our study demonstrates that even by comparing the extremes of phenotypes no association between the *TNFR2* genotype and disease severity can be detected in Caucasian patients with sporadic RA.

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Chapter 4

Association of the *PTPN22* C1858T single nucleotide polymorphism with rheumatoid arthritis phenotypes in an inception cohort

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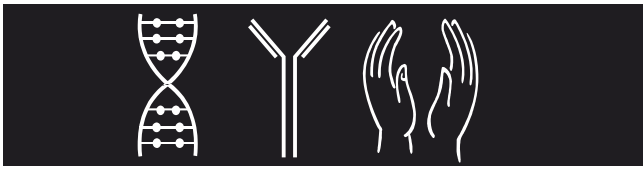

A missense SNP (rs2476601, C1858T) in *PTPN22,* which encodes a tyrosine phosphatase, has been associated with multiple autoimmune diseases including RA (1-3). This SNP results in the substitution of a conserved arginine with tryptophan at codon 620 (R620W) in the protein's SH-3-binding domain. The 1858T risk allele, which results in disruption of the interaction between PTPN22 and the C-Src kinase, Csk, (3) potentially alters these proteins' function as negative regulators of T-cell activation. In this study, we investgate the association of the *PTPN22* C1858T SNP with RA, undifferentiated arthritis (UA) and both quantitative (rate of joint destruction) and qualitative (autoantibody status and remission or progression) RA characteristics.

All RA cases (n=416) were participants in the Leiden Early Arthritis Clinic (EAC), a population-based inception cohort of recent onset arthritis described by van Aken et al (4), and fulfilled ACR criteria for RA at one year follow-up. EAC participants who could not be properly classified at one year (n=265) were categorized as UA cases. All patients and unrelated control individuals (described in reference 5) were Dutch whites. Appropriate institutional review boards approved the protocol.

The C1858T SNP and HLA-DRB1 alleles were genotyped as described and genotyping accuracy was > 99.8% (1,6). An individual was considered positive for the shared epitope (SE) if they carried at least one copy of any of the following HLA-DRB1 alleles: 0101, 0102, 0401, 0404, 0405, 0408, 1001 or 1402. X-rays of hands and feet were taken at baseline, six months, one year and annually thereafter. The χ^2 test or Fisher's exact test, unconditional logistic regression and tests for trend were used for statistical analysis. Reported p-values are two-tailed; values less than 0.05 were considered significant.

PTPN22 C1858T genotypes were generated for 416 RA cases, 265 UA cases, and 891 controls, and were in Hardy-Weinberg equilibrium in all groups. The frequency of the *PTPN22* risk allele in our control group was similar to previously reported allele frequencies in US white controls (0.091 and 0.087 respectively)(1). We observed a higher frequency of the 1858T risk allele in RA cases compared to controls (0.119 vs. 0.091; p=0.0257) (Table 1). Genotypic analysis showed an increase in risk of RA associated with having one or two copies of the 1858T allele (OR=1.37; p=0.0336). In agreement with previous reports (1-2), TT homozygotes (OR=1.95) appeared to be at greater RA risk than TC heterozygous individuals (OR=1.34) when compared to CC homozygotes.

Next we investigated this SNP in the patients stratified according to autoantibody status (Table 1). Compared to the controls, the 1858T allele frequency was elevated in both RF positive (p=0.0091) and CCP positive (p=0.0132) RA patients but not in patients negative for these autoantibodies. Genotypic analysis showed that carriers of the 1858T allele were

Table 1. *PTPN22* C1858T SNP case-control analysis[a]

	Frequency		Genotype			CT	TT		CT + TT	
Study Group	(T)	P[b]	CC	CT	TT	OR (95% CI)	OR (95% CI)	P_{Trend}[c]	OR (95% CI)	P[d]
Controls (n=891)	0.091		736	148	7					
RA cases (n=416)	0.119	0.0257	323	87	6	1.34 (1.00-1.80)	1.95 (0.65-5.86)	0.0838	1.37 (1.03-1.82)	0.0336
Rheumatoid Factor										
Positive (n=249)	0.131	0.0091	189	55	5	1.45 (1.02-2.05)	2.78 (0.87-8.86)	0.0319	1.51 (1.08-2.11)	0.0173
Negative (n=167)	0.102	0.5288	134	32	1	1.19 (0.78-1.82)	0.79 (0.10-6.43)	0.7058	1.17 (0.77-1.78)	0.4636
Anti-CCP[f]										
Positive (n=197)	0.132	0.0132	149	44	4	1.47 (1.00-2.15)	2.82 (0.82-9.76)	0.0447	1.53 (1.06-2.21)	0.0237
Negative (n=153)	0.092	0.9734	125	28	0	1.11 (0.71-1.74)		0.8936	1.06 (0.68-1.66)	0.7858
UA (n=265)	0.126	0.0163	201	61	3	1.51 (1.08-2.11)	1.57 (0.40-6.12)	0.0492	1.51 (1.09-2.10)	0.0141

[a]SNP = single nucleotide polymorphism; OR = odds ratio; 95% CI = 95% confidence interval; RA = rheumatoid arthritis; CCP = cyclic citrullinated peptide; UA = undifferentiated arthritis.

[b]Test of significant difference in allele frequency compared to control group.

[c]Test of linear trend for genotypic ORs.

[d]Test of significant genotypic OR, 1858T carriers vs. non-carriers.

[e]One year diagnosis.

[f]Data were not available for all cases.

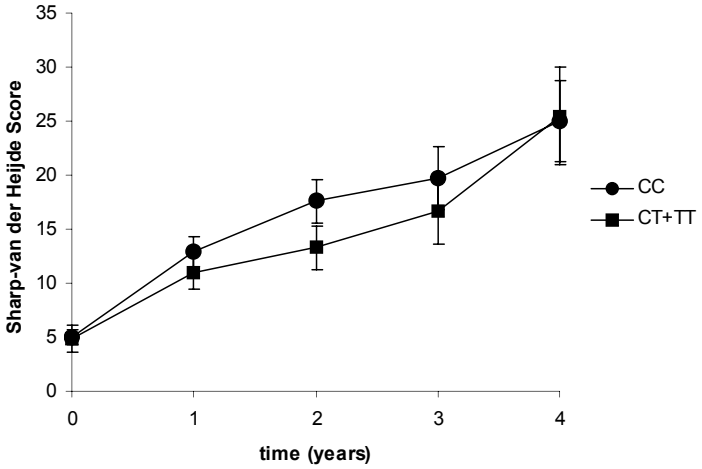

Figure 1. Sharp-van der Heijde scores (mean ± SEM) over time among RA patients, by *PTPN22* 1858T carrier status (CT+TT vs. CC). Radiological data (X-rays of hands and feet) of 220 CC and 68 CT+TT patients were available at baseline. Respectively, data for 254 and 72 patients at year 1, 178 and 56 patients at year 2, 117 and 36 patients at year 3, and 101 and 27 patients at year 4 were analyzed.

at increased risk for RF positive (p=0.0173) and anti-CCP positive RA (0.0237). This was not the case for autoantibody negative patients suggesting an association between the *PTPN22* 1858T risk allele and autoantibody production in RA. Consistent with previous studies (1-2), *PTPN22* C1858T genotype frequencies were similar in HLA-DRB1 shared epitope (SE)-positive and SE-negative cases (0.125 vs. 0.105; p=0.4022), suggesting that the *PTPN22* risk allele acts independently of HLA-DRB1 susceptible alleles to influence RA risk.

Allele frequencies of C1858T were similar in 45 RA patients who achieved remission (defined as absence of arthritis with no use of DMARDs for at least one year) versus 319 patients with persistent inflammation (0.10 vs. 0.118, p=0.707)(7). Mean baseline and yearly Sharp-Van der Heijde scores of X-rays of hand and feet of RA patients with different *PTPN22* genotypes (Figure 1) were also similar in 1858T carriers and non-carriers (8). These data suggest no association of the *PTPN22* risk allele with rate of joint destruction.

The 1858T allele frequency was also elevated in UA cases compared controls (0.126 vs. 0.091; p=0.0163) (Table 1). Genotypic analysis indicated that carriers of the 1858T allele were at significantly higher risk of UA (OR=1.51).

These results support the role of the *PTPN22* C*1858*T SNP as a common, HLA-independent, genetic risk factor for RA. Moreover, this study also replicated the dosage effect when comparing CT and TT risk genotypes (1-3). We confirm the predominant *PTPN22*

association with RF positive RA (1-2) and provide the first evidence that this SNP is also associated with anti-CCP positive RA. These findings support the hypothesis that this variant may predispose individuals to autoimmunity by facilitating the production of certain disease-associated autoantibodies (1-2). Although no association between the *PTPN22* C1858T SNP and the level of RA joint destruction and remission was observed in this study, the sample-size is too small to rule out the possibility of a type II error. Our results also indicate that the 1858T allele is a risk factor for UA. In conclusion, our data suggest the *PTPN22* 1858T variant acts as a susceptibility allele for autoantibody positive RA but does not appear to influence RA severity.

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Chapter 5

The RAGE G82S polymorphism is not associated with rheumatoid arthritis independent of HLA-DRB1*0401

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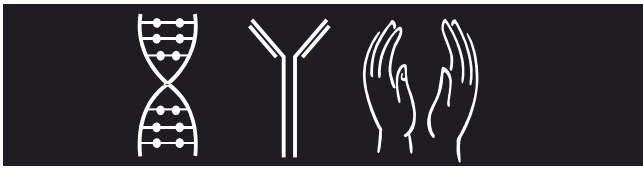

The receptor for advanced glycation endproducts (RAGE) has been shown to play a role in several pathologies including Rheumatoid Arthritis (RA) (1). RAGE binding of ligands upregulated in RA synovial tissue, fluid and serum, can lead to increased cell activation, including migration, hyperplasia and increased cytokine production. Several animal studies have described a possible role for RAGE in the onset and severity of arthritis. In these animal studies, blockade of the receptor showed suppression of arthritis, while administration of RAGE ligands induced arthritis in healthy mice (2,3). In addition, increased levels of RAGE ligands have been found in RA patients and correlate with disease severity (4-6).

Previous studies indicated that a gain of function mutation of RAGE correlates with RA. Linkage of RAGE with the HLA-DRB1-DQ region, a region known to associate with RA susceptibility and severity, could account for this correlation. To dissect the possible confounding effects of the HLA-DRB1 region for the possible association of RAGE with RA, we investigated the correlation of a gain of function mutation in RAGE, to HLA-DRB1 alleles and RA.

377 consecutive RA patients of the Leiden Early Arthritis Clinic, an inception cohort for patients with recent onset arthritis (7) (mean age 48 ± 17 years SD, 55% female) and 535 non-RA controls of the same cohort (mean age 57 ± 16 SD, 67% female) were included in the analysis. All RA patients fulfilled the 1987 criteria of the American College of Rheumatology. The study was approved by the local Ethics Committee and written informed consents were obtained from all patients and controls according the declaration of Helsinki. HLA genotyping was available for all patients and controls and in addition for typing of the RAGE G82S polymorphism a PCR was performed, followed by an overnight digestion with *Alu* I. The 82S polymorphism resulted in the formation of an extra *Alu*I restriction site.

A previous report by Hoffman et al (2) described that the RAGE 82S polymorphism correlated with susceptibility for rheumatoid arthritis. However, RAGE is in strong linkage disequilibrium with HLA-DR4 (8), particularly HLA-alleles encoding a common 'shared epitope' within the HLA-DRB1 allele. In the population studied by Hoffman et al, a linkage was found with DR1*0401 and after correction for this allele, the correlation between the RAGE 82S polymorphism and susceptibility for RA was lost. These data were not conclusive, since the number of patients and controls positive for the RAGE 82S polymorphism were very low (5 out of 95 and 2 out of 134 respectively). Here, we identified 46 out of 377 RA patients and 36 out of 535 controls harbouring the RAGE 82S polymorphism. We found an association between the RAGE 82S polymorphism and RA without correction for HLA alleles. However, in patients, RAGE 82S was in linkage (defined as $P<0.01$) with DRB1*0401 (odds ratio (OR) 6.5 $P<0.0001$). In controls RAGE 82S was in linkage

Table 1. Frequencies of patients and controls positive or negative for RAGE 82S or HLA-DRB1*0401 and statistic calculation by the method described by Svejgaard et al.

	RAGE 82S	DRB1*0401		Patients	Controls			
	+	+		36	20			
	+	–		10	16			
	–	+		78	60			
	–	–		253	439			
Comparison	Patients		Controls			OR (CI.)	P	Test
RAGE 82S$^+$ vs. RAGE 82S$^-$	*82S+*	*82S-*	*82S+*	*82S-*				
	46	331	36	499		1.99 (1.19-3.12)	0.0045	RAGE associated?
DRB1*0401$^+$ vs. DRB1*0401$^-$	*DRB1*0401+*	*DRB1*0401-*	*DRB1*0401+*	*DRB1*0401-*				
	114	263	80	455		2.47 (1.76-3.45)	<0.001	DR0401 associated?
82S$^+$ /0401$^+$ vs. 82S$^-$ /0401$^+$	*82+/0401+*	*82-/0401+*	*82+/0401+*	*82-/0401+*		1.38 (0.70-2.77)	0.32	RAGE associated in DR0401+?
	36	78	20	60				
82S$^+$ /0401$^-$ vs. 82S$^-$ /0401$^-$	*82+/0401-*	*82-/0401-*	*82+/0401-*	*82-/0401-*		1.08 (0.45-2.57)	0.84	RAGE associated in DR0401-?
	10	253	16	439				
82S$^+$ /0401$^+$ vs. 82S$^+$ /0401$^-$	*82-/0401+*	*82-/0401-*	*82-/0401+*	*82-/0401-*				
	36	10	20	16		2.88 (1.00-8.45)	0.029	DR0401 associated in RAGE+1?
82S$^-$ /0401$^+$ vs. 82S$^-$ /0401$^-$	*82+/0401+*	*82+/0401-*	*82+/0401+*	*82+/0401-*				
	78	253	60	439		2.26 (1.53-3.32)	<0.001	DR0401 associated in RAGE-1?
Association between RAGE and DR0401 in patients	*patients 82S+*	*patients 82S-*	*patients 0401+*	*patients 0401-*				
	36	10	78	253		11.68 (5.28-26.4)	<0.001	Linkage disequilibrium in patients?
Association between RAGE and DR0401 in controls	*controls 82S+*	*controls 82S-*	*controls 0401+*	*controls 0401-*				
	20	16	60	439		9.15 (4.26-19.74)	<0.001	Linkage disequilibrium in controls?

with DRB1*0401 (OR 4.43, P<0.00001) and *0901 (OR 5.05, P=0.002). Correction for presence or absence of these HLA alleles was done by the Svejgaard method (9). When the association of RAGE 82S with RA was corrected for the absence or presence of DRB1*0901, the association between RAGE and RA remained present. Conversely, after correction for the presence/absence of HLA DRB1*0401 the association between RAGE 82S and RA was lost (Table 1), indicating that RAGE is not associated with RA independently of HLA DRB1*0401 in this cohort. Also in logistic regression analysis with DRB1*0401 and RAGE as possible explanatory variables and the presence of RA as dependent variable, only DRB1*0401 was independently associated with RA (OR 2.5, P<0.001).

In conclusion, considering the size of our study, it is unlikely that the RAGE 82S polymorphism is associated with RA independently of HLA DRB1*0401.

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Chapter 6

Understanding the genetic contribution to rheumatoid arthritis

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ABSTRACT

Purpose of review. Identification of the genetic variants that mediate risk for susceptibility and severity of Rheumatoid Arthritis (RA) will allow development of new drug targets as well as increase the ability to predict disease course. Technical and methodological progress has fuelled the progress in this field.

Recent findings. The second risk factor for RA was identified, the PTPN22 polymorphism. This genetic variant regulates the threshold of T-cell activation. Intriguingly this variant is a risk factor for diabetes as well. Moreover it was shown that multiple genetic variants in one pathway (both in a transcription factor, RUNX-1 as in the transcription factor binding site of RUNX1 in the SLC22A4 gene) can each confer very small risks but by gene-gene interactions can confer a 9-fold risk to RA. Phenotype-genotype interactions were described by the enhanced prevalence of an RA-specific autoantibody (anti-CCP antibodies) in RA patients that harbour the RA-associated HLA class II genes, the shared epitope alleles. An environmental risk factor for RA, smoking was demonstrated to confer its risk especially in RA patients that have both the shared epitope alleles as the RA-specific anti-CCP antibodies.

Summary. Two new pathways (T-cell Receptor signalling and a haematopoietic-specific signal transduction pathway) have been discovered that allow future pharmacological interventions. The description of the new genetic risk factors and the interaction with environmental triggers as well as phenotypic features is gradually expanding our prognostic abilities to predict disease susceptibility and course.

INTRODUCTION

The completion of human genome sequencing and the technological revolution in genotyping is driving projects that aim to understand the genetic contribution to virtually every common disease by identifying sequence variants associated with these disorders. The motivation to study the genetic contribution is two-fold. First, identification of new critical pathways in disease pathogenesis leads to the identification of new drug targets leading to more optimal treatments. Second, the increased understanding of pathogenesis will lead to better and more focussed treatment protocols. The current clinical prediction models have insufficient power to provide patients with individualized treatments. Given the fact that the efficacy of treatments is proven at the group level by randomized controlled clinical trials, many patients are over- or under treated. It is hoped for that prediction of disease outcome by genetic risk factors may lead to more individualized treatment protocols.

For rheumatoid arthritis (RA) historically sound epidemiological data (the different prevalence of RA in various populations, migration studies, familial clustering and twin studies) demonstrated the genetic contribution to susceptibility of RA (1). The comparison between the concordance rates in monozygotic twins and the prevalence in the respective populations revealed that about 50% of the variation in occurrence of disease is caused by genetic factors (2). Moreover large initiatives from Japan, Europe, United Kingdom and the USA are present in which multicase families are available and genome wide searches are being performed. Finally, both the resources (large inception cohorts) and the tools (well developed disease activity measurements and outcome measurements) are available in the field of arthritis to take maximum advantage of the automated genotyping technologies which enable the systematic ascertainment of sequence variants, in the form of single-nucleotide polymorphisms (SNPs), for the genomes of large numbers of individuals. During the last two years this has led to remarkable progress in understanding the genetic contribution to RA. This review is divided in four sections

- Progress following linkage data
- Progress from candidate genes from previously identified linkage regions in RA
- Progress from candidate genes
- Progress on phenotypic-genotypic interactions

PROGRESS FOLLOWING LINKAGE DATA

For the pan-European, the Japanese, the North American Rheumatoid Arthritis and the UK consortium, genome scans have previously been performed with an average marker

spacing ranging from 10 to 12 centimorgans (cM). The French group published last year a scan with a mean marker spacing of 3.3 cM, resulting in an average distance between any RA susceptibility gene and its nearest marker of 0.8 cM (3). 19 non-HLA regions showed suggestive evidence for linkage ($P < 0.05$). Nine of these overlapped with regions suggested in other published RA genome scans. In order to provide an estimate what the error rate of this approach is, an assessment of the significance of the number of regions with suggestive evidence for linkage was obtained by using 10,000 computer simulations with the null hypothesis of absence of any true RA gene region. The probability of observing 19 non-HLA peaks by chance was 3.7%, which provided convincing evidence that these peaks contained at least 1 true non-HLA RA gene region. Since a mean ± SD of about 11 ± 4 false-positive peaks were expected, the number of true RA linkage peaks was estimated to be 8 ± 4.

The UK group explored the utility of SNPs for linkage analysis. A whole-genome screen of 157 families with multiple cases of RA was performed using 11,245 genomewide SNPs (4). The SNP analysis detected HLA*DRB1, the major RA susceptibility locus ($P = .00004$), with a linkage interval of 31 cM, compared with a 50-cM linkage interval detected by the previously published 10-cM microsatellite scan in the same cohort. Moreover, four additional loci were detected at a nominal significance level ($P < .05$) in the SNP linkage analysis. The authors concluded that a dense SNP map was very suitable for performing linkage analysis in RA. This approach allowed loci to be defined more precisely.

PROGRESS FROM CANDIDATE GENES FROM PREVIOUSLY IDENTIFIED LINKAGE REGIONS IN RA

The Japanese consortium (5) set out by densely mapping candidate regions that were originally defined by whole genome scans. For the previously defined 1p36 region, the Japanese investigators reasoned that the unique specificity of anti-CCP antibodies for RA might be caused by differential citrinullation caused by mutations in enzymes involved in citrinullation. The 1p36 gene region contained all the known genes that encode peptidylarginine deiminases citrullinating (PAD) enzymes, the PAD genes (PAD types 1–4) in a region of 0.3 megabase (Mb). A case control study using in 830 RA patients and 736 healthy Japanese controls identified an association between haplotypes (combinations of SNPs on one chromosome that tend to inherit together) of the gene encoding PAD4 with increased susceptibility to RA. The difference between two haplotypic variants was four SNPs in exons, with three subsequent amino acid substitutions. The RA-susceptible PAD4 variant was shown to produce a more stable transcript than the non-susceptible variant, implying an increased production of PAD4 by the RA-susceptible variant. Circumstantial evidence for a role of PAD4 in RA was the detection of PAD4 in synovial tissue. Although

this observation may provide insight in the generation of anti-CCP antibodies with the (not yet proven suggestion) that enhanced production of citrinullated antigens leads to a higher chance of developing anti-CCP antibodies, it is not known whether this is specific for the Japanese population. Data from both France Caucasians as well as Caucasians from the UK showed no association with PAD4 haplotypes and RA (6,7). More specific data on the PAD4 (8) gene revealed that this gene is extremely variable at least in the white Caucasian population. Therefore the jury is still out whether the observed association in the Japanese population is specific for this population or whether associations exist in other populations as well. This last option is important because this may indicate that the amount of citrinullated antigens can be a new target for therapy.

With respect to the progress on the 1p36 region it is not yet known what proportion of this linkage peak can be explained by PAD4 gene variants. Another candidate gene in this region is the TNF-receptor type II gene that has a polymorphism causing the amino acid substitution M to R at position 196. Initially the UK group reported an association but in French families this association between different variants of the TNF-RII gene could only be replicated in a subset of multicase RA families (9,10). Finally by taking advantage of the spectrum of phenotypes in a large inception cohort from The Netherlands, it was found that either in RA patient that developed complete remission or in those with the worst progression to destructive disease and in healthy controls the genotype distribution was equal (11). Thus in conclusion it is most likely that variants in the TNF-RII gene are not relevant for susceptibility or severity of RA.

The HLA Class II molecules are the most powerful recognized genetic factors for RA that contributes to at least 30% of the total genetic effect. The HLA-DRB1 alleles *0101, *0102, *0401, *0404, *0405, *0408, *1001, *1402 share a conserved amino acid sequence (QKRAA, QRRAA or RRRAA) at position 70-74 in the third hypervariable region of the DRβ1 chain. These residues constitute an α-helical domain forming one side of the antigen presenting binding site. The Shared Epitope hypothesis postulates that the shared epitope motif itself is directly involved in the pathogenesis of RA by allowing the presentation of an arthritogenic peptide. Extensive evidence exists showing associations between the shared epitope encoding alleles and susceptibility to RA as well as severity of RA (12-14). Homozygosity for the shared epitope is associated with a higher risk to develop RA (15), and with more severe radiological destruction (13). Regional differences in HLA prevalence and association with RA exist. Associations between HLA-DRB1*0401 and *0404 and RA were first described in Western Americans and in the Northern Europe population. HLA-DRB1*1402 was associated with RA in Native Americans (16) and associations with HLA-DRB1*0101 and *1001 were reported in Indian and Mediterranean patients (17,18). On the other hand no associations were found in Greeks (19). Extra-articular manifestations of RA such as rheumatoid nodules are described to occur more often in shared epitope positive pa-

tients (20). Homozygosity for HLA-DRB1*0401 as well as homozygosity for two different shared epitope encoding HLA-DRB1 alleles conferred a higher risk to develop extra-articular manifestations than heterozygosity (20). A relationship of vasculitis with 3 genotypes containing a double dose of the shared epitope, specifically HLA-DRB1*0401/*0401, *0401/*0404 and *0101/*0401 has been observed as well (21).

Although it is accepted that the shared epitope encoding HLA-DRB1 alleles are associated with RA, a more controversial issue is the question whether predisposition to RA is also conferred by HLA-DQ alleles. Support for a role for HLA-DQ comes from studies in mice (22,23) and humans (24,25). The concerning HLA-DQ alleles are the DQ3 and DQ5 heterodimers. As they both are in strong linkage with some shared epitope alleles, the individual contribution of the HLA-DQ alleles is difficult to discern. Recently the HLA region has been fine-mapped (4). The highest linkage peak was located exactly at the DRB1 locus, however considering the wideness of the linkage peak haplotype associations cannot be excluded (4). Therefore, no definite evidence is available pinpointing RA susceptibility to either HLA-DR or HLA-DQ alleles.

Besides the above-mentioned predisposive effects of HLA-DRB1 alleles, there are also reports on protective effects by certain HLA-DRB1 haplotypes. These haplotypes contain, instead of the shared epitope, another common anchor-region consisting of the amino acids DERAA. The HLA-DRB1 alleles that express the DERAA sequence (DRB1*0103, *0402, *1102, *1103, *1301, *1302, *1304) have been shown to protect against RA (24-26). However, these studies have been performed with relatively few RA patients carrying the DERAA haplotype (24,25). There is also evidence that patients carrying the DERAA sequence have less erosive disease (27,28). It is not known whether the effect of the DERAA encoding HLA-DRB1 alleles is truly protective or is due to the effect of the concomitant absence of shared epitope encoding HLA-DRB1 alleles (non-predisposition). More clarity will come from currently performed studies in which a large number of patients with early RA have been followed for 4 years. As in this study subgroups of patients with the same amount of shared epitope alleles were compared, the effects of DERAA could be differentiated from non-predisposition. It was observed that the DERAA haplotype conferred a lower risk to develop RA and was associated with a lower rate of joint destruction.

PROGRESS FROM CANDIDATE GENES FROM PREVIOUSLY IDENTIFIED LINKAGE REGIONS IN AUTOIMMUNE DISEASE IN GENERAL

Linkage data to select candidate genes can be alternatively used by searching for genomic regions that overlap in the scans reported for several diseases such as arthritis, diabetes, asthma, atopic dermatitis, osteoporosis, and inflammatory bowel disease (29). One of the

regions is the 5q31.1–q33.1 region. By very dense SNP mapping using a similar strategy as the PAD4 identification in the Japanese population, the Japanese group performed linkage disequilibrium (LD) mapping using single nucleotide polymorphisms (SNPs) in a case-control approach (30). In 820 RA patients and 620 controls a risk for RA of 1.3 was identified for a risk allele in the organic cation transporter gene SLC22A4. The expression of SLC22A4 is specific to haematological and immunological tissues and SLC22A4 is highly expressed in the inflammatory joints of mice with collagen-induced arthritis. Intriguingly the identified SNP affects the transcriptional efficiency of SLC22A4 in vitro by altering the binding affinity of a haematopoietic transcription factor, called RUNX1. Next SNPs in this transcription factor were analysed as well. An association was observed with the minor allele in the RUNX1-gene conferring a small but significant risk (1.3) in the comparison between 820 RA patients and 620 controls. Intriguingly, the biological data suggest that these two SNPs would have a cumulative effect given that a transcription factor with less binding capacity has to bind to a disrupted transcription factor binding site, thus resulting in overall loss of function. Indeed in the analysis of the data from individuals who were genotyped for both SNPs (719 cases and 441 controls), it was observed that the genotype that was homozygous with respect to the susceptibility alleles of both genes showed a high odds ratio of 9 (95% confidence interval 2–39), whereas the genotype that was homozygous with respect to the susceptible allele of *SLC22A4* and heterozygous with respect to *RUNX1* showed a moderately high odds ratio of 2.5 for disease. The data have been replicated in two other diseases (psoriasis and SLE) and studies are underway in cohorts of RA patients of different ethnic background. This is a nice example how gene-gene interactions may explain complex traits while at the same time the crude OR of the gene variant for the disease is quite low.

PROGRESS FROM CANDIDATE GENES

The methodology of searching genes can be divided in the unbiased approach of linkage analysis and subsequent fine mapping. In this method a linkage hot spot is covered with a grid of markers in order to search systematically for linkage disequilibrium (LD, the non-random association of genes across the genome) and haplotypes. Next the original region is narrowed and the disease gene is identified. For multifactorial diseases like RA the power to detect a risk gene for a common disease with a relative risk of two by LD mapping necessitates data on transmission in 5000 affected and 5000 non-affected. Given the fact that these numbers are not available the choice of a candidate gene has subjective elements because the genes that are intuitively logical will be tested first (see above-mentioned examples of PAD4 and TNF-RII as candidate genes). A less biased approach is in genome-wide association studies. Patients and controls are unrelated and therefore

more recombinations have taken place, leading too much smaller regions in which non-random association of genes is present. Thus a positive result is less likely to be caused by linkage of recombining neighboring genes that explain the observed linkage pattern. The obvious curse is that selection of candidate genes is biased by limited knowledge. An interesting alternative was explored by Begovich et al. (31) who tested the association of SNPs with putative functional consequences in different sets of patients and controls. This yielded a large number of putative functional polymorphisms distributed differently in patients and controls. In a second set of 463 patients and 926 controls, all from Caucasian origin, a risk allele of a haematopoietic-specific protein tyrosine phosphatase, PTPN22 was identified in 17% of the controls and 28% of the RA patients. The risk allele changed the function of the protein that functions as negative regulator of T-cell activation, leading to T-cells with a lower threshold for T-cell activation. This mutation is apparently leading to autoimmune disease in general since this mutation also conferred risk for diabetes in both an American and an Italian population (32). These data are a nice example of the power of the technique of whole genome SNP scanning but also emphasize the power of replication to diminish the number of false positives (33). Although this approach has considerable power to detect risk genes, real-positives may be falsely excluded if risk is only conferred in the context of gene-gene interactions such as the RUNX1 pathway genes.

Progress from other candidate genes that were selected by presumed importance is still preliminary. On the telomeric portion of the HLA region is a gene located which is called "inhibitor of NF-kappa B-like gene" or I-kappa-BL. In spite of initial reports of a positive association of a SNP located at –62, a large Spanish study in two cohorts failed to detect any association (34).

Haplotypes as defined by microsatellites (the IL10R-CA repeat) of the IL10 gene has been previously associated with RA in several ethnic populations with severe RA (35). Using a SNP –A2849G that tags this haplotype, Lard et al were not able to find this association in incident cases of RA, but observed that this haplotype was associated with higher rates of joint destruction in a cohort-followed overtime as well as with higher titers of autoantibodies (36). Recent data indicate the importance of IL10 promoter haplotypes for the outcome of transplantation indicating it relevance for immune-mediated diseases (37). Moreover it has been demonstrated by a robust assay that haplotypes of the IL10 can be transcribed at a different rate implying a biological basis for the observed associations (38). However, currently the relation between the different haplotypes and susceptibility to RA is still unclear. For the association of IL10 haplotypes gene-gene interactions have been suggested by the observation that the association of IL10 genotypes is only present in female patients and not in male patients (39). Finally IL10 haplotypes were associated with treatment responses to TNF-blocking agents (40).

For a large number of genes suggested to be relevant in the pathophysiology of RA association was observed in only one study without replication such as Beta-adrenergic receptor gene SNPs, RANKL, ICAM-1, VEGF, PDCD-1 and IL1-RA gene or data were published of lack of association with RA such as CTLA-4, CCR5, Mannose-Binding-Lectin, Toll-like receptor 2 or 4 gene variants (41-50).

PROGRESS FROM PHENOTYPIC-GENOTYPIC INTERACTIONS

Apart from the relevance of gene-gene interactions, progress has been made with respect to identification of gene-environment interactions. The risk conferred by the HLA region has been put in a different perspective given the significant interaction between smoking and presence of the shared epitope (SE) of HLA-DR as risk factors for seropositive RA, but not at all for seronegative RA (51). Thus the major genetic risk factor for RA is only active in a certain subgroup of RA (RF positive), and the magnitude of this genetic risk factor is to a large extent influenced by the presence of an environmental risk factor (here smoking).

Another clear genotypic-phenotypic association was reported for the presence of CCP-antibodies and SE-HLA alleles (52). This has put into functional perspective by the observation that immunity to citrinullated antigens is influenced by the better fit of antigens after citrinullation in the HLA binding groove of the HLA-DR4 allele (one of the shared epitope alleles) (53).

CONCLUSION

The field of genetics has shown remarkable progress. For the coming years it is expected that the technical breakthroughs with respect to rapid and massive genotyping in large cohorts will lead to the further elucidation of the risk genes. It has become clear that replication of results, preferably in independent cohorts is essential for reliable data. Given the extensive collaborations that have been formed, this is not foreseen to be a major problem. The major challenges will be to identify genetic variants that confer small risks by it selves but by affecting a pathway by a number of genetic variants are of great relevance in the elucidation of the genetic causes of RA.

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Chapter 7

Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins

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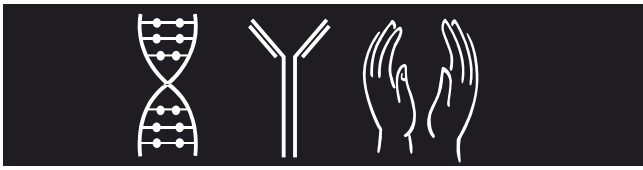

ABSTRACT

Objective. The main genetic risk factor for rheumatoid arthritis (RA), the HLA region, has been known for 25 years. Previous research has demonstrated, within the RA population, an association between HLA-DRB1 alleles carrying the shared epitope (SE) and antibodies directed against cyclic citrullinated peptides (anti-CCP antibodies). We undertook this study to make the first comparison of SE allele frequencies in the healthy population with those in RA patients who do or do not harbor anti-CCP antibodies.

Methods. HLA-DRB1 typing was performed in 408 RA patients from the Leiden Early Arthritis Clinic (the Leiden EAC; a Dutch population-based inception cohort in which disease course was followed up over time), in 423 healthy Dutch controls, and in 720 affected members of 341 US multiplex (sibpair) families of Caucasian origin from the North American RA Consortium (NARAC) with well-established disease and fulfilling the American College of Rheumatology classification criteria for RA. The presence of anti-CCP antibodies was determined by enzyme-linked immunosorbent assay.

Results. For the Leiden EAC, the odds ratio (OR) describing the association of 2 copies of the SE allele with anti-CCP positivity (using no copies of the SE allele in the healthy control group as the referent) was 11.8 ($P < 0.0001$), while the OR for 1 SE allele was 4.4 ($P < 0.0001$). No association with the SE was observed in the Dutch anti-CCP-negative RA patients. For the NARAC families, linkage and association analysis revealed the SE to be associated only with anti-CCP-positive disease and not with anti-CCP-negative disease. Stratified analyses indicated that anti-CCP antibodies primarily mediated association of the SE with joint damage or disease persistence.

Conclusion. HLA-DRB1 alleles encoding the SE are specific for disease characterized by antibodies to citrullinated peptides, indicating that these alleles do not associate with RA as such, but rather with a particular phenotype.

INTRODUCTION

A definition of the rheumatoid arthritis (RA) phenotype has been developed through consensus procedures. The current RA classification criteria were developed by expert clinicians who examined the features of "classic" RA cases and analyzed their sensitivity and specificity using clinical judgment as the gold standard. As is the case with most complex disorders defined by consensus criteria, there is large variation in the phenotype. Despite the phenotypic heterogeneity encompassed by this disease definition, the genetic contribution is estimated to be 50-60% (1), with the HLA region having the largest impact on genetic risk. In particular, HLA-DRB1 alleles encoding a common amino acid sequence (the shared epitope SE) in the third hypervariable region of the DRB1 molecule have been identified as risk alleles for RA (2). The functional significance implied by the location of this SE sequence along the rim of the peptide-binding groove has stimulated efforts to search for the putative RA antigen.

A considerable proportion of RA patients have autoantibody responses, especially rheumatoid factor (RF). More recently, another autoantibody response has received much attention. These antibodies are directed against cyclic citrullinated peptides (CCPs) and are highly specific for RA (3,4). Intriguingly, these antibodies may be detected years before disease onset (5,6), are stable over time (7), and are associated with joint destruction, the hallmark of RA (8,9).

The immune response to citrullinated antigens may occur in the context of the SE, because conversion of arginine to citrulline at the peptide side-chain position interacting with the SE significantly increases peptide-major histocompatibility complex affinity and leads to the activation of CD4-positive T cells in mice transgenic for one of the SE alleles (10). Previous research has demonstrated, within the RA population, an association between HLA alleles and anti-CCP antibodies, but no comparison has been made between the HLA profile in the healthy population and that in RA patients who do or do not produce anti-CCP antibodies (8,9,11). Such a comparison may be important, since different genetic contributions associating with different phenotypes point to different etiopathologies.

PATIENTS AND METHODS

Patients

Four hundred eight Dutch RA patients were participants in the Leiden Early Arthritis Clinic (EAC), a population-based inception cohort described previously in detail (12). All RA patients fulfilled the 1987 revised criteria of the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) (13) and were of Caucasian

origin. The study protocol included collection of serologic, radiographic, and clinical data upon study entry and yearly thereafter. Informed patient consent was obtained, and the local medical ethics committee approved the study. Radiographs of the hands and feet were obtained for all EAC patients at baseline and at each subsequent annual visit and were scored for damage using the modified Sharp/van der Heijde method (14). Controls consisted of a random panel of 423 healthy unrelated Dutch individuals.

Patients with RA who had clinical remission of disease were defined as follows: at cohort entry they had 1) symmetric arthritis of the small joints, 2) morning stiffness of at least 1 hour duration, 3) a diagnosis of definite or probable RA according to the ACR criteria, and 4) they had achieved long-term and complete remission of disease (no signs of arthritis in the absence of disease-modifying drugs). Patients were not included in the remission group when their disease had been in remission for <1 year without the use of disease-modifying drugs. All other patients were classified as having persistent RA.

The US sample consisted of multiplex (sibpair) families of Caucasian origin from the North American RA Consortium (NARAC) with well-established disease and fulfilling the ACR classification criteria, as described previously (15). Radiographs of the hands and feet of all affected individuals were obtained at the time of study entry, unless films obtained within 2 years prior to entry were available for review. To document the presence of erosive disease, all radiographs were read by a single radiologist who was blinded to the patients' clinical and genetic information.

Serology and genotyping

Anti-CCP titers were determined based on a second-generation enzyme-linked immunosorbent assay (ELISA) (either the Immunoscan RA Mark 2 [Euro-Diagnostica, Arnhem, The Netherlands], in the case of the Leiden EAC, or an ELISA manufactured by Inova Diagnostics [San Diego, CA], in the case of the NARAC). HLA-DRB1 typing and subtyping were performed using polymerase chain reaction-based methods. The following alleles were classified as SE positive: DRB1*0101, *0102, *0104, *0401, *0404, *0405, *0408, *0413, *0416, *1001, and *1402 (2). Genotyping of NARAC families for 27 microsatellite markers on chromosome 6 was performed using markers from the Marshfield set8A combo list (available online at http://www.marshfieldclinic.org/research/genetics/sets/combo.html) with additional markers in the HLA complex, as described previously (15).

Statistical analysis

Nonparametric linkage analysis was performed using the Multipoint Engine for Rapid Likelihood INference (MERLIN) statistical package (16). Analysis of the anti-CCP-positive families (575 affected members in 271 families comprising 317 sibpairs) was restricted to anti-CCP-positive siblings (i.e., anti-CCP-negative siblings were excluded). Analysis of the anti-CCP-negative families (145 affected members in 70 families comprising 75 sibpairs)

was restricted to anti-CCP-negative patients and their siblings with negative or low titers of anti-CCP antibodies (<49), provided that at least 1 sibling in the family had anti-CCP-negative disease. Chi-square tests and odds ratios (ORs) were used to assess the statistical significance and magnitude of associations for categorical outcomes.

With regard to the issue of multiple testing, statistical significance levels accounted for the fact that we examined the following 4 hypotheses in this study: 1) whether the SE and anti-CCP antibodies are associated, 2) whether the SE and anti-CCP antibodies are associated differently in RA patients and in controls, 3) whether the SE and anti-CCP antibodies are associated with remission, and 4) whether the SE and anti-CCP antibodies are associated with joint destruction. Although one may argue that these 4 hypotheses are probably not completely independent based on previous data, we considered results to be statistically significant if the *P* values were less than or equal to 0.01.

RESULTS

As shown in Table 1, the presence of the SE allele was strongly associated with anti-CCP positivity. There was also evidence of a dose effect, with increasing copies of the SE allele being associated with increasing risk for RA. For the Leiden EAC, the OR describing the association of 2 copies of the SE allele with anti-CCP positivity (using no copies of the SE allele in the healthy control group as the referent) was 11.8 ($P < 0.0001$), while the OR for 1 SE allele was 4.4 ($P < 0.0001$). When no copies of the SE allele in the EAC was used as the referent, the OR describing the association of 2 copies of the SE allele with anti-CCP positivity was 8.6 ($P < 0.0001$), while the OR for 1 SE allele was 3.4 ($P < 0.0001$). Interestingly, however, no association with the SE was observed in the Dutch anti-CCP-negative RA patients (for 2 copies of the SE allele versus healthy Dutch controls, OR 1.4, $P = 0.3$; for 1 copy of the SE allele versus healthy Dutch controls, OR 1.3, $P = 0.2$), indicating that the SE does not associate with RA as such, but rather with a defined anti-CCP phenotype.

Table 1. Distribution of SE and anti-CCP positivity*

		Dutch EAC RA patients			
		Anti-CCP positive (n=195)		Anti-CCP negative (n=213)	
SE	Dutch controls (n=423), no (%)	No (%)	OR (95% CI)	No (%)	OR (95% CI)
+/+	26 (6)	49 (25)	11.79 (6.58-21.13)	16 (8)	1.38 (0.71-2.67)
+/–	153 (36)	107 (55)	4.37 (2.88-6.65)	88 (41)	1.29 (0.91-1.82)
–/–	244 (58)	39 (20)	1.0	109 (51)	1.0

***** The following alleles were classified as shared epitope (SE) positive: DRB1*0101, *0102, *0104, *0401, *0404, *0405, *0408, *0413, *0416, *1001, and *1402 (4). EAC = Early Arthritis Clinic; RA = rheumatoid arthritis; CCP = cyclic citrullinated peptide; OR = odds ratio; 95% CI = 95% confidence interval.

Other autoantibodies such as IgM RF have been reported to be associated with the SE as well. However, a comparison of the distribution of copies of the SE allele among RA patients who were RF negative and anti-CCP positive (10 SE+/+ patients, 21 SE+/- patients, 4 SE-/- patients) with the distribution among those who were RF positive and anti-CCP negative (4 SE+/+ patients, 11 SE+/- patients, 22 SE-/- patients) yielded a strikingly significant difference ($P < 0.0001$). Thus, the SE appears to be associated primarily with anti-CCP autoantibodies, but not with RF autoantibodies.

Analysis of NARAC families revealed a strong association between the SE and anti-CCP positivity. Specifically, the OR describing the association of 2 copies of the SE allele with anti-CCP positivity was 7.7 ($P < 0.0001$), and the OR for 1 SE allele was 3.5 ($P < 0.0001$), both relative to NARAC patients with no copies of the SE allele.

Results of nonparametric linkage analysis in NARAC families highlighted the strong relationship between the HLA region and anti-CCP positivity. Figure 1 shows the linkage analysis for 27 microsatellite markers on chromosome 6 among 720 affected members of 341 families. Among the 271 anti-CCP-positive families, the maximum logarithm of odds (LOD) score in the region was 10.63, in contrast to the maximum LOD score of 1.12 among the 70 anti-CCP-negative families. Anti-CCP-negative families were defined by anti-CCP-negative patients and their siblings with negative or low titers of anti-CCP antibodies (<49), provided that at least 1 sibling in the family had anti-CCP-negative disease. Therefore, sensitivity analyses employing stricter definitions of anti-CCP-negative families (e.g., all affected individuals within a family must be anti-CCP negative) were performed, and these revealed a similar pattern (data not shown). The level of 49 was chosen due to the fact that titers in the range of 25-49 are considered intermediate based on analyses of sensitivity versus specificity (3,17).

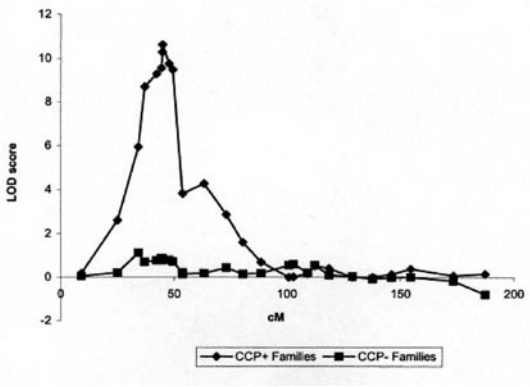

Figure 1. Results of nonparametric linkage analysis of chromosome 6 in anti-CCP-positive and anti-CCP-negative Caucasian multiplex families with well-established rheumatoid arthritis. The 2 curves correspond to anti-CCP-positive families (575 affected members of 271 families) and anti-CCP-negative families (145 affected members of 70 families). LOD = logarithm of odds.

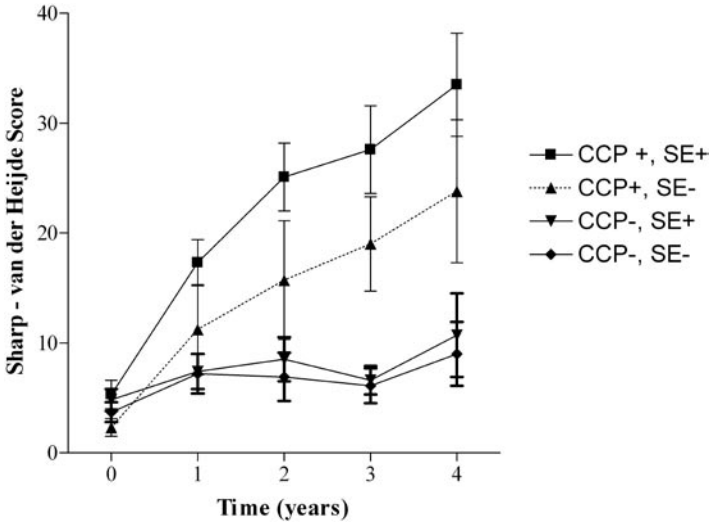

Figure 2. Progression of joint damage (in Sharp/van der Heijde units) according to the presence of the SE and anti-CCP antibodies. 74 patients were anti-CCP positive and SE positive, 18 were anti-CCP positive and SE negative, 30 were anti-CCP negative and SE positive, and 34 were anti-CCP negative and SE negative. Radiographs of the hands and feet were obtained at baseline and at each subsequent annual visit and were scored for damage using the modified Sharp/van der Heijde method. Shown are mean ± SEM.

The prospective design of the EAC cohort allowed us to examine the relative contributions of anti-CCP status and the SE to the rate of joint destruction (Figure 2). Large differences were observed between anti-CCP-positive and anti-CCP-negative patients. No apparent association was observed between SE positivity and progression of joint damage in anti-CCP-negative patients. In contrast, radiographic severity scores were higher among anti-CCP-positive patients who were SE positive than among those who were SE negative. Similar results were observed for the second hallmark of RA, chronic inflammation. The presence of anti-CCP antibodies yielded an OR of 9.5 ($P < 0.0001$) for persistent disease compared with RA patients who had clinical remission of their disease. In stratified analyses of the NARAC population, the number of copies of the SE allele was associated with the presence of erosive disease among patients with anti-CCP-positive disease ($P < 0.02$), but not among those with anti-CCP-negative disease.

DISCUSSION

Defining the phenotype for multifactorial diseases often occurs through consensus procedures. The current RA classification criteria were developed by expert clinicians who examined the features of "classic" RA cases and analyzed their sensitivity and specificity using clinical judgment as the gold standard. It is anticipated that phenotype definitions

based on specific biologic characteristics such as anti-CCP antibody production will facilitate the search for genetic risk factors, leading to greater understanding of underlying disease mechanisms.

It has been previously reported that the SE correlates strongly with another autoantibody, RF (18). Given the fact that RF is less specific for RA than anti-CCP, it is not surprising that the SE was more strongly associated with anti-CCP than with RF. Indeed, comparison of the distribution of copies of the SE allele among RF-negative, anti-CCP-positive patients with the distribution of copies of the SE allele among RF-positive, anti-CCP-negative patients revealed a much stronger association of the SE with anti-CCP antibodies. Although anti-CCP autoantibody titers were not available for the healthy controls in the current study, many previous studies have documented the absence of anti-CCP antibody production among healthy individuals (3-8,9). Further, previous research indicates that a double dose of the SE allele is relatively rare among controls. Finally, even among RA patients with a double dose of the SE allele, approximately one-third was anti-CCP negative. Thus, it is highly unlikely that anti-CCP antibodies are present in healthy controls.

Another intriguing observation is that the proportion of anti-CCP-positive RA patients is clearly less in inception cohorts with incident cases of arthritis than in groups of RA patients who have been selected for chronic, well-established disease (2,5,8), as was true for NARAC patients. Our analysis demonstrated differences in disease course for anti-CCP-positive disease compared with anti-CCP-negative disease. The strong tendency to achieve longstanding remission in anti-CCP-negative disease is compatible with the fact that cohorts of patients with longstanding disease are selected for anti-CCP positivity. The phenotypic data on joint destruction are compatible with the hypothesis that the presence of the SE is not only a risk factor for anti-CCP-positive disease but is also associated with more destructive disease. Although the current study had insufficient statistical power to examine associations of the SE with the rate of joint destruction among subgroups defined according to anti-CCP antibody production, this was due in part to the low rate of joint destruction in anti-CCP-negative patients. Thus, additional studies will be required to more definitively address this question.

Prior to this study, we defined 4 hypotheses to be tested and conservatively considered them as independent tests, and we therefore used a threshold for statistical significance of $P \leq 0.01$. Although one may argue that this threshold is too conservative, this decision did not alter the main findings in this study, since most of the results were associated with P values that were either much smaller than 0.01 or much larger than 0.05.

A strength of the current study was the demonstration of an association between the SE and anti-CCP-positive RA in 2 independent cohorts and using 2 analytic methods, association and linkage. Moreover, a recent study of juvenile RA demonstrated that anti-CCP-positive patients were 5 times more likely to be DR4 positive compared with anti-CCP-negative patients. Intriguingly, a strong association was observed between HLA-DR4

and the presence of anti-CCP antibodies in healthy children as well (4 of 688 children tested positive for anti-CCP antibodies, and all 4 children carried the DR4 allele) (19). Unfortunately, HLA typing in that study did not allow for determination of SE status, but those data also support the theory that a better fit in the SE of citrullinated antigens can give rise to the anti-CCP antibody response.

In summary, we propose that refinement of the RA phenotype (as defined by the ACR criteria) to "disease characterized by the presence of antibodies to citrullinated peptides" will facilitate the search for the mechanisms underlying the pathophysiology of the disease. Indeed, despite the fact that the most prominent genetic risk factor for RA has been known for 25 years, our current data illustrate that the SE is not associated with disease in a substantial proportion of RA patients, but rather that it is associated with disease in RA patients who have anti-CCP antibodies. Thus, our data suggest the presence of distinct pathways underlying disease induction/progression in anti-CCP-positive and anti-CCP-negative RA, since these 2 phenotypes exhibit different genetic associations.

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Chapter 8

HLA-DR3 is associated with anti-CCP antibody negative rheumatoid arthritis

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ABSTRACT

Objectives. Recent data have shown that the most prominent and longest known genetic risk factors for rheumatoid arthritis (RA), HLA-DRB1 shared epitope alleles, are only associated with RA that is characterized by the presence of antibodies against cyclic citrullinated peptide (anti-CCP antibodies) and not with anti-CCP-negative RA. We undertook this study to investigate whether anti-CCP-negative RA is associated with other HLA-DRB1 alleles.

Methods. HLA typing was performed for 377 patients from the Leiden Early Arthritis Clinic who were diagnosed as having RA within the first year of followup (206 anti-CCP-positive patients and 171 anti-CCP-negative patients), 235 patients who, after 1 year, had undifferentiated arthritis (UA) (28 anti-CCP-positive patients and 207 anti-CCP-negative patients), and 423 healthy control subjects. Odds ratios (ORs) with 95% confidence intervals (95% CIs) for HLA-DRB1 allele frequencies were determined for all patient groups compared with the healthy control group.

Results. HLA-DR3 was more frequently present in the anti-CCP-negative RA group than in the control group (OR 1.84, 95% CI 1.26-2.67). This was not the case for anti-CCP-positive RA (OR 0.92, 95% CI 0.60-1.40). HLA-DR3 was also more frequently present in anti-CCP-negative UA patients (OR 1.59, 95% CI 1.10-2.28), but not in anti-CCP-positive UA patients (OR 0.68, 95% CI 0.17-1.92).

Conclusions. HLA-DR3 is associated with anti-CCP-negative arthritis and not with anti-CCP-positive arthritis. These data show that distinct genetic risk factors are associated with the presence of anti-CCP antibodies in RA and indicate that different pathogenetic mechanisms underlie anti-CCP-positive and anti-CCP-negative RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a multifactorial autoimmune disease with a complex genetic background. As in other autoimmune diseases, an association between RA and the HLA complex has long been observed in many different populations and is thought to account for approximately one-third of the genetic component of RA susceptibility (1). There is extensive evidence for the association between certain frequently occurring HLA-DRB1 alleles, the so-called "shared epitope" (SE)-encoding alleles (DRB1*0101, *0102, *0104, *0401, *0404, *0405, *0408, *0413, *0416, and *1001), and susceptibility to RA (2). These SE alleles encode for a common amino acid sequence in the third hypervariable region of the DRB1 molecule (QKRAA, QRRAA, or RRRAA).

In recent years, many studies on antibodies against cyclic citrullinated peptide (CCP) showed that these antibodies are highly specific and predictive for RA (3), that they can be detected years before onset (4), and that they are associated with joint destruction (5). Furthermore, the presence or absence of these antibodies seems to be a stable trait (6). Anti-CCP antibodies are detected in SE-positive as well as in SE-negative RA patients. Carriership of SE alleles in RA is associated with the presence of anti-CCP antibodies (7). Interestingly, when we recently compared anti-CCP-positive and anti-CCP-negative RA patients with healthy controls, we found that HLA-DRB1 alleles encoding the SE were only associated with RA in the presence of anti-CCP antibodies and were not associated with anti-CCP-negative RA (8). These data indicate that the SE-encoding alleles are not associated with RA as such, but rather with anti-CCP-positive RA. These observations indicate that distinct phenotypic manifestations of the disease are associated with distinct genetic risk factors. They also raise the question of whether anti-CCP-negative RA is associated with HLA-DRB1 alleles other than SE-encoding alleles. We therefore investigated the possible associations of particular HLA-DRB1 alleles with anti-CCP-positive RA and anti-CCP-negative RA. To verify the results and to study whether the results were specific for RA, we also performed the same analysis in a group of patients with undifferentiated arthritis (UA).

PATIENTS AND METHODS

Study population

In 1993 an Early Arthritis Clinic (EAC) was started at the Department of Rheumatology of the Leiden University Medical Center, as described previously (9). The population studied here consisted of 377 patients who, within the first year of follow-up, fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for RA (10) and 235 patients who, after 1 year of follow-up, could

not be properly classified according to one of the ACR criteria sets and were therefore categorized as having UA. For every patient within the cohort, routine diagnostic laboratory screening was performed, including measurements of IgM-rheumatoid factor (IgM-RF). Informed patient consent was obtained, and the study was approved by the local medical ethics committee. Four hundred twenty-three healthy Dutch individuals served as controls. The control subjects were normal healthy donors of both sexes who were randomly selected and were ages 55 years and younger.

HLA genotyping

HLA class II alleles were determined in all patients and controls. The HLA-DRB1 (sub)typing was performed by polymerase chain reaction using specific primers and hybridization with sequence-specific oligonucleotides.

Anti-CCP autoantibodies

Serum antibodies directed against CCP were assessed with a commercial enzyme-linked immunosorbent assay (Immunoscan RA, Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). Anti-CCP antibodies were measured in serum collected within 4 months after the first visit (94%) or, when serum was not available within this time period, in the first stored serum sample available thereafter.

Statistical analysis

Odds ratios (ORs) were calculated using the Epi Info Statcalc computer program (Centers for Disease Control and Prevention, Atlanta, GA) to compare HLA-DR allele frequencies between the patient groups and the control population. ORs were reported with 95% confidence intervals (95% CIs), which excluded the value of 1 in case of statistical significance. Exact confidence limits were used as described by Mehta et al (11). For HLA-DR3, both allele frequencies and genotype frequencies were compared between the patient groups and the control population using the same methods described above.

RESULTS

To find possible associations of HLA-DRB1 alleles with anti-CCP-positive or anti-CCP-negative RA, we analyzed HLA-DRB1 allele frequencies and the presence of anti-CCP antibodies in 377 RA patients of the Leiden EAC. Two hundred six of the RA patients had anti-CCP antibodies and 171 were anti-CCP negative. Other patient characteristics are presented in Table 1. After we determined HLA-DRB1 allele frequencies, we calculated ORs and 95% CIs for both patient groups compared with a control group of 423 healthy individuals (see Table 4).

Table 1. Baseline characteristics of the 377 rheumatoid arthritis (RA) patients and 235 undifferentiated arthritis (UA) patients within the study.

	RA	UA
Age, mean (range) years	57 (14-92)	48 (16-88)
Female, %	66	58
IgM rheumatoid factor positive, %	55	14
Anti-CCP* antibody positive, %	55	12
Presence of erosions on radiographs of hands and feet**, %	35	18
Duration of symptoms, median (range) weeks	19 (0.6–104)	23 (0.14-104)

* CCP = cyclic citrullinated peptide
** radiological data were available for 58% of the 377 RA patients and for 51% of the 235 UA patients

Table 2. Association of HLA-DR3 alleles with anti-CCP positive or negative rheumatoid arthritis (RA) and undifferentiated arthritis (UA)

DR3	**anti-CCP+ RA N=206**		**anti-CCP- RA N=171**		**anti-CCP+ UA N=28**		**anti-CCP- UA N=207**		**Co N=423**
	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)
Genotype frequency									
DR3/DR3	0 (0)	-	5 (2.9)	3.64 (0.77-18.62)	0 (0)	-	4 (1.9)	2.3 (0.4-12.52)	4 (0.9)
DR3/x	39 (18.9)	1.02 (0.65-1.59)	49 (28.7)	1.83 (1.18-2.82)	4 (14.3)	0.73 (0.18-2.21)	55 (26.6)	1.62 (1.07-2.45)	78 (18.4)
x/x	167 (81.1)	1.0	117 (68.4)	1.0	24 (85.7)	1.0	148 (71.5)	1.0	341 (80.6)
DR3/DR3 or DR3/x	39 (18.9)	0.97 (0.62-1.51)	54 (31.6)	1.92 (1.25-2.92)	4 (14.3)	0.69 (0.17-2.10)	59 (28.5)	1.66 (1.10-2.48)	82 (19.4)
Allele frequency	39 (9.5)	0.92 (0.60-1.40)	59 (17.3)	1.84 (1.26-2.67)	4 (7.1)	0.68 (0.17-1.9)	63 (15)	1.59 (1.10-2.28)	86 (10.2)

RA= rheumatoid arthritis; UA= undifferentiated arthritis; Co=controls; CCP= cyclic citrullinated peptide; OR= odds ratio; 95% CI= 95% confidence interval.
ORs were calculated comparing double-dose DR3 carriers (DR3/DR3), single-dose DR3 carriers (DR3/x) or at least single-dose DR3 carriers with carriers of no DR3 alleles (x/x) in patients versus controls and comparing allele frequencies in all groups versus controls.

As described previously, the SE alleles DRB1*0401, *0404, *0405, and *0408 were associated with predisposition for anti-CCP-positive RA, as were DR9 and DR10. Interestingly, HLA-DR3 was associated only with predisposition for anti-CCP-negative RA (OR 1.84, 95% CI 1.26-2.67) (Table 2). This association was not found for anti-CCP-positive RA, indicating that HLA-DR3 is only associated with RA in the absence of anti-CCP antibodies.

To confirm these findings in another group of patients and to address the question of whether the association is only found in anti-CCP-negative RA or whether it is also present in another form of arthritis, we also analyzed the association of HLA-DR3 with UA. In a group of 235 patients who, 1 year after their first visit, were categorized as having

Table 3. HLA-DR3 allele frequencies in rheumatoid arthritis with and without anti-CCP antibodies and IgM-RF.

Population	HLA-DR3 allele frequency		
	N (DR3 alleles/ total alleles)	%	OR (95% CI)
RA			
anti-CCP + ; RF +	32 / 342	9.4	0.91 (0.58-1.42)
anti-CCP + ; RF -	7 / 70	10.0	0.98 (0.37-2.24)
anti-CCP – ; RF +	16 / 74	21.6	2.44 (1.25-4.53)
anti-CCP – ; RF –	43 / 268	16.0	1.69 (1.11-2.54)
Controls	86 / 846	10.2	1.0

RA= rheumatoid arthritis; CCP= cyclic citrullinated peptide; RF= rheumatoid factor; OR= odds ratio; 95% CI= 95% confidence interval

ORs were calculated comparing allele frequency of the diseased group with allele frequency in the healthy control group

UA (Table 2), HLA-DR3 was more frequently present in anti-CCP-negative patients (n = 207) than in healthy controls (OR 1.59, 95% CI 1.10-2.28), suggesting that HLA-DR3 is not specifically associated with anti-CCP-negative RA, but rather with anti-CCP-negative arthritis. No association was observed between HLA-DR3 and anti-CCP-positive UA (n = 28) (OR 0.68, 95% CI 0.17-1.92) (Table 2). Analysis of HLA-DR3 allele frequencies in UA patients thus confirmed the results found in RA patients and indicated that association with HLA-DR3 also occurs in anti-CCP-negative UA. Analysis of whether HLA-DR3 is associated with development of RA in patients who presented initially with anti-CCP-negative UA did not show that HLA-DR3 increased the risk for developing RA (data not shown), indicating that HLA-DR3 is not a prognostic risk factor for the development of RA in this group of patients.

Since the presence of anti-CCP antibodies is linked to the presence of RF and since RF is associated with the SE (12), we next compared the frequencies of HLA-DR3 in IgM-RF-positive and IgM-RF-negative RA patients with that in controls. Indeed, HLA-DR3 was also associated with RF-negative RA (OR 1.66, 95% CI 1.13-2.43). However, after dividing the study population into those with and without anti-CCP antibodies, the association was lost for the anti-CCP-positive group (Table 3). Anti-CCP-negative, RF-positive RA patients and anti-CCP-negative, RF-negative RA patients both harboured HLA-DR3 significantly more frequently than did control subjects (OR 2.44, 95% CI 1.25-4.53 and OR 1.69, 95% CI 1.11-2.54, respectively). In contrast, anti-CCP-positive, RF-positive RA patients and anti-CCP-positive, RF-negative RA patients did not (OR 0.91, 95% CI 0.58-1.42 and OR 0.98, 95% CI 0.37-2.24, respectively) (Table 3). These data indicate that anti-CCP status, rather than RF status, is the predominant disease trait associated with HLA-DR3.

Table 4. HLA-DRB1 allele frequencies in 206 anti-CCP positive RA patients, 171 anti-CCP negative RA patients and 423 healthy controls.

DRB1	anti-CCP positive RA (N=412)			anti-CCP negative RA (N=342)			Controls (N=846)	
	N	%	OR (95% CI)	N	%	OR (95% CI)	N	%
DR1								
01(00)	33	8.0		31	9.1		6	0.7
0101	28	6.8		13	3.8		92	10.9
0102	0	0		3	0.9		2	0.2
0103	0	0		1	0.3		1	0.1
Total	*61*	*14.8*	*1.28 (0.89-1.83)*	*48*	*14.0*	*1.20 (0.81-1.76)*	*101*	*11.9*
DR3								
03(00)	0	0		0	0		2	0.2
0301/ [a]	9	2.2		5	1.5		0	0
0301	30	7.3		53	15.5		84	9.9
0302/ [b]	0	0		1	0.3		0	0
Total	*39*	*9.5*	*0.92 (0.60-1.40)*	*59*	*17.3*	***1.84 (1.26-2.67)***	*86*	*10.2*
DR4								
04(00)	9	2.2		2	0.6		0	0
0401	84	20.4	**2.56 (1.80-3.63)**	33	9.6	1.07 (0.67-1.66)	77	9.1
0402	0	0		1	0.3		2	0.2
0403	2	0.5	0.34 (0.04-1.53)	2	0.6	0.41 (0.04-1.85)	4	0.5
0403/ [c]	0	0		0	0		8	0.9
0404/ [d]	0	0		0	0		18	2.1
0404	25	6.1	**3.02 (1.69-5.49)**	12	3.5	1.89 (0.89-3.94)	2	0.2
0408	7	1.7		3	0.9		3	0.4
0405	5	1.2	**10.4 (1.15-492)**	0	0	-	1	0.1
0406	1	0.2		0	0		0	0
0407	1	0.2		4	1.2		8	0.9
Total	*134*	*32.5*	***2.83 (2.12-3.79)***	*57*	*16.7*	*1.18 (0.82-1.67)*	*123*	*14.5*
DR7								
07(00)	6	1.5		5	1.5		91	10.8
0701	28	6.8		27	7.9		0	0
Total	*34*	*8.3*	*0.75 (0.48-1.14)*	*32*	*9.4*	*0.86 (0.54-1.33)*	*91*	*10.8*
DR8								
08(00)	2	0.5		2	0.6		16	1.9
0801	3	0.7		1	0.3		10	1.2
0803	0	0		0	0		1	0.1
Total	*5*	*1.2*	***0.37 (0.11-0.99)***	*3*	*0.9*	***0.27 (0.05-0.88)***	*27*	*3.2*
DR9								
09(00)	2	0.5		1	0.3		10	1.2
0901	11	2.7		5	1.5		0	0
Total	*13*	*3.2*	***2.72 (1.09-7.00)***	*6*	*1.8*	*1.49 (0.44-4.57)*	*10*	*1.2*
DR10								
10(00)	8	1.9		2	0.6		4	0.5
1001	12	2.9		1	0.3		0	0
Total	*20*	*4.9*	***10.7 (3.56-43.4)***	*3*	*0.9*	*1.86 (0.27-11.1)*	*4*	*0.5*

DRB1	anti-CCP positive RA (N=412)			anti-CCP negative RA (N=342)			Controls (N=846)	
	N	%	OR (95% CI)	N	%	OR (95% CI)	N	%
DR11								
11(00)	8	1.9		6	1.8		5	0.6
1101/ [e]	2	0.5		6	1.8		79	9.3
1101	8	1.9	**0.29 (0.14-0.55)**	6	1.8	**0.41 (0.21-0.75)**	0	0
1104	2	0.5		2	0.6		0	0
1102/ [f]	0	0	0.34 (0.01-2.82)	1	0.3	1.24 (0.20-5.84)	6	0.7
1102	1	0.2		2	0.6		0	0
Total	*21*	*5.1*	***0.45 (0.26-0.75)***	*23*	*6.7*	***0.61 (0.36-0.99)***	*90*	*10.6*
DR12								
12(00)	6	1.5		4	1.2		7	0.8
1201	1	0.2		1	0.3		16	1.9
1202	0	0		1	0.3		4	0.5
Total	*7*	*1.7*	*0.52 (0.19-1.25)*	*6*	*1.8*	*0.54 (0.18-1.36)*	*27*	*3.2*
DR13								
13(00)	0	0		3	0.9		6	0.7
1301/ [g]	2	0.5		9	2.6		116	13.7
1301	4	1.0		13	3.8		0	0
1302	13	3.2		14	4.1		1	0.1
1303	2	0.5		4	1.2		3	0.4
Total	*21*	*5.1*	***0.31 (0.18-0.50)***	*43*	*12.6*	*0.82 (0.55-1.20)*	*126*	*14.9*
DR14								
14(00)	6	1.5		9	2.6		0	0
1401	0	0		1	0.3		27	3.2
1404	1	0.2		0	0		0	0
Total	*7*	*1.7*	*0.52 (0.19-1.25)*	*10*	*2.9*	*0.91 (0.39-1.97)*	*27*	*3.2*
DR15								
15(00)	33	8.0		33	9.6		128	15.1
1501	16	3.9		17	5.0		1	0.1
Total	*49*	*11.9*	*0.75 (0.52-1.08)*	*50*	*14.6*	*0.95 (0.65-1.37)*	*129*	*15.2*
DR16								
16(00)	1	0.2		2	0.6		5	0.6
Total	*1*	*0.2*	*0.41 (0.01-3.68)*	*2*	*0.6*	*0.99 (0.09-6.08)*	*5*	*0.6*
Total alleles	412	100		342	100		846	100

RA= rheumatoid arthritis; UA= undifferentiated arthritis; CCP= cyclic citrullinated peptide; OR= odds ratio; 95% CI= 95% confidence interval.

ORs were calculated comparing allele frequencies of the diseased group with allele frequencies in the healthy control group. HLA typings without subtyping are presented as x(00). Subtypings without conclusive result are presented as

[a] 0301/ for subtyping HLA-DRB1*0301, *0304 or *0305; [b] 0302/ for subtyping HLA-DRB1 *0302 or *0303 or *0307; [c] 0403/ for subtyping HLA-DRB1*0403, *0406 or *0407; [d] 0404/ for subtyping HLA-DRB1*0404, *0408 or *0419; [e] 1101/ for subtyping HLA-DRB1*1101 or *1104; [f] 1102/ for subtyping HLA-DRB1*1102 or *1103; [g] 1301/ for subtyping HLA-DRB1*1301 or *1302.

DISCUSSION

The data presented herein show that distinct genetic risk factors are associated with distinct subtypes of RA as defined by the presence of anti-CCP antibodies. SE-encoding alleles are associated with anti-CCP-positive RA and not with anti-CCP-negative disease. In contrast, anti-CCP-negative disease is associated with HLA-DR3, while this association is not found in anti-CCP-positive RA. Although HLA-DR3 or SE expression is not required for the development of anti-CCP-negative or anti-CCP-positive RA, respectively, our findings are important because they indicate that distinct pathogenic mechanisms may underlie anti-CCP-positive and anti-CCP-negative RA.

In a previous study of a relatively small number of patients (n = 44), it was found that HLA-DR3 frequencies in RA patients differ from the frequencies observed in controls (13). Likewise, in a group of 85 Arab RA patients, HLA-DR3 conferred a risk for RA susceptibility (14), and a more recent study showed a significantly increased frequency of HLA-DR3 in patients with synovitis of recent onset (15). In a Caucasian population of 167 RA patients, an association with HLA-DR3 was observed after excluding SE alleles from the analysis (16). Our findings represent an extension of those findings by establishing that HLA-DR3 is associated only with a particular subset of RA. Our analysis also confirmed that particular HLA-DRB1 alleles, such as DR8, DR11, and DR13, protect against RA (17). We now show that these alleles tend to be associated with protection against both anti-CCP-positive and anti-CCP-negative disease. Therefore, our data indicate that the HLA alleles conferring protection do so independently of the anti-CCP status, while the alleles that predispose to RA are associated with distinct RA phenotypes (anti-CCP-positive or anti-CCP-negative RA).

It is debatable, however, whether the association of HLA-DR3 with anti-CCP-negative RA (and UA) is attributable to the HLA-DR3 gene itself. Other genetic factors in high linkage disequilibrium with HLA-DR3 may also underlie the observed association. HLA-DR3 is known to be part of a conserved ancestral haplotype (A1;B8;DRB1*03, also known as the 8.1 haplotype (18) that occurs frequently in Caucasian individuals and has been reported to be associated with RA (19). Jawaheer et al described an additional genetic risk factor present within the major histocompatibility complex (MHC) that is part of this conserved haplotype (20). Their finding concerned a certain allelic combination of tumor necrosis factor (TNF) polymorphisms and another polymorphism on the HLA class III region of chromosome 6. Likewise, the class III MHC TNF-lymphotoxin region was described as appearing to influence susceptibility to RA separately from the HLA-DR region (21), and a microsatellite marker (MIB*350) that is also part of an ancestral haplotype associated with DRB1*0301 was described as a risk factor for RA independently of DRB1*0301 (22).

In summary, HLA-DR3 is associated with anti-CCP-negative RA and UA and not with anti-CCP-positive RA or UA. The data presented herein indicate that separate genetic risk

factors are associated with different phenotypes, which suggests that various pathogenetic mechanisms underlie anti-CCP-positive and anti-CCP-negative disease.

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Chapter 9

Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients that carry HLA-DRB1 shared epitope alleles

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ABSTRACT

Objectives. To study the gene-environment interaction between tobacco-exposure (TE) and shared-epitope (SE) alleles on autoantibodies in rheumatoid arthritis (RA) and undifferentiated arthritis (UA).

Methods. From incident cases of arthritis (n=1305), patients that did not fulfill any classification criteria at the two weeks visit, UA (n=486), as well as patients that fulfilled the ACR criteria for RA (n=407) were identified. IgM Rheumatoid Factor (RF), anti-cyclic-citrullinated peptide (CCP) antibodies and HLA-DRB1 alleles were determined.

Results. In RA an interaction was found between TE and SE for the presence of anti-CCP antibodies, as the odds ratio (OR) for anti-CCP antibodies of patients having both TE and SE was higher than the summed ORs of patients having only TE or SE (OR TE+SE- 1.07, TE-SE+ 2.49, and TE+SE+ 5.27, all relative to TE-SE-). A similar effect was found for RF, but stratification revealed that the interaction primarily associates with the anti-CCP antibody response. In patients with UA at two weeks or with persistent UA after one year no interaction between TE and SE was observed for the presence of autoantibodies.

Conclusions. TE increases the risk factor for anti-CCP antibodies only in SE-positive RA-patients. The gene-environment interaction between smoking and SE leading to autoantibodies is specific for RA and is not observed in UA.

INTRODUCTION

In the search for the etiology of Rheumatoid arthritis (RA) genetic predisposition, environmental- and dietary risk factors may present clues for pathogenesis (1-7). The most important genetic risk factor for the development of RA is the presence of HLA Class II alleles that share the conserved amino acid sequence called the Shared Epitope (SE) (8). These SE-residues constitute a part of the antigen presenting binding site. The Shared Epitope hypothesis postulates that the shared epitope motif itself is directly involved in the pathogenesis of RA by allowing the presentation of a peptide to arthritogenic T-cells. The most prominent environmental risk factor for RA is smoking; smokers have increased levels of Rheumatoid Factor (RF) (9-11), are more prone to develop RA (11-14) and develop more severe disease (15-17). Interaction between environmental and genetic risk factors points to the existence of disease-specific pathogenic pathways involved in disease induction or progression.

For RA Padyukov et al recently described a gene-environment-interaction between smoking and SE that provides risk (OR 2.8; 95% CI 1.6-4.8) for RF-positive but not RF-negative RA in a large cohort of 858 RF-positive and 1048 RF-negative patients with RA (18). Recently, we identified in two large cohorts from both the USA and from Europe by different genetic-epidemiological methods (association and linkage) that HLA-DRB1 alleles are only a risk factor for RA patients that have anti-CCP antibodies and not in the absence of anti-CCP antibodies, suggesting different pathogenic pathways for anti-CCP positive and negative RA (19).

This study investigates whether the gene-environment interaction smoking-shared epitope is also present for the anti-CCP antibody response and whether this interaction was more pronounced for the development of RF compared to the development of anti-CCP antibodies. Secondly, this study aimed to assess whether the interaction smoking and SE is specific for patients with RA or is also present in undifferentiated arthritis (UA). To this end patients with arthritis that did not fulfill any classification criteria at presentation and patients with persistent UA at one-year follow-up were used. These patients have a spontaneous remission rate of about 50% (20) and might have differences in the underlying pathogenesis. If the interaction between smoking and SE that leads to autoantibody formation is specific for the pathogenesis of RA, it is hypothesized that such an interaction will not be seen in patients with an undifferentiated arthritis of which clinical follow-up has learned that these patients have not developed RA.

PATIENTS AND METHODS

Patients

For this study, the Leiden Early Arthritis Clinic (EAC), a population-based inception cohort of patients with newly diagnosed early arthritis was used (for further reading see (21)). RA was diagnosed according to the American College of Rheumatology (ACR) criteria of 1987(22). Patients who could not be properly classified according to one of the ACR-criteria at 2 weeks follow-up were categorized as having UA (20). This population of UA patients was further divided in patients that (1) had developed RA, (2) remained unclassified (persistent UA) or (3) had developed other rheumatic diseases such as spondylarthropathies, osteoarthritis, gout, reactive arthritis at one year follow-up (Figure 1).

At inclusion in the EAC-cohort, for each patient the smoking status (cigarettes, cigars) was registered as past, current or never smokers. Current and past smokers were classified as tobacco exposure positive (TE+) and never smokers as tobacco exposure negative (TE-)(11). Baseline laboratory parameters included C-reactive protein (CRP), IgM rheumatoid factor (ELISA as previously described 23), anti-CCP antibodies (ELISA, Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands and Axis-Shield, Dundee, UK) and HLA-Class II alleles. The HLA-DRB1 (sub-)typing was performed by polymerase chain reaction using specific primers and hybridisation with sequence-specific oligonucleotides. Shared epitope alleles were: DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *0410 and *1001 (24). Patients homozygous and heterozygous for shared epitope were both classified as SE positive. Missing data for the whole cohort of 1305 patients ranged for several items

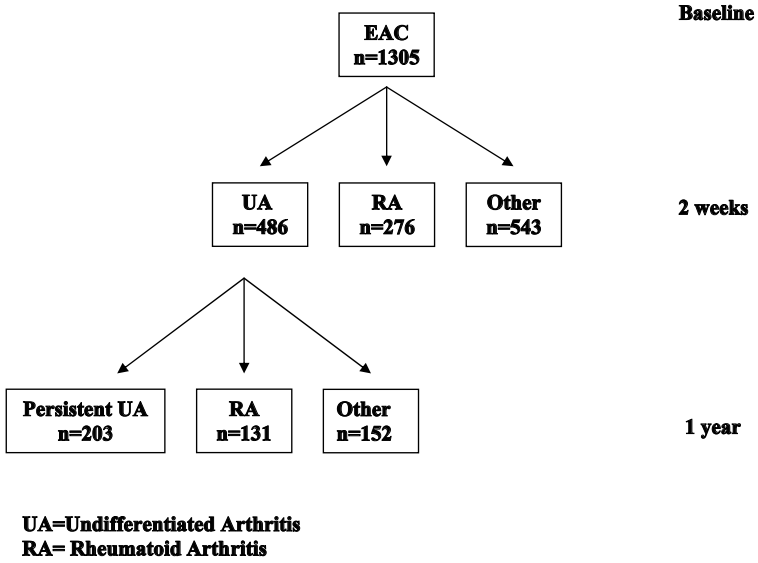

Figure 1. Flow chart early arthritis patients.

SE, TE anti-CCP and RF between 0%-20%. An analysis of the baseline values of patients with missing data-points revealed no differences to those patients without.

Statistical analysis

Odds ratios were calculated for the primary outcome measures RF and anti-CCP antibodies. Stratified analysis was performed for both anti-CCP-positive and anti-CCP negative as well as RF-positive and RF-negative strata. Chi-square analysis was performed on 2 by 4 tables.

RESULTS

Patient characteristics

Between 1993 and 2003, 1305 patients were included in the EAC-cohort. At two weeks follow-up, 486 patients did not fulfill any classification criteria and were thus classified as UA. At this time 276 patients fulfilled the ACR criteria for RA. Of the 486 UA patients, after one year follow-up 131 patients were diagnosed as having RA. In 203 patients the diagnosis remained UA (persistent UA) and in the other 152 patients another rheumatic disorder such as spondylarthropathy, osteoarthritis, psoriatic arthritis, etc was identified (Figure 1). The total number of patients from the cohort that was identified as having RA at one year was 407. From the patients classified as persistent UA at one year, 15% developed RA during further follow-up (K.Verpoort, unpublished data).

Baseline patient characteristics of patients that at two weeks presented with RA or UA are given in Table 1; the data on the UA patients are presented for both the UA patients

Table 1. Patient characteristics at baseline of patients that presented with RA, patients that presented with UA and developed RA after 1 year and patients that presented with UA and had other diagnoses than RA after one year follow-up.

	RA at 2 weeks (n=276)	UA→RA (n=131)	UA→non RA (n=355)	p*
Age yr (mean)	58	56	48	<0.001
Female (%)	66	64	51	n.s.
CRP (mg/l, mean)	35	29	21	0.036
IgM RF + (%)	65	52	13	<0.001
Anti-CCP + (%)	54	51	7	< 0.01
Shared Epitope + (%)	68	63	49	0.046
Tobacco exposure + (%)	47	52	50	n.s.

Shared Epitope + means presence of 1 or 2 shared epitope alleles.
Tobacco exposure + means current and past smokers as indicated in the medical history.
*P values were determined for UA→RA versus UA→non RA.
Comparison of RA at 2 weeks versus UA→RA revealed no significant differences.

that developed RA, as the UA patients that had persistent UA after one-year follow-up. UA patients that did not develop RA were younger at presentation than the patients that developed RA (mean 48 years, vs. 56 years, $p<0.001$). The UA patients that developed RA after one year had at baseline higher levels of CRP, RF and anti-CCP antibodies and were more often SE-positive compared to the UA-patients that had persistent UA or developed other rheumatologic diagnosis (Table 1). No differences were observed between the 131 RA patients that developed RA after 1 year and the 276 patients with RA that were diagnosed at the two weeks visit.

Interaction tobacco exposure and shared epitope in RA

In RA and UA patients the interaction between SE and TE was analyzed. Outcome parameters were RF and anti-CCP antibodies.

In all RA patients, no effect of TE on the RF status was seen in SE negative patients in contrast to a clear effect in SE positive patients. The OR for positive RF was 1.47 for TE+ SE- patients, 1.35 for TE- SE+ patients and 3.23 for TE+SE+ patients all relative to TE-SE- patients (Table 2), showing an interaction between TE and SE for the development of RF.

In SE negative RA patients, no effect of TE was seen for positive anti-CCP antibodies in contrast to a clear effect in the SE positive group. The OR for positive anti-CCP antibodies was 1.07 for TE+ SE- patients, 2.49 for TE+ SE- patients and 5.27 for TE+SE+ patients, again all relative to TE-SE- patients. As the odds ratio for anti-CCP antibodies of patients having both TE and SE was higher than the summed OR's of patients having only TE or SE, an interaction was found between TE and SE for the presence of anti-CCP antibodies. The difference between the TE- SE+ patients and the TE+ SE+ patients was significant both for IgM-RF and for anti-CCP (Table 2).

Presuming that RF-positive and anti-CCP positive patients partly overlap each other, a stratified analysis was done for both RF-positive and RF-negative and anti-CCP positive and negative patients. The results, shown in Table 2, demonstrate that when stratified for the presence/absence of anti-CCP antibodies, no significant interaction is found between TE and SE in relation to the presence of RF. When stratified for RF, in the RF negative group an interaction between TE and SE was observed for the development of anti-CCP antibodies. These data suggest that the interaction between TE and SE primarily associates with positive anti-CCP antibodies and not with positive RF.

Interaction tobacco exposure and shared epitope in UA

In the whole group of patients who presented with UA, the combination of TE and SE did not significantly increase the risk for the presence of positive RF or anti-CCP antibodies (Table 3). The OR for positive anti-CCP antibodies in TE- SE+ patients with UA was increased compared to TE-SE- (OR 3.83: 95% CI 1.33-12.53), but addition of TE to the

Table 2. Odds ratios for developing RF and anti-CCP antibodies in the presence of Tobacco Exposure (TE) and/or shared epitope alleles (SE) in all Rheumatoid Arthritis patients at 1 year.

	TE	SE	RF +	RF-	OR	95% CI	p
	–	–	23	31	1.00	-	-
	+	–	25	23	1.47	0.62-3.45	0.33
	–	+	54	54	1.35	0.66-2.75	0.37 *
	+	+	72	30	3.23	1.54-6.81	<0.001 *
							0.002 π
	TE	**SE**	**anti-CCP +**	**anti-CCP -**	**OR**		**p**
	–	–	18	34	1.00	-	-
	+	–	17	30	1.07	0.43-2.65	0.87
	–	+	58	44	2.49	1.18-5.31	0.01 #
	+	+	67	24	5.27	2.37-11.80	<0.001 #
							<0.001 π
anti-CCP	**TE**	**SE**	**RF +**	**RF-**	**OR**	**95% CI**	**p**
+	–	–	14	4	1.00	-	-
+	+	–	17	0	∞	∞	0.10
+	–	+	46	12	1.10	0.22-4.42	0.88 ¶
+	+	+	54	13	1.19	0.24-4.68	0.79 ¶
							0.23
–	–	–	7	27	1.00	-	-
–	+	–	8	22	1.40	0.38-5.32	0.57
–	–	+	6	38	0.61	0.12-2.40	0.41 ¶
–	+	+	7	17	1.59	0.39-6.34	0.45 ¶
							0.39 π
RF	**TE**	**SE**	**anti-CCP +**	**anti-CCP -**	**OR**	**95% CI**	**p**
+	–	–	14	7	1.00	-	-
+	+	–	17	8	1.06	0.26-4.34	0.92
+	–	+	46	6	3.83	0.91-16.07	0.03 ¶
+	+	+	54	7	3.86	0.96-15.11	0.02 ¶
							0.02 π
–	–	–	4	27	1.00	-	-
–	+	–	1	22	0.31	0.01-3.47	0.28
–	–	+	12	38	2.13	0.56-9.97	0.22 ¶
–	+	+	13	17	5.16	1.28-24.71	0.01 ¶
							0.04 π

* TE-SE+ versus TE+SE+: OR 2.4 (95%CI 1.3-4.4, p=0.002)
# TE-SE+ versus TE+SE+: OR 2.1 (95%CI 1.1-4.1, p=0.02)
¶ Comparison TE-SE+ versus TE+SE+ not significant
π P-value of Chi-square analysis of 2 by 4 table

Table 3. Odds ratios for developing RF and anti-CCP antibodies in the presence of Tobacco Exposure (TE) and/or shared epitope alleles (SE) in patients with Undifferentiated Arthritis (UA).

3A. All UA patients at two weeks

TE	SE	IgM RF +	IgM RF –	OR	95% CI	p
–	–	12	55	1.00	-	-
+	–	13	48	1.24	0.47-3.29	0.63
–	+	19	60	1.45	4.60-3.59	0.34 ¶
+	+	18	54	1.53	0.62-3.82	0.31 ¶
						0.75 π
TE	**SE**	**anti-CCP +**	**anti-CCP –**	**OR**	**95% CI**	**p**
–	–	6	54	1.00	-	-
+	–	9	48	1.69	0.49-6.19	0.35
–	+	20	47	3.83	1.33-12.53	0.01 ¶
+	+	21	47	4.02	1.40-13.09	0.01 ¶
						<0.01 π

3B. Subgroup of UA patients who develop RA within one year

TE	SE	IgM RF +	IgM RF –	OR	95% CI	p
–	–	6	12	1.00		
+	–	8	14	1.14	0.26-5.24	0.84
–	+	15	22	1.36	0.37-5.44	0.61 ¶
+	+	19	13	2.92	0.76-11.90	0.08 ¶
						0.87 π
TE	**SE**	**anti-CCP +**	**anti-CCP –**	**OR**	**95% CI**	**p**
–	–	8	10	1.00		
+	–	8	13	0.77	0.18-3.33	0.69
–	+	19	16	1.48	0.41-5.46	0.50 ¶
+	+	21	9	2.92	0.74-11.66	0.08 ¶
						0.12 π

3C. Subgroup of persistent UA patients at one year

TE	SE	IgM RF +	IgM RF –	OR	95% CI	p
–	–	6	37	1.00		
+	–	5	32	0.96	0.21-4.20	0.95
–	+	9	34	1.63	0.46-6.17	0.39 ¶
+	+	4	33	0.75	0.14-3.48	0.67 ¶
						0.62 π
TE	**SE**	**anti-CCP +**	**anti-CCP –**	**OR**	**95% CI**	**p**
–	–	1	36	1.00		
+	–	2	34	2.12	0.10-128	0.54
–	+	8	27	10.67	1.26-486	0.01 ¶
+	+	4	32	4.50	0.41-227	0.16 ¶
						0.03 π

¶ Comparison TE-SE+ versus TE+SE+ not significant

π P-value of Chi-square analysis of 2 by 4 table

presence of SE did not significantly increase the risk of having anti-CCP antibodies (OR 4.02 relative to OR of 3.83, table 3A).

Subsequently, we assessed whether in the subgroup UA patients that developed RA (n=131) smoking combined with presence of SE increased the risk of having anti-CCP antibodies. Therefore the non-smoking SE negative UA patients that developed RA were compared to smoking SE negative UA patients that developed RA patients (OR 0.77), as well as to non-smoking SE-positive patients with UA that developed RA (OR 1.48) and finally to smoking, SE-positive patients with UA that developed RA (OR 2.92). In this smaller group of RA patients a trend for the interaction of SE and TE to increase the risk of having anti-CCP antibodies was observed. Calculations for outcome RF showed similar results (Table 3B).

The same calculations were repeated for patients with persistent UA (n=203). Although the number of anti-CCP positive UA patients is low, no effect of tobacco exposure combined with SE on the risk of having anti-CCP antibodies or RF was observed (Table 3C). Thus, the interaction of SE and tobacco exposure was found for the presence of RF and for anti-CCP antibodies in patients with RA and in those UA patients that develop RA within one year but not in patients with persistent UA.

DISCUSSION

A strong gene-environment interaction between TE and SE for the presence of autoantibodies was observed. Intriguingly, this gene-environmental interaction was only present in patients with RA and not observed in patients with (persistent) UA. Stratified analysis for the different autoantibody responses IgM RF and anti-CCP showed that the interaction is primarily for the anti-CCP response.

A recent Swedish study demonstrated that a gene-environment interaction between SE and smoking results in an elevated risk specifically for RF-positive RA (18). These data were replicated as well as extended in the present study. Replication by a separate group in a separate cohort minimizes the risk that the current findings are false positive (25). Both the Swedish and the current data demonstrate the lack of a relation between smoking and autoantibodies in SE-negative RA, indicating that this interaction is preferential for a given pathogenetic pathway in SE-positive RA. To specify this pathogenetic pathway with regard to the specificity of the autoantibody response, the stratified analysis for anti-CCP positivity yielded no additional effect of smoking on risk to develop RF. In contrast the stratified analysis for RF indicated that smoking more than doubled the risk in the SE-positive patients to develop anti-CCP antibodies. These data suggest that the gene-environment interaction between smoking and SE leading to autoantibodies is primarily

associated with the anti-CCP response. Apart from the specificity of this gene-environment interaction, our group has recently described that SE is only a risk factor for anti-CCP positive RA and not for anti-CCP negative RA (19). The current data do not allow the analysis of smoking as a risk factor for anti-CCP positive RA versus anti-CCP negative RA because no data of smoking in a matched group of the general population are known. However given the fact that SE alone is not a risk factor for anti-CCP negative RA (19), the gene-environment interaction between smoking and SE leading to anti-CCP antibodies seems characteristic for anti-CCP positive RA. Indeed no effect of smoking was observed in the SE-negative patients (see Table 2). These data are in line with our previously reported hypothesis that different pathogenetic pathways operate in anti-CCP negative RA as compared to anti-CCP positive RA. The demonstration of these pathogenetic pathways is difficult because it is not known which proteins are citrinullated as a result of smoking, nor is it known if or how smoking breaks normal tolerance to citrinullated self-proteins.

In this study the diagnosis persistent RA was defined as the presence of arthritis that did not fulfill any of the classification criteria after one-year follow-up. This may lead to some misclassification because a small proportion of UA patients only develop RA after a longer time period. However, in previous analysis of this cohort this concerned only less than 15% of the patients with persistent UA at one year. More importantly, no interaction between smoking and shared epitope was observed at all in the persistent UA group.

A weakness of the current study is that the information on TE is limited to patient history taking and patients were not asked about the number of pack-years smoking. Therefore no conclusion on the minimal exposure can be drawn.

In summary, smoking was confirmed to be a risk factor for anti-CCP antibodies in the presence of shared epitope alleles in patients with RA. In UA no interaction between TE and SE was demonstrated for the presence of autoantibodies.

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Chapter 10

The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-CCP antibodies and are not an independent risk factor to develop rheumatoid arthritis

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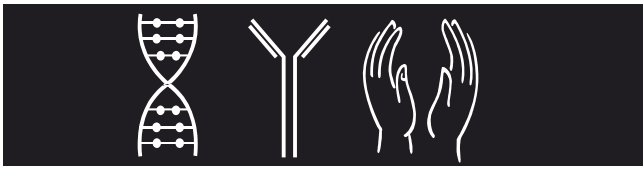

ABSTRACT

Objectives. The Shared-Epitope (SE)-containing HLA-DRB1-alleles represent the most significant genetic risk factor for rheumatoid arthritis (RA). Recent studies showed that the SE-alleles only associate with RA that is characterized by the presence of anti-Cyclic Citrullinated Peptide (CCP)-antibodies, and not with anti-CCP-negative disease. Here we studied whether the SE-alleles contribute to the development of anti-CCP-positive RA, or rather associates with the presence of anti-CCP-antibodies. Therefore the influence of SE-alleles and anti-CCP-antibodies on the progression from recent onset undifferentiated arthritis (UA) to RA is investigated.

Methods. From the Leiden Early Arthritis Cohort, an inception cohort of patients with recent onset arthritis, the patients with UA at the 2-week visit were selected (n=570). SE-alleles, rheumatoid factor (RF) and anti-CCP-antibody levels were determined. Progression to RA or other diagnoses was monitored.

Results. 177 UA patients developed RA during 1-year follow-up, whereas 393 patients remained unclassified or developed other diagnoses. The SE-alleles correlated with the presence of anti-CCP-antibodies, but, after stratification for anti-CCP-antibodies, not with the presence of RF. Both in SE-positive and SE-negative UA-patients, the presence of anti-CCP-antibodies was significantly associated with the development of RA. More intriguingly however, no apparent contribution of the SE-alleles was found on the progression to RA when stratified for the presence of anti-CCP-antibodies. In anti-CCP-positive patients the presence of SE-alleles was associated with significantly higher levels of anti-CCP-antibodies, suggesting that the SE-alleles act as classical immune response genes.

Conclusions. The SE-alleles do not independently contribute to the development of RA from UA, but rather to the development of anti-CCP-antibodies.

INTRODUCTION

The most important genetic risk factor for rheumatoid arthritis (RA) are the HLA-Class II alleles. Especially the HLA-DRB1 alleles encoding for the shared epitope (SE) confer a higher risk to develop RA (1). The shared epitope hypothesis postulates that the shared epitope motif (a conserved amino acid sequence in the peptide binding pocket of the DRB1 molecule) is directly involved in the pathogenesis of RA by allowing the presentation of an arthritogenic peptide to T cells (2). Recently, it was observed by two different methods (linkage and association analysis) that the SE-alleles are only a risk factor for RA that is characterised by the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies and that the SE-alleles are not associated with anti-CCP-negative RA (3). Anti-CCP antibodies are highly specific for RA, can be detected years before the first clinical manifestation of RA (4) and are reported to be a good predictor for the development of RA (5). Because the contribution of the SE-containing HLA-alleles to the pathogenesis of RA is not well understood, the novel information on the association of SE-alleles with anti-CCP-positive disease (3) led us to evaluate the hypothesis that the SE-alleles are mainly a risk factor for anti-CCP-antibodies, rather than for (anti-CCP-positive) RA. To this end, we took advantage of a well-characterized inception cohort and studied the patients with an early arthritis that at presentation could not be classified according to the ACR-criteria (undifferentiated arthritis). Analysis of the clinical evolution, in combination with genetic and serological risk factors, of these patients prone to develop RA allows insight in the factors that are associated with progression towards RA. Accordingly this permits the analysis of the contribution of the SE-alleles to the development of RA after stratification for anti-CCP antibodies.

PATIENTS AND METHODS

Study population

For this study, the Leiden Early Arthritis Clinic (EAC) was used (see for description ref 6). In short, the EAC was started in 1993. Patients were referred to the EAC when arthritis was suspected and were included in the cohort when arthritis was found at physical examination. At baseline blood samples were taken. At present more than 1900 patients are included. Two weeks after inclusion, 313 patients had the diagnosis RA according to the 1987 ACR criteria and 570 patients had an arthritis that could not be classified according to one of the ACR criteria and were therefore called undifferentiated arthritis (UA). After one year of follow-up, the disease status of all UA patients was examined in order to determine whether they had developed RA according to the ACR-criteria. It might be possible that some patients did not fulfil the ACR criteria for RA at one year but developed RA at a

later time point. Inherent to the design of an inception cohort the duration of follow-up differs within the study population. However, at the moment of analysis the majority of patients (94%) has been followed for more than one year (mean follow-up 8 years, SD 3 years) and only 9% of the patients that did not classify as RA at one year developed RA later on in the disease course.

Laboratory investigations

Baseline laboratory parameters (determined using blood samples that were taken at inclusion) included IgM rheumatoid factor (RF, ELISA) and anti-CCP2 antibodies (ELISA, Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands). The cut-off level for anti-CCP antibody positivity was according to the manufacturers instruction set at a level of 25 arbitrary units. The HLA-DRB1 (sub)typing was performed by polymerase chain reaction using specific primers and hybridisaton with sequence specific oligonucleotides. The shared epitope alleles were HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408,*0410 and *1001. Of 438 from the 570 UA patients both data on SE-alleles and anti-CCP antibodies were available.

Statistical analysis

Odds ratios were calculated and proportions were compared by the chi-square test. Differences in means between groups were analysed using the Mann Whitney test or t-test when appropriate. The question whether SE-alleles and anti-CCP antibodies both independently contribute to progression of RA was investigated with a stratification procedure, as well as with logistic regression analysis. In this logistic regression analysis the disease outcome was entered as dependent variable and anti-CCP antibodies and SE-alleles as possibly explanatory variables; with a backward selection procedure the significant independent variables were selected. For all tests, p-values of < 0.05 were considered significant.

RESULTS AND DISCUSSION

Outcome of UA

Of 570 patients with UA at the two weeks visit, 177 developed RA during the first year of follow-up, 99 patients developed other rheumatologic diseases (reactive arthritis, psoriatic arthritis, SLE, etc) and 294 patients remained unclassified (persistent UA). For further analysis the patients with persistent UA and with other rheumatologic diagnosis were described as the non-RA group. Characteristics of the patients that developed RA and the non-RA group are depicted in Table 1. In univariate analysis, the presence of SE-alleles, RF and anti-CCP antibodies were all associated with significantly higher odds ratio's to develop RA (OR 1.8, 6.3 and 8.5 respectively, Table 1).

Table 1. Baseline characteristics of patients with undifferentiated arthritis at two weeks that did and did not develop RA during the first year of follow-up

	RA (n=177)	non-RA (n=393)	P	OR (95%CI)
Age (yr, mean ± SD)	56.3 ± 15.3	48.6 ± 16.9	<0.001	–
Gender F/M	121/56	208/185	0.001	1.9 (1.3-2.8)
SE positive*	100 (63%)	158 (49%)	0.005	1.8 (1.2-2.6)
Anti-CCP positive#	83 (51%)	38 (11%)	<0.001	8.5 (5.2-13.7)
RF positive	84 (47%)	56 (14%)	<0.001	6.3 (4.1-9.7)

* SE data was missing in 17 of the UA-RA patients and in 68 of the UA-non-RA patients
# Anti-CCP antibody data was missing in 15 of the UA-RA patients and 49 of the UA-non-RA patients

Association between SE-alleles and presence of auto-antibodies

To determine whether the SE-alleles correlate with RF, with anti-CCP antibodies or with both auto-antibodies, the associations between SE-alleles and anti-CCP and the associations between SE-alleles and RF were investigated in the 570 UA patients. In univariate analysis, the SE-alleles were associated with both RF and anti-CCP antibodies (OR 1.7, 95%CI 1.1-2.7, p=0.01 and OR 3.1, 95%CI 2.1-5.3, p<0.001 respectively). As anti-CCP positivity is correlated with RF positivity, the association between SE-alleles and anti-CCP antibodies was assessed in both the RF-positive and RF-negative patients. In RF-negative patients the presence of SE-alleles was associated with a higher odds ratio to develop anti-CCP antibodies (OR 2.9, 95%CI 1.2-6.9, p<0.01). Similarly, in RF-positive patients the presence of SE-alleles conferred a higher odds ratio to have anti-CCP antibodies (OR 5.6, 95% CI 2.1-14.6, p<0.001). These data indicate that the SE-alleles are associated with the presence of anti-CCP antibodies independent of the presence or absence of RF. Next it was assessed whether the SE-alleles are associated with RF, independent of the anti-CCP antibodies. In both the anti-CCP positive and anti-CCP negative patient groups, the SE-alleles were not associated with the presence of RF (p=0.9 and 0.2 respectively), indicating that after correction for the presence or absence of anti-CCP antibodies the SE-alleles do not confer risk to RF positivity. In conclusion, the SE-alleles are primarily correlated with the presence of anti-CCP antibodies but not with the presence of RF.

SE-alleles and anti-CCP antibodies in progression from UA to RA

Subsequently, the influence of SE-alleles on the progression from UA to RA was examined. Univariate analysis assessing the association between patient characteristics and disease outcome revealed that the presence of SE-alleles and anti-CCP antibodies at baseline were both associated with the development of RA (see Table 1). However, as the presence of SE-alleles and anti-CCP antibodies are correlated, the individual effect of SE-alleles on the development of RA was determined by stratification for anti-CCP antibodies. Both in the anti-CCP positive and in the anti-CCP negative UA-patients, the presence of SE-alleles was not associated with the development of RA (Table 2). These data are important as they

Table 2. Number of UA-patients that during one year of follow-up did not and did develop RA, stratified for baseline anti-CCP antibodies and SE-alleles

		non-RA n (%)	RA n (%)	P	OR (95%CI)
Stratification for anti-CCP antibodies					
anti-CCP –	SE –	142 (55)	37 (53)		
	SE +	118 (45)	33 (47)	0.8	1.1 (0.6-1.9)
anti-CCP+	SE –	8 (26)	21 (27)		
	SE +	23 (74)	56 (73)	0.9	0.9 (0.3-2.6)
Stratification for SE-alleles					
SE –	anti-CCP –	142 (95)	37 (64)		
	anti-CCP +	8 (5)	21 (36)	<0.001	10.1 (3.9-27.1)
SE +	anti-CCP –	118 (84)	33 (39)		
	anti-CCP +	23 (6)	56 (61)	<0.001	8.7 (4.5-17.0)

indicate that the SE-alleles do not correlate with RA development in patients with UA when stratified for the presence/absence of anti-CCP antibodies.

To assess the effect of the anti-CCP antibodies independent of SE alleles, the risk to develop RA was determined in SE-positive and SE-negative UA-patients separately (Table 2). This analysis showed that in the SE-positive as well as in the SE-negative UA patients the presence of anti-CCP antibodies was significantly associated with the development of RA (OR 8.7 and 10.1 respectively).

In a logistic regression analysis with a backward selection procedure entering the disease outcome (RA versus non-RA) as dependent variable and the SE-alleles and anti-CCP antibodies as possibly explanatory variables, the anti-CCP antibodies were the only independent factor that significantly associated with the development of RA with an odds ratio of 9.2 ($p<0.001$). This odds ratio resulting from multivariate analysis is not importantly different from the odds ratio for anti-CCP antibodies on the development of RA as observed in univariate analysis (OR 8.5, see Table 1).

In conclusion, these data show that after stratification for SE-alleles the anti-CCP antibodies confer a high risk to develop RA, whereas after stratification for anti-CCP antibodies the SE-alleles are not associated with progression to RA. Together these data indicate that the SE-alleles primarily predispose to the presence of anti-CCP antibodies and are not an independent risk factor for the development of RA.

Association between SE-alleles and anti-CCP antibody level

In classic studies performed in mice on the genetical background associated with antibody production it has been shown that major histocompatibility alleles act as Immune Response genes (Ir-genes) that control the magnitude and specificity of antibody production in a dominant fashion (7). In these mice the magnitude of the antibody response of the first generation offspring was comparable to the magnitude of the high responding parent, denoting that in mice homozygositiy for MHC-genes did not improve the level of antibody

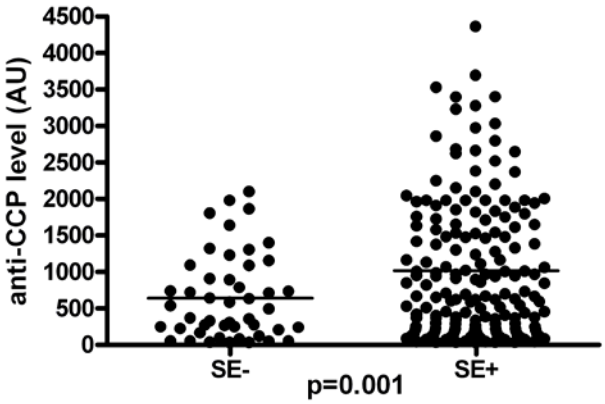

Figure 1. **Levels of anti-CCP antibodies (arbitrary units) in anti-CCP positive RA patients without and with SE-alleles.** The median anti-CCP antibody level is depicted. The mean anti-CCP antibody levels for the anti-CCP positive RA patients were: mean 1041 (SEM 134, n=46) for carrying two SE-alleles, 1029 (SEM 86, n=123) for carrying 1 SE-allele and 652 (SEM 86, n= 46) for carrying no SE-alleles. The median anti-CCP antibody levels for the subgroup anti-CCP positive UA patients that progressed to RA were: 699 (IQR 278-1282, n=13) for carrying 2 SE-alleles, 927 (IQR 251-1970, n=43) for carrying 1 SE-allele and 358 (IQR 169-1424, n=21) for carrying no SE-alleles.

production compared to heterozygosity (7). As the current data revealed that the SE-alleles are associated with anti-CCP antibodies, we wished to investigate whether the characteristics of the SE-alleles resemble such a classic Ir-gene. Thus we wished to analyse whether the level of anti-CCP antibodies present in serum was correlated to the presence of SE-alleles. To this end the correlation between the presence of SE-alleles and level of anti-CCP antibodies was assessed in all anti-CCP positive patients that at one-year follow-up had the diagnosis RA. Of a total number of 490 RA patients (313 RA diagnosed at two weeks follow-up and 177 patients that progressed from UA to RA during the first year of follow-up), 233 patients had anti-CCP antibodies of which 73% harboured SE-alleles. The anti-CCP antibody levels of anti-CCP-positive SE-positive and anti-CCP-positive SE-negative patients are depicted in Figure 1. SE-positive patients had a significantly higher level of anti-CCP antibodies (n= 169, mean 1032, SEM 72 arbitrary units) than SE-negative patients (n= 46, mean 652, SEM 86 arbitrary units, p=0.001). Patients carrying one SE-allele displayed a significantly higher level of anti-CCP antibodies (n=123, mean 1029; SEM 86 arbitrary units) compared to patients without SE-alleles (p=0.002). Patients with two SE-alleles did not have a significantly higher anti-CCP level (n=46, mean 1041, SEM 134 arbitrary units) compared to patients carrying one SE-allele (p=0.94). Thus, the current data show that in anti-CCP-positive patients the presence of SE-alleles is associated with higher levels of anti-CCP antibodies and indicate that the presence of one or two SE-alleles does not result in an apparent difference in anti-CCP antibody level. This observation is compatible with the notion that the SE-alleles are Ir-genes for the development of anti-CCP antibodies.

CONCLUSION

Recently, we reported that the SE-alleles were only associated with anti-CCP-positive RA and not with anti-CCP-negative disease, indicating that the SE-alleles are not associated with RA as such but rather with a distinct phenotype of the disease. We now extend these findings by showing that the SE-alleles are not an independent risk factor for the development of RA after correction for anti-CCP-antibody status. The SE-alleles were however associated with the presence of anti-CCP antibodies. Moreover, the presence/ absence of SE-alleles was correlated with the levels of anti-CCP antibodies, suggesting that the SE-alleles act as classic Ir-genes for the development of anti-CCP antibodies. Although no formal conclusions on causality can be drawn from this association study, these findings suggest that anti-CCP antibodies mediate the association between SE-alleles and RA. It would be of interest to replicate the findings of the present study by following the development of anti-CCP antibodies and RA in healthy asymptomatic persons with and without SE-alleles. Nevertheless, the present data constitute an important refinement of the long-known association between HLA and RA by indicating that the SE-alleles do not primarily associate with RA, but rather with anti-CCP-positivity.

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Chapter 11

Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis

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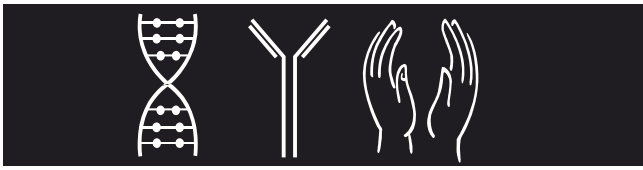


ABSTRACT

Antibodies to citrullinated proteins (anti-CCP) are highly specific for rheumatoid arthritis (RA) and precede the onset of disease symptoms, indicating a pathogenetic role for these antibodies in RA. Recently we showed that distinct genetic risk factors are associated with either anti-CCP positive and anti-CCP-negative disease. These data are important as they indicate that distinct pathogenic mechanisms are underlying anti-CCP positive and negative disease. Likewise, these observations raise the question whether anti-CCP positive and negative RA are clinically different disease entities. Therefore, we investigated whether RA patients with anti-CCP antibodies have a different clinical presentation and disease course compared to patients without these auto-antibodies. In a cohort of 454 incident patients with RA, 228 patients were anti-CCP positive and 226 patients anti-CCP negative. The early symptoms, tender and swollen joint count and C-reactive protein level at inclusion, as well as the swollen joint count and radiological destruction during 4 years follow-up were compared for the 2 groups. There were no differences in morning stiffness, type, location and distribution of early symptoms, patients' rated disease activity and C-reactive protein at inclusion between RA patients with and without anti-CCP antibodies. The mean tender and swollen joint count for the different joints at inclusion was similar. At follow-up, patients with anti-CCP antibodies had more swollen joints and more severe radiological destruction. Nevertheless, the distribution of affected joints, for swelling, bone erosions and joint space narrowing, was similar. In conclusion, the phenotype of RA patients with or without anti-CCP antibodies is similar with respect to clinical presentation but differs with respect to disease course.

INTRODUCTION

Autoantibodies directed to citrullinated proteins (e.g. anti-cyclic-citrullinated peptide, anti-CCP antibodies) are highly specific serological markers for rheumatoid arthritis (RA) that are thought to be directly involved in the disease pathogenesis (1). Citrullinated proteins are not exclusively located in synovial tissue of RA patients, but can also be found in synovium samples of patients with other inflammatory joint diseases (2), suggesting that the specificity of anti-CCP antibodies for RA is not due to the expression of citrullinated proteins but might be the result of an abnormal humoral response. Intriguingly this antibody response may occur years before any clinical symptoms, as shown by the presence of anti-CCP antibodies several years before the clinical onset of arthritis (3,4). Furthermore, a proportion of RA patients do not harbour anti-CCP antibodies, suggesting that the presence of anti-CCP antibodies is not obligatory for the development of arthritis or that the pathogenic mechanisms underlying anti-CCP positive and negative RA are different.

These observations inspired subsequent research addressing the question whether RA patients with anti-CCP antibodies are different from those who are anti-CCP negative. Very recently, we demonstrated in two independent Caucasian populations that the shared epitope encoding HLA-DBR1 alleles associate with RA in patients with anti-CCP antibodies but not in patients without these antibodies (unpublished data, 5). These findings are important as they indicate that the shared epitope alleles are not associated with RA as such, but rather with a particular phenotype of the disease.

Given the findings suggesting a pathophysiological role for anti-CCP antibodies in RA and the reported immunogenetical differences between anti-CCP positive and negative patients, it is conceivable that anti-CCP positive and negative RA are different disease entities and have thus different phenotypical properties. Anti-CCP antibodies have been suggested to be associated with more severe radiological outcome (5,6). However, to our knowledge a detailed description of the distribution and degree of early symptoms and signs in both patient groups has not been published. Nevertheless, such an analysis is relevant as it might provide novel insight into the putative pathogenic role of anti-CCP antibodies in the aetiology of the disease. Therefore, in this study we set out to determine whether anti-CCP antibody positive and negative RA patients differ in different aspects of their phenotype, either the early symptoms of disease, the findings at physical examination at initial presentation or the acute phase reactant C-reactive protein at initial presentation. Moreover, we expanded the data on the influence of anti-CCP antibodies on disease course during 4-year follow-up for the distribution and extend of both inflammation (swollen joints) and radiological joint destruction. We show that the phenotype

of RA patients with or without anti-CCP antibodies is similar with respect to clinical presentation but differs with respect to disease course.

PATIENTS AND METHODS

Patients

In 1993 an Early Arthritis Clinic (EAC) was started at the department of Rheumatology of the Leiden University Medical Center, the only referral center for Rheumatology in a health care region of about 400 000 inhabitants in the western part of the Netherlands (7). General practitioners were encouraged to refer patients directly when arthritis was suspected. Patients referred could be seen within two weeks and were included in the program when the physician's examination of the patients revealed arthritis and the symptoms had lasted less than 2 years. At the first visit the rheumatologist answered a form with questions about the initial symptoms as reported by the patient (type of initial joint symptoms, localization and distribution of initial joint symptoms, presence of morning stiffness). Patients rated their global assessment of disease activity on a visual analogue scale (0-100). The Health Assessment Questionnaire, a self-assessed questionnaire asking about the ability of the patient to perform several daily activities over the past week, was used to obtain an index of disability. A tender joint count and swollen joint count (8,9) was performed on entering the study and yearly thereafter. For the tender joint count, each joint was scored on a 0-3 scale with 3 being maximal tenderness (0= no tenderness, 1= pain on pressure, 2= pain and winced and 3= winced and withdrew). For the swollen joint count the individual joints were scored on a 0-1 scale (0 = no swelling, 1= swelling). At inclusion from every patient blood samples were taken for routine diagnostic laboratory screening including C-reactive protein and were stored to determine antibodies to CCP2 at a later time point. The anti-CCP2 antibody ELISA (Immunoscan RA Mark 2; Eurodiagnostica, Arnhem, The Netherlands) was performed according to the manufacturer's instructions with a cut off value of 25 units. At present more than 1600 early arthritis patients are included in the EAC-cohort and have a follow-up of at least one year. 454 patients fulfilled the diagnosis of RA according to criteria of the 1987 American College of Rheumatology one year after inclusion in the study. The treatment of the patients in our longitudinal cohort study is characterized by a secular trend. The 122 RA patients (61 anti-CCP negative and 61 anti-CCP positive) included between 1993 and 1995, were treated initially with analgesics and subsequently with chloroquine or salazopyrine if they had persistent active disease (delayed treatment); the 135 (70 anti-CCP negative and 65 anti-CCP positive) RA patients included between 1996 and 1998, were promptly treated with either chloroquine or salazopyrine (early treatment) (for further description see 10). The 197 RA patients (97 anti-CCP negative and 100 anti-CCP positive) included after 1998

were promptly treated with either methotrexate or salazopyrine (early treatment). The rheumatologists that treated the patients were not aware of the anti-CCP status of their patients because anti-CCP antibodies were not routinely determined at inclusion but were assessed for research purposes years after inclusion using stored serum samples. Patients gave their informed consent and the local Ethical Committee approved the protocol.

Radiograpic progression

Radiographs of hands and feet were made at baseline, at one year and yearly thereafter. For 138 patients a complete radiological follow-up was available for 4 years. Inherent to an inception cohort, not all included patients had already completed 4 years of follow-up. Radiographs were scored using the Sharp-van der Heijde method (11). The rheumatologist that scored the radiographs was blinded to the clinical data and unaware of the study question. The distribution of radiological destruction of the small joints was studied by comparing the erosion score and joint space narrowing score of the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints of the hands.

Statistical analysis

Differences in means between groups were analysed with the Mann-Whitney test or t-test when appropriate. Proportions were compared using the chi square test. In the analysis of the tender joint count and the swollen joint count, for each joint location, the scores for the left and right joints were summed. Furthermore, the scores for the individual metacarpophalangeal joints were summed, as well as the scores for the metatarsophalangeal joints and the interphalangeal joints of the hands and feet. For the 138 RA patients with complete 4 year radiological follow-up, the swollen joint count, erosion score and joint space narrowing score were determined for the individual metacarpophalangeal and proximal interphalangeal joints of the hands at inclusion and 2 and 4 years follow-up, and expressed in mean with 95% confidence interval (95% CI). The distribution and degree of radiological destruction and swelling of these joints was studied by comparing the variance of these scores for the individual joints. The 95% CI was used as a measure of variance; as the number of observations in this study is constant (138 patients at all time points during 4 years follow-up), the extent of the confidence interval reflects the degree of variance. Correlations between joint swelling and erosion score or joint space narrowing score were determined for each MCP and PIP joint of the hands using the Spearman correlation test. The statistical Package for Social Sciences (SPSS) version 12.0 (SPSS, Chigaco, IL) was used to analyze the data. In all tests, p values less than 0.05 were considered significant.

RESULTS

Early symptoms of disease

In total 454 patients fulfilled the ACR-criteria for RA; 228 of these patients had anti-CCP antibodies and 226 patients had no anti-CCP antibodies at inclusion. Patient characteristics and the type, localization and distribution of initial disease symptoms are depicted in Table 1. In both groups, 13% of patients reported to have no morning stiffness. In the patients that experienced morning stiffness the mean duration in the anti-CCP negative and anti-CCP positive patients was similar: 118 minutes and 123 minutes respectively. In both groups symptoms started with pain and swelling, predominantly symmetrical and in the small joints of the hands and feet. In the statistical analysis without correction for multiple testing, one difference in initial presentation between the two groups was observed: in anti-CCP positive patients symptoms started more often at both upper and lower extremities than in anti-CCP negative patients (20% vs. 11% respectively, $p<0.05$).

Table 1. Characteristics of the early symptoms in rheumatoid arthritis patients with and without anti-CCP antibodies.

	Anti-CCP– (N=228)	Anti-CCP+ (N=226)
Female n (%)	147 (64%)	150 (66%)
Age at inclusion Mean ± SD	57 ± 17	55 ± 16
Morning stiffness No n (%)	30 (13%)	30 (13%)
If yes, minutes (mean±SD)	118 ± 138	123 ± 128
Type of initial joint symptoms: n (%) #		
- pain	208 (91%)	205 (91%)
- swelling	146 (64%)	135 (60%)
- stiffness	106 (46%)	85 (38%)
- function loss	64 (28%)	57 (25%)
- redness or increased surface temperature of joints	19 (8%)	26 (12%)
Localization of initial joint symptoms: n (%)		
- small joints of hands and/or feet	105 (46%)	112 (50%)
- large joints	54 (24%)	50 (22%)
- both small and large joints	63 (28%)	59 (26%)
- unknown	6 (2%)	5 (2%)
Localization of initial joint symptoms: n (%)		
- upper limbs	114 (50%)	86 (38%)*
- lower limbs	72 (32%)	77 (34%)
- both upper and lower limbs	25 (11%)	45 (20%)*
- unknown	18 (8%)	18 (8%)
Localization of initial joint symptoms: n (%)		
- symmetric	145 (64%)	130 (58%)
- asymmetric	71 (31%)	83 (37%)
- unknown	10 (4%)	13 (6%)
VAS patients' rated global disease activity (0-100)	51.3 ± 39.9	46.7 ± 28.2
HAQ-score (mean ± SD)	1.0 ± 0.7	1.0 ± 0.7

Patients can have both swelling and pain at the start of the symptoms and therefore total can add to more than 100%. * P< 0.05, anti-CCP+ vs. anti-CCP–

Given the marginal p-value, that was not significant after correction for multiple testing, this finding was not considered a relevant difference. The mean patients' rated global disease activity on a VAS was not significantly different between the two groups. Likewise, the functional ability measured by a HAQ-score was similar in both groups. In conclusion, there are no fundamental differences in the early symptoms of disease between anti-CCP positive and anti-CCP negative RA patients.

Findings at physical examination at initial presentation

In each of the 454 patients a tender joint count and swollen joint count was performed at inclusion. The mean tender joint count per joint is presented in Table 2. There were no significant differences between RA patients with and without anti-CCP antibodies. Table 3 reveals the mean scores for joint swelling for both anti-CCP positive and anti-CCP negative patients, showing no statistical significant differences between the two groups. Thus, anti-CCP positive or negative RA patients cannot be distinguished at presentation by physical examination.

Table 2. Tender joint count (mean ± SD) at inclusion in rheumatoid arthritis patients with and without anti-CCP antibodies.

	Anti-CCP- (N=228)	Anti-CCP+ (N=226)
Temporomandibular joints	0.01 ± 0.41	0.08 ± 0.36
Sternoclavicular joints	0.23 ± 0.76	0.12 ± 0.47
Acromioclavicular joints	0.31± 0.63	0.55 ± 0.79
Shoulder joints	0.85 ± 1.5	0.86 ± 1.4
Elbow joints	0.42 ± 0.99	0.35 ± 0.81
Wrist joints	0.94 ± 0.94	0.80 ± 0.93
Metacarpophalangeal joints	4.3 ± 4.3	3.5 ± 3.4
Proximal interphalangeal joints of the hands	3.2 ± 3.6	3.3 ± 3.4
Distal interphalangeal joints of the hands	1.3 ± 2.4	1.2 ± 2.2
Hip joints	0.18 ± 0.73	0.11 ± 0.54
Knee joints	0.54 ± 0.88	0.59 ± 0.90
Ankle joints	0.41 ± 0.92	0.53 ± 1.1
Subtalar joints	0.31 ± 0.72	0.52 ± 0.76
Midtarsal joints	0.21 ± 0.40	0.18 ± 0.58
Metatarsophalangeal joints	4.2 ± 3.4	4.1 ± 3.7
Interphalangeal joints of the feet	0.91 ± 1.8	1.4 ± 3.2
Total Ritchie articular index score	10.4 ± 8.2	10.2 ± 8.0

Tenderness was scored per joint on a 0-3 scale; 0 = no tenderness, 1 = pain at pressure, 2 = pain and winced and 3 = winced and withdrew. The scores for the metacarpophalangeal joints were summed, as the scores for metatarsophalangeal joints and the interphalangeal joins of the hands and feet. The scores for the left and right joints were summed. The summed scores were divided by the total numbers of patients; the resulting means (± SD) are presented. There were no statistical differences between patients with and without anti-CCP antibodies.

Table 3. Joint swelling (mean ± SD) at inclusion in rheumatoid arthritis patients with and without anti-CCP antibodies.

	Anti-CCP- (N=228)	Anti-CCP+ (N=226)
Temporomandibular joints	0.01 ± 0.10	0.02 ± 0.18
Sternoclavicular joints	0.08 ± 0.34	0.04 ± 0.22
Acromioclavicular joints	0.06 ± 0.24	0.03 ± 0.17
Shoulder joints	0.08 ± 0.30	0.12 ± 0.40
Elbow joints	0.22 ± 0.54	0.20 ± 0.49
Wrist joints	1.0 ± 0.89	1.0 ± 0.90
Metacarpophalangeal jointss	3.2 ± 3.0	2.2 ± 2.2
Poximal interphalangeal joins of the hands	2.6 ± 3.1	2.0 ± 1.8
Distal interphalangeal joints of the hands	0.32 ± 0.60	0.21 ± 0.60
Knee joints	0.46 ± 0.74	0.49 ± 0.74
Ankle joints	0.31 ± 0.67	0.34 ± 0.63
Subtalar joints	0.24 ± 0.61	0.21 ± 0.55
Metatarsophalangeal joints	1.6 ± 2.2	1.8 ± 2.4
Interphalangeal joints of the feet	0.06 ± 0.24	0.18 ± 0.58
Total number of swollen joints	10.0 ± 7.2	8.6 ± 5.5

Swelling was scored for each joint on a 0-1 scale, 0 = no swelling, 1 = swelling. The scores for the metacarpophalangeal joints were summed, as the scores for metatarsophalangeal joints and the interphalangeal joins of the hands and feet. The scores for the left and right joints were summed. The summed scores were divided by the total numbers of patients; the resulting means (± SD) are presented. There were no statistical differences between patients with and without anti-CCP antibodies.

Acute phase reactant at initial presentation

The mean C-reactive protein level was 29.5 mg/L (SD 31.5) in the anti-CCP negative RA patients and 35.6 mg/L (SD 37.8) in the anti-CCP positive patients, and was not significantly different between the two groups (p=0.08).

Swollen joints at follow-up

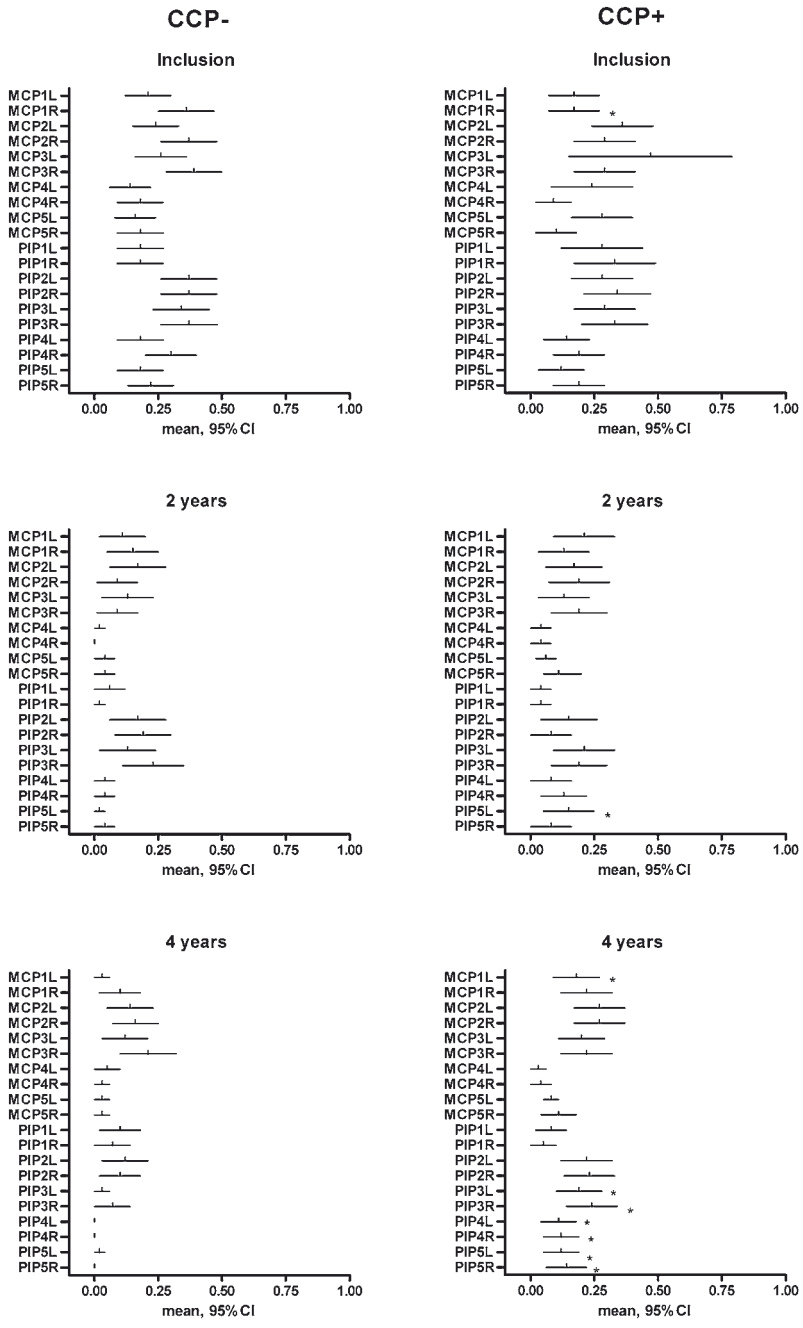
In the 138 early arthritis patients with complete radiological follow-up for 4 years the swollen joint count was assessed yearly. These patients had a mean age at inclusion of 53.7 ± 13.9 years, 67% (93 patients) were women and 74 patients (54%) were anti-CCP positive. The total number of swollen joints decreased during follow-up. At inclusion, in the anti-CCP negative patients the mean (±SD) number swollen joint was 10.0 ± 7.2; at 2 and 4 years follow-up the mean (±SD) number swollen joints was respectively 4.1 ± 6.7 and 3.1 ± 4.2. The mean (±SD) number swollen joints in the anti-CCP positive group was at inclusion 8.6 ± 5.5; this decreased to 5.2 ± 7.5 and 5.3 ± 6.8 at 2 and 4 years follow-up respectively. At 4 years follow-up the total number of swollen joints was significantly higher in the RA patients with anti-CCP antibodies (p=0.01).

In addition, the scores for the individual MCP and PIP joints of the hands were compared. Overall the pattern of inflammation of the individual small joints is similar in CCP-negative and CCP-positive RA as is depicted by the means and 95% CI of the swollen joint count in Figure 1. Several individual joints had significant higher scores in the anti-CCP positive patients compared to the anti-CCP negative patients; this concerned at inclusion the 1st MCP joint at the right side, at 2 years follow-up the 5th PIP joint at the left side and at 4 years follow-up the 1st MCP, 3rd PIP, 4th PIP and 5th PIP joints at the left side and 3th PIP, 4th PIP and 5th PIP joints at the right side ($p<0.05$). Furthermore Figure 1 shows that in both anti-CCP positive and negative RA patients, the second and third MCP joints were more frequently swollen than other MCP joints. Likewise, in both groups the second and third PIP joints were more frequently affected than the other PIP joints. In conclusion, the pattern of inflammation of the individual small joints of the hand seems similar in anti-CCP positive and negative patients, however, particular at 4 years follow-up some MCP and PIP joints are significantly less frequently swollen in anti-CCP negative RA patients.

Radiographic progression

In the 138 RA patients with complete 4 years radiological follow-up, the total Sharp-van der Heijde scores between the RA patients with and without anti-CCP antibodies were compared (Figure 2). At 2 and 4 years follow-up anti-CCP positive patients had significantly higher radiological scores than anti-CCP negative patients ($p<0.001$).

The distribution of the radiological destruction in the MCP and PIP joints of the hands was further investigated. The erosion scores and joint space narrowing scores of the MCP and PIP joints are depicted in Figure 3. As the most pronounced radiological destruction was present in anti-CCP positive patients, the erosion scores and joint space narrowing scores are shown for the RA patients with anti-CCP antibodies. Figure 3 shows that at all time points, of all MCP joints, the second MCP joints had the highest erosion score, followed by the third MCP joints. Concerning the PIP joints, the highest erosion scores were present in the third and fourth PIP joints. Figure 3 further reveals that the second and third MCP joints are the MCP joints with the highest joint space narrowing scores at all time points during follow-up. The joint space narrowing scores of the PIP joints differ less, but there are slightly higher scores for the third and fourth PIP joints. The erosion scores and joint space narrowing scores for the patients without anti-CCP antibodies revealed the same distribution as for the anti-CCP positive RA patients (data not shown). In the anti-CCP negative patients the values for the mean and 95%CI were lower than in the anti-CCP positive patients, which is in concordance with the finding of lower total Sharp-van der Heijde scores in anti-CCP negative RA patients. Correlations between joint swelling and erosion score and between joint swelling and joint space narrowing score were determined for each MCP and PIP joint at 4 years follow-up. For all PIP joints and



For each joint swelling was scored on a 0 -1 scale, 0=no swelling, 1=swelling;
*p<0.05

Figure 1. Joint swelling (mean and 95%CI) of the metacarpophalangeal and proximal interphalangeal joints of the hands at inclusion, 2 and 4 years follow-up in rheumatoid arthritis patients with and without anti-CCP antibodies.

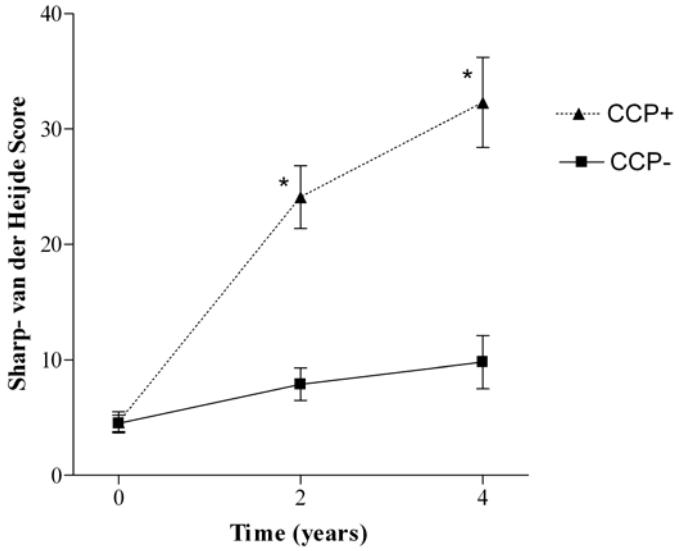


Figure 2. Total Sharp-van der Heijde scores (mean ± SEM) at inclusion, 2 and 4 years follow-up in rheumatoid arthritis patients with and without anti-CCP antibodies.

for all MCP joints, except the fourth MCP joints, the erosion score was significantly correlated with joint swelling ($p<0.05$). The joint space narrowing scores were significantly correlated with joint swelling in all MCP joints except the fourth MCP joint ($p<0.05$). This implies that at that time point the joints that were the most swollen were also the joints with the most severe radiological destruction.

DISCUSSION

This study shows that the phenotype of RA patients with or without anti-CCP antibodies does not differ at clinical presentation. In a large prospective early arthritis cohort we observed neither a significant difference in the reported first symptoms nor in the signs found at the physical examination at initial presentation between anti-CCP positive and negative patients. However, during follow-up anti-CCP positive RA patients have more swollen joints and show more radiological destruction than anti-CCP negative RA patients. Remarkable is that at follow-up, in spite of the difference in magnitude of disease characteristics, the distribution of swollen joints and the distribution of radiological joint space narrowing and bone erosions remains similar for RA patients with and without anti-CCP antibodies. This implies that, although different associations with known risk factors are reported for anti-CCP positive and negative RA patients, the presence or absence of anti-CCP antibodies is not associated with a distinguishable clinical phenotype at presentation of disease.

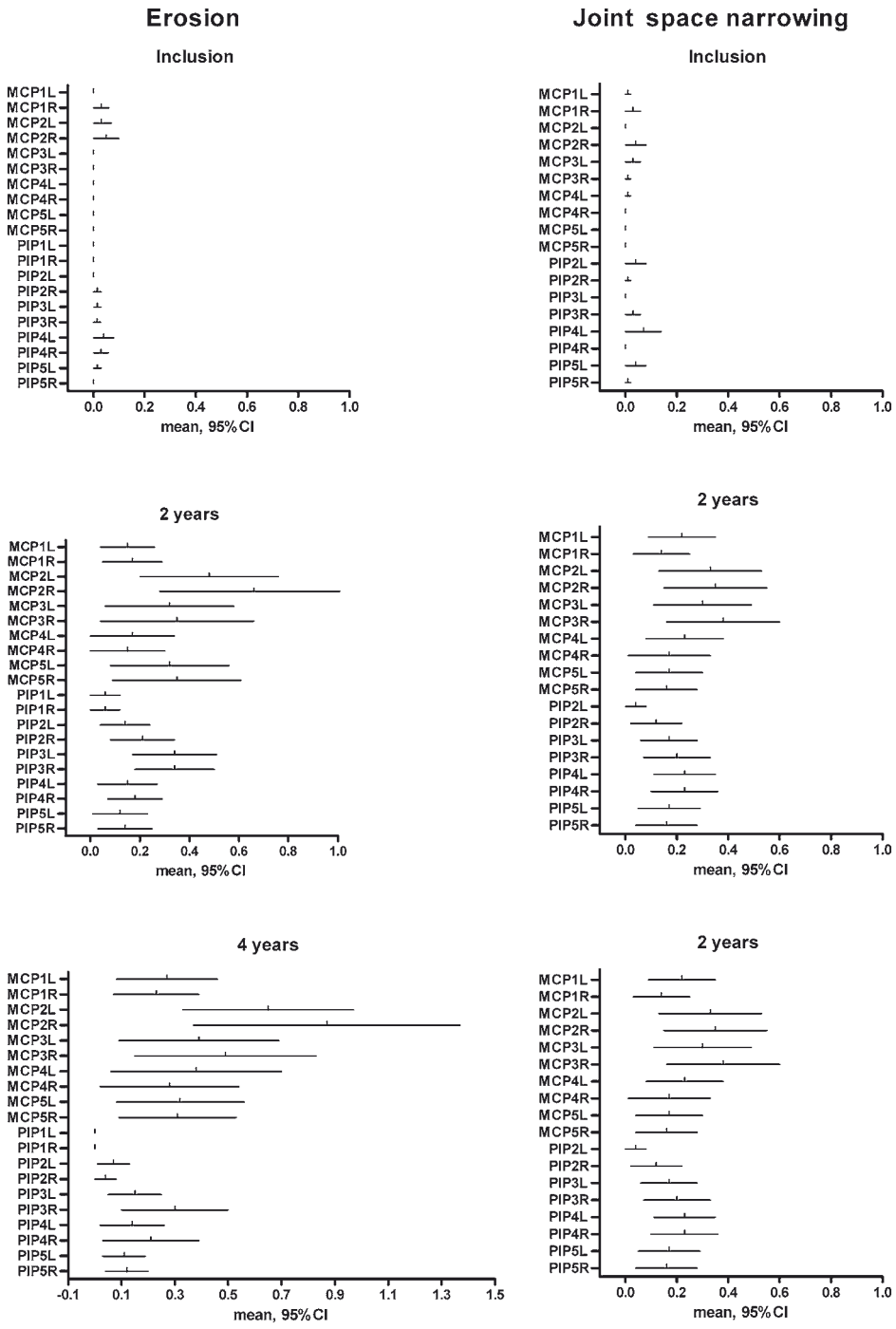


Figure 3. Erosion and joint space narrowing scores of the metacarpophalangeal and proximal interphalangeal joints of the hands (means and 95%CI) at inclusion, 2 and 4 years follow-up in rheumatoid arthritis patients with anti-CCP antibodies.

Pathophysiologically, this may have implications. It was recently observed that the prominent genetic risk factor HLA-Class II alleles only associate with susceptibility to RA in the presence of anti-CCP antibodies but not with RA in the absence of these antibodies (unpublished data, 5). In mice it has been shown that citrullination of arginine in a peptide can lead to a higher binding affinity of that peptide for HLA-DRB*0401, an important shared epitope allele (12), allowing peptide-specific T-cell induction. It can be speculated that also in humans citrullination may improve antigen presentation to CD4 positive T-cells and that the genetical background (presence of shared epitope alleles) provide the basis for a citrulline-specific immune reaction. It was demonstrated that anti-CCP antibodies occur years before disease onset (3,4). This last observation suggests that in anti-CCP positive RA patients the induction of disease occurs years before presentation, however the current study shows that the age of onset of clinical disease is similar in RA patients with and without anti-CCP antibodies. The risk factors such as HLA-alleles differ between anti-CCP negative RA and anti-CCP positive RA (5). Although differences in risk factors presume different pathophysiological pathways for anti-CCP positive and negative RA, the initial phenotypical presentation of both patient groups is similar and characterised by a symmetric poly-arthritis of the same small joints. At follow-up the clinical phenotype remains comparable with regard to joint distribution, but the anti-CCP positive patients have more inflamed joints and once there is inflammation also more rapid joint destruction. This leads to a pathophysiological model in which one or more triggers lead to arthritis in similar joints in anti-CCP positive and negative patients. Subsequently during inflammation antigens are citrullinated and in the presence of anti-CCP antibodies inflammation is aggravated, resulting in more severe radiological destruction. Further studies are needed to add insight into the pathogenic role of circulating anti-CCP antibodies in anti-CCP positive RA and to unravel risk factors associated with anti-CCP negative RA.

In a study by Kastbom et al (13) several baseline disease characteristics of anti-CCP positive and negative RA patients were compared. This study observed no significant differences in baseline total swollen joint count, C-reactive protein levels or DAS28 score between RA patients with and without anti-CCP antibodies, but showed a positive correlation between the number of fulfilled ACR-criteria and the frequency of anti-CCP positivity (13). Furthermore, in this study anti-CCP positive individuals were more often treated with disease modifying antirheumatic drugs than anti-CCP negative patients (13). Although in the present study secular trends in the initial treatment strategies with disease modifying antirheumatic drugs were present, these trends yielded the same effect for the anti-CCP positive and negative RA patients. Furthermore, the rheumatologists that treated the patients were not aware of the anti-CCP status of their patients. Therefore, the more severe disease course in patients with anti-CCP antibodies is not likely to be due to either a

more delayed treatment of these patients or confounding by treatment adapted to the anti-CCP status. We cannot exclude that during follow-up the anti-CCP positive patients that had more inflamed joints received more aggressive treatment. However, in case of a more aggressive treatment during follow-up in anti-CCP positive patients, this did not prevent the development of more severe radiological destruction in the RA patients with anti-CCP antibodies. The finding that the swollen joint count decreased during follow-up is probably due to the fact that at inclusion patients were not treated with disease modifying antirheumatic drugs.

The sensitivity of anti-CCP2 antibodies for RA is reported to vary between 39% and 80% (14,15). The present study measured anti-CCP2 levels at inclusion (a very early stage of the disease) and reports a relatively low percentage (50%) of RA patients with anti-CCP antibodies. As CCP measurements were not repeated during follow-up, we cannot exclude that some RA patients that were anti-CCP negative at inclusion have become anti-CCP positive on a later stage in the disease. A relatively low prevalence of anti-CCP antibodies in early arthritis patients has been described before (14).

The present study shows that the second and third MCP joints have the highest erosion scores, as well as the highest joint space narrowing scores and are, of all MCP joints, most frequently swollen. Although the present study was not designed to study the correlation between inflammation and destruction, the observed similarity in joints that are affected by swelling, erosions and joint space narrowing supports the concept that in general the mechanisms leading to clinical inflammation and radiological destruction are related.

This study includes a detailed description on the distribution of affected joints in RA and shows that the MCP joints of the second and the third digits are most frequently inflamed and destructed. Although to our experience rheumatologists generally feel that the joints of the second and third digits are more frequently inflamed than other joints of the hands, to our knowledge this phenotypic characterisation has not been frequently described.

CONCLUSION

The present study shows that, although separate risk factors for anti-CCP positive and negative RA were recently described, the clinical presentation of RA patients with or without anti-CCP antibodies is not different. Patients with anti-CCP antibodies develop a more severe disease course with more radiological destruction compared to RA patients without these auto-antibodies. Nonetheless, also at follow-up the distribution of affected joints is similar.

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Chapter 12

The invasiveness of fibroblast-like synoviocytes is an individual patient characteristic associated with the rate of joint destruction in patients with rheumatoid arthritis

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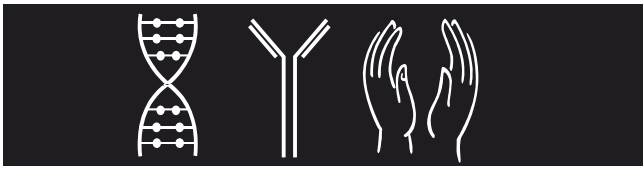

ABSTRACT

Objectives. Rheumatoid arthritis (RA) is characterized by inflammation and destruction of synovial joints. Fibroblast-like synoviocytes (FLS) harvested from synovial tissue from patients with RA can invade normal human cartilage in severe combined immunodeficiency disease (SCID) mice and matrigel in vitro. This study was undertaken to investigate the association of these in-vitro characteristics with disease characteristics in patients with RA.

Methods. Synovial tissue samples of 72 RA and 50 OA patients were obtained; from 7 patients with RA samples of different joints were collected. The FLS invasiveness in Matrigel matrix was studied; the intra-individual and inter-individual differences were compared. Of the patients with the FLS that exhibit the most extreme differences in in-vitro ingrowth (most and least invasive FLS) the X-rays of hand and feet were collected and read according to the Sharp-van der Heijde method to determine the relationship between in vitro invasion data and estimated yearly joint damage progression.

Results. FLS from patients with RA were more invasive than FLS from patients with OA ($P < 0.001$). The mean intra-individual variation in FLS invasion was much less than the mean inter-individual variation (mean ± SD 1067 ± 926 and 3845 ± 2367 for intra-individual and inter-individual variation, respectively; $P = 0.035$), which shows that the level of FLS invasion is a patient characteristic. The mean ± SEM Sharp score on radiographs of the hands or feet divided by the disease duration was 4.4 ± 1.1 units per year of disease duration in patients with the least invasive FLS ($n = 9$), which was much lower compared with the 21.8 ± 3.1 units per year of disease duration in patients with the most invasive FLS ($n = 9$) ($P < 0.001$).

Conclusions. The ex vivo invasive behaviour of FLS from patients with RA is associated with rate of joint destruction and is a patient characteristic given the much smaller intra-individual than inter-individual variation.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease which predominantly targets the synovial joints ultimately leading to joint destruction. The destructive process is suggested to be mediated, at least in part, by fibroblast-like synoviocytes (FLS) from the synovium, because in a severe combined immunodeficiency disease (SCID) mouse co-implementation model it was demonstrated that FLS of patients with RA attach to and invade in normal cartilage (1). Moreover, many groups have observed that in RA, FLS show characteristics of transformed cells like anchorage independent growth (2), insensitivity to apoptosis, and increased proliferation. Processes that are associated with the FLS-change from normal to aggressive behaviour are phosphorylation of the signal transducer and activator of transcription (STAT3) protein and elevated levels of the pro-oncogene c-myc (3). Whether these features are non causal associations or a causal factor is not yet known. FLS in culture express large amounts of proteases which can degrade extracellular matrix components such as collagens. One family of proteases that is expressed by FLS are the matrix metalloproteinases (MMPs). FLS express MMP-1, -3, -9 and -10 and expression of these MMPs correlate with invasion (4). Other protease families that are expressed in RA FLS are the cathepsins and ADAMs (a disintegrin and metalloproteinase). FLS from patients with RA express several oncogenes at higher levels than FLS from normal controls. Oncogenes that are upregulated in RA are c-myc (11), Ras (5) and p53 (6,7). These data have led to the suggestion that aspects of behaviour of FLS in RA resemble malignant tissue. However whether the transformed behaviour is of relevance for the disease characteristics of RA and if so for which characteristics has not been studied. The present study investigates the association of in vitro characteristics of FLS with disease characteristics in RA patients. We particular adressed the questions 1) whether the degree of FLS invasion is comparable in different joints of the same patient, i.e. is the FLS invasiveness a characteristic occurring at multiple joints of the same patient or rather a random process, and 2) whether the degree of in vitro invasion is correlated with the degree of radiological destruction.

MATERIALS AND METHODS

Patients and synovium

Synovial tissue was obtained from 122 patients (72 with RA and 49 with OA) at joint replacement surgery or synovectomy. 69% of the patients with RA were female and the mean age was 60 years ± 14. The samples were obtained from knee (53 patients), elbow (17 patients), shoulder (12 patients), hip (26 patients), ankle (7 patients), wrist (4 patients) and foot (1 patient). All patients with RA met the criteria of the American College of Rheumatology. Tissue was harvested by an orthopaedic surgeon and collected in ster-

ile phosphate buffered saline (PBS). Connective tissue and fat were removed and tissue was digested with collagenase IA (1 mg/ml; Sigma, St Louis, MO, USA) for at least two hours at 37°C. Cells were separated from the tissue using a 200 mm filter (NPBI, Emmer-Compascuum, The Netherlands) and cultured in 75 cm2 culture flasks (Cellstar, Greiner, Alphen aan de Rijn, The Netherlands) with Iscove's modified Dulbecco's medium (IMDM; Biowitthaker, Verviers, Belgium) supplemented with glutamax (GibcoBRL, Paisley, UK), penicillin and streptomycin (Boehringer Mannheim, Germany), and 10% fetal calf serum (FCS; GibcoBRL, Paisley, UK) at 37°C and 5% CO2. When the cells had grown to confluence they were detached with 0.25% trypsin and split in a 1:3 ratio. For invasive growth analysis passage 1 or 2 FLS were used. Light microscopy and Giemsa staining indicated that more than 95% of cells were FLS. As a control for the possible contamination of macrophages in the cell source, we reasoned that after several passages the macrophages will be gone. Thus the invasiveness of FLS from several patients was tested for several passages. This revealed stable invasiveness for several passages. Moreover, FACS staining of a randomly chosen subset of the samples revealed absence of CD14-positive cells.

In vitro invasion assay

Invasiveness of FLS was measured as described previously (4). Briefly, Transwells (6.5 mm diameter, 8.0 mm pore width; Costar, Cambridge, NY, USA) were coated with paraffin to avoid meniscus formation. Hereafter, the transwells were pre-incubated with 100 ml IMDM for 30 minutes at 37ºC. Transwells were coated overnight with 100 ml of 0.375 mg/ml Matrigel (Matrigel basement membrane matrix; Becton Dickinson, USA) in IMDM under sterile conditions in a laminar flow cabinet. The next day the Matrigel-coated wells were incubated with 100 ml IMDM for 1 hour at 37ºC. Cells were harvested as described above and after removal of the medium, 200 ml of 100,000 FLS/ml in IMDM was seeded in the inner compartment of the transwell system. In the outer compartment, 900 ml IMDM/10% FCS/10% human serum was pipetted and the cells were incubated for 3 days at 37ºC and 5% CO_2. After 3 days, the cells were fixed with 2% glutaraldehyde in PBS for 30 minutes at room temperature. After removal of the glutaraldehyde and subsequent washing with PBS, the cells were stained with a crystal violet solution for 30 minutes at room temperature. The cells were thoroughly washed with PBS and the cells that did not invade through the transwell membrane were removed together with the matrix by cleaning the inner wells of the transwell system with a cotton bud. The number of cells that had grown through the matrix and the transwell membrane were counted under a light microscope. All experiments were carried out in duplicate.

Radiological destruction and FLS characteristics

To compare the association between the degree of FLS invasiveness and joint destruction, the radiographs of hands and feet of the patients with the most invasive FLS (9 patients)

and least invasive FLS (9 patients) were scored according to the Sharp/van der Heijde method (8). The person that scored the radiographs was unaware of the clinical data and study question. The total erosion and joint space narrowing scores, as well as the total Sharp-van der Heijde scores, were divided by the disease duration from date of diagnosis to determine the radiological progression per year (9).

Statistical analysis

Differences in invasiveness of FLS between patients, differences between intra-individual and inter-individual variation and the radiological scores of the patients with the most and less invasive disease were compared with the Mann-Whitney test.

RESULTS

FLS were isolated from these tissues and cultured. When the cells had grown to confluency, the cells were harvested and tested for invasiveness as described before (4). RA FLS were significantly more invasive than OA FLS in this assay ($p < 0.001$ mean ± SD 2884±2326 and 4573±2502 for OA and RA respectively; Figure 1). These results are in line with previous studies (1;4).

Then it was studied whether the invasiveness of FLS from different joints operated at different times exhibit the same invasive characteristics. From 7 patients with RA, two

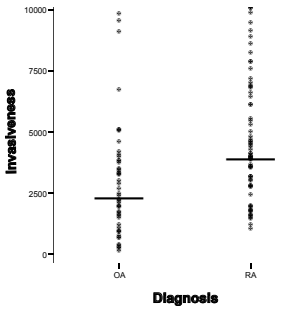

Figure 1

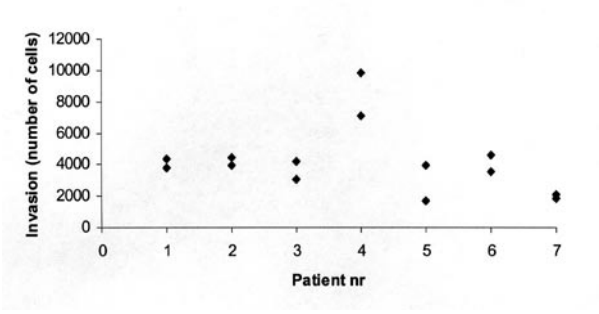

Figure 2

Figure 1. In vitro invasiveness of FLS from patients with RA (72) and OA (50). Each dot represents one individual. Invasiveness was measured using a transwell system coated with matrigel. The number of invasive cells was determined by counting the number of cell under the filter.

Figure 2. In 7 RA patients in vitro invasiveness of FLS was obtained from two different joints. Synovial samples were obtained at different times, and invasiveness was measured using a transwell system coated with matrigel. The number of invasive cells was determined by counting the number of cell under the filter. Tissue samples were obtained from hip (1), shoulder (3), elbow (6), wrist (3), ankle (1), knee (1).

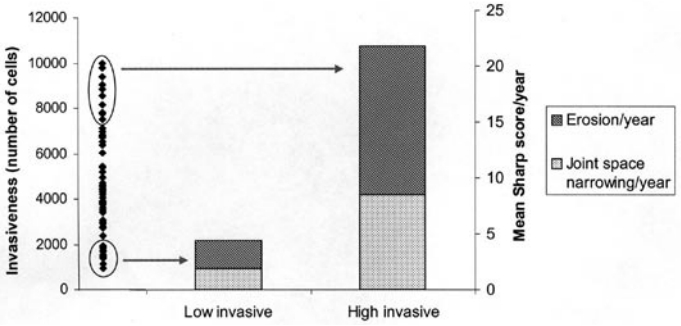

Figure 3. Association between in vitro invasiveness of FLS and estimated yearly radiological progression. From the nine most invasive and the nine least invasive RA patients the radiographs of hand and feet were scored according to the Sharp-van der Heijde method. The total Sharp-van der Heijde scores were divided by disease duration to determine the yearly radiological progression.

different samples were obtained from two different joints. The differences in invasiveness within the different samples of individual patients was significantly less than the differences in invasiveness between patients (p= 0.035; median difference ± SD: 1067 ± 926 and 3845± 2367 for intra- and inter-individual variation respectively). The results are shown in Figure 2. From the group of OA patients, three patients were operated twice. This group was too small to analyse the differences between intra-individual and inter-individual variation. Thus, the variation within patients is smaller than the variation between patients.

Next it was adressed whether the *in vitro* invasiveness of FLS was correlated with radiological joint destruction in RA. It has been published that the estimated yearly progression rate obtained by dividing the Sharp-van der Heijde score of radiographs of hands and feet by the disease duration correlated well with the observed destruction rate in observational studies with multiple measurements (9). Therefore, we assessed the association between invasiveness of FLS and radiological joint destruction. The Sharp-van der Heijde scores from the nine most invasive and the nine least invasive FLS were determined and the estimated yearly progression rates were compared (Figure 3). For the patients with the most invasive FLS the total Sharp-van der Heijde score per year disease duration is 21.8 (SEM 3.1), with an erosion score of 13.3 (SEM 1.7) and a narrowing score of 8.5 (SEM 1.6). For the patients with the least invasive FLS, the total Sharp-van der Heijde score per year disease duration is 4.4 (SEM 1.1), with an erosion score of 2.5 (SEM 0.7) and a narrowing score of 1.9 (SEM 0.5). No difference in disease duration was observed between patients with most and least invasive FLS (15.9 ± 11.5 versus 15.0 ± 7.3 years respectively; p=0.85). These results show a strong association between invasiveness of FLS and radiologically estimated yearly rate of joint destruction (p<0.001).

DISCUSSION

This study shows that in RA the intra-individual variation in FLS invasiveness is much less than the inter-individul variation, indicating that the invasive behaviour is a characteristic of an individual RA patient. Furthermore, this study shows for the first time that the in vitro FLS invasiveness is associated with radiological joint destruction. Patients with the least invasive FLS have significantly lower Sharp-van der Heijde scores per year disease duration compared to the patients with the most invasive FLS. This suggests that the invasive behaviour of FLS is of relevance for the pathogenesis of RA.

The finding of a rather large variation in rate of invasion of FLS between patients implies that the mechanism or processes underlying invasive behaviour differs between individuals. The mechanism that leads to transformation of FLS is not fully understood.

Previous studies have shown a myriad of alterations in the behaviour of FLS in RA. One very striking change in FLS is the expression of oncogenes (10). Oncogenes that are upregulated in RA are c-myc (11 ;12), Ras (5), p53 etc. Inhibition of the Ras pathway reduced expression of MMP-1 and MMP-3. Inhibition of both Ras and c-myc pathways also reduced invasion into normal human cartilage in the SCID mouse coimplantation model (13). And it is demonstrated that down-regulation of p53 influences proliferation and invasion of RA FLS (14;15). Transformation of FLS is different in different individuals, suggesting that a genetic component plays a role.

This study shows that the transformed behaviour of FLS in patients with RA is a patient characteristic and is strongly associated with clinical joint destruction. We used FLS from passages 1 and 2 in this study. Although no macrophages were detected in these samples by light microscopy and Giemsa staining, and no CD14-positive cells were detected in a randomly chosen subset of the samples, we cannot exclude unambiguously that macrophages contaminated the cells. However, the experiments in which FLS from different passages and from different donors were compared yielded similar levels of invasiveness, which is evidence against major artefacts from macrophages. Further research should elucidate the exact mechanism of transformation of FLS and the role of a genetic component.

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Chapter 13

Variation in radiological joint destruction in rheumatoid arthritis differs between monozygotic and dizygotic twins and pairs of unrelated patients

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Currently, the research on genetic factors associated with rheumatoid arthritis (RA) flourishes and the number of genetic factors found to associate with RA susceptibility has expanded in the latest years. Besides the long-known HLA class II alleles, a R620W SNP in the PTPN22 gene has been identified and replicated in several different populations (1). A number of other SNPs also confer risk to RA; some of them await replication or gave distinct results in different populations (2). The total genetic contribution to RA susceptibility is estimated by comparing monozygotic and dizygotic twins and is reported to account for 50-60% of the population liability to the disease (3). As the HLA-Class II alleles account for about one third of the genetic contribution, it is expected that in the nearby future more genetic factors will be identified. Currently most attention is paid on the role of genetics in RA susceptibility. So far the contribution of genetics to RA severity is not known. The recognition of genetic factors associating with RA severity might be helpful in predicting the disease course and may lead to the identification of pathways involved in joint destruction. To get more insight in the role of genetics in RA severity, the present report investigates the variability in radiological joint destruction between monozygotic and dizygotic twins and random pairs of unrelated RA patients.

This study includes the monozygotic (n=20) and dizygotic (n=8) twins with RA that were enrolled in the North American RA Consortium (NARAC). The unrelated RA patients were included in the Leiden Early Arthritis Cohort (EAC) (4). The median disease duration at the time of the radiograph was 9 years in dizygotic twins and 10 years in monozygotic twins. The monozygotic twins were all rheumatoid factor positive (see Table 1). To match for differences in era of disease onset and autoantibody status, the unrelated RA patients selected from the EAC cohort were rheumatoid factor positive and included in the cohort in the early or mid '90 years. This resulted in 40 persons with available radiographs made after 8 years of disease; these patients were by the computer assigned in 20 random pairs. All subjects were Caucasian and had radiographs of the hands scored according to the Sharp-Van der Heijde method (5) by a rheumatologist that was unaware of the clinical data (intraclass correlation coefficient for repeated readings 0.96, 95% confidence interval 0.83-0.99). To correct for differences in disease duration, for every subject the Sharp score was divided by the disease duration at the time of the radiograph, yielding the estimated radiological progression per year (6). To determine the variation in joint destruction, for every pair of RA patients, the difference in radiological progression per year was calculated. Subsequently, to evaluate the variation in joint destruction in the different groups of patients, the mean difference and variance in radiological progression per year were compared between monozygotic and dizygotic twins and unrelated patients with RA.

Patient characteristics and results on radiological joint destruction scores are presented in Table 1. Both monozygotic and dizygotic twins were significantly younger than the pairs

Table 1. Patient characteristics and radiological joint destruction scores of monozygotic and dizygotic twins with RA and pairs of unrelated RA patients

	Monozygotic twin (n=20)	Dizygotic twin (n=8)	Unrelated RA patients random pairs (n=40)
Gender, % female	70	63	65
Age at diagnosis, mean ± SD	37.1 ± 11.4	31.5 ± 17.3	53.8 ± 12.3#
Disease duration at radiograph, median	10.5	9.0	8.0
Rheumatoid factor positivity, %	100	63	100
Estimated radiological progression per year ¶, mean ± SEM	2.8 ± 0.6	1.7 ± 0.8	3.3 ± 0.6
Difference in Sharp scores per year within pairs, mean	1.7	2.4	4.3 *
variance	3.3	6.1	16.1

# p< 0.001 unrelated RA patients versus monozygotic as well as dizygotic twins

* p= 0.037 unrelated pairs of RA patients versus monozygotic twins

¶ Sharp score of the hands divided by the disease duration at the time of the radiograph

of unrelated patients (p< 0.001 Mann Whitney test). The mean estimated radiological progression per year was not significantly different among the groups of monozygotic twins, dizygotic twins and unrelated RA patients. However, the variation in joint destruction was the highest within the pairs of unrelated RA patients (mean difference 4.3 Sharp points per year), followed by the dizygotic twins (mean difference 2.4 Sharp points per year) and the smallest difference was observed within the monozygotic twins (1.7 Sharp points per year), with a significant difference between the monozygotic twins and unrelated RA patients (p=0.037, Mann Whitney test). The finding of an increasing variation in degree of joint destruction comparing respectively monozygotic twins, dizygotic twins and unrelated pairs of RA patients supports the notion of a role for genetic factors in RA severity.

The current report indicates that genetic factors are associated with the severity of joint destruction during the disease course of RA. This study has some limitations. First, as the number of dizygotic twins was low, the heritability could not be properly calculated. Classic twin studies compare monozygotic and dizygotic twins and are based on the principle that the environmental factors within twin pairs are (mostly) comparable and that the contribution of genetic factors to covariance within dizygotic twins is half that of monozygotic twins. Unrelated patients are not used for quantifying the genetic contribution, as these patients differ in genetic as well ass environmental factors. Nevertheless, as RA starts and progresses at adult age, environmental factors are presumably also different within twin pairs. Therefore, comparison of mono- with dizygotic twins as well as twins with unrelated patients may be relevant. The current report added data on unrelated pairs of

RA patients to dizygotic and monozygotic twin data and observed an increase in variation in joint destruction parallel to a decrease in genetic similarity, indicating that genetics factors are important for the severity of joint destruction in RA.

It might be a concern that twins and unrelated RA patients, although both Caucasian, came from different continents. However, comparison of Sharp scores of American and Dutch early RA patients not treated with disease-modifying antirheumatic drugs (DMARDs) revealed similar scores (7,8).

The most important risk factor for RA severity is the autoantibody status. The unrelated RA patients were matched for rheumatoid factor comparable to the monozygotic twins. Matching on anti-CCP status was not possible as anti-CCP antibodies were determined in only a part of the twins. As the presence of autoantibodies associates with the presence of HLA-shared epitope alleles (9), it is likely that by matching for autoantibodies patients were also partly corrected for differences in shared epitope status. This might have underestimated the difference in variation due to genetic factors.

Given the hypothetical effect of the difference in age of onset between the twins and unrelated RA patients on the rate of joint destruction, the Sharp scores of EAC patients <35 and >60 years were compared, showing no significant difference during 4 years follow-up.

The patients included in this study were diagnosed with RA in the ‘80 years or beginning of the ‘90 years. We did not provide detailed descriptions of treatment history of all patients and cannot exclude differences in treatment. Nevertheless, in the mentioned time era’s therapy with DMARDs was started in a relatively late stage and medications of choice were among others antimalarials, of which is known that their ability to halt disease progression is limited. Therefore, we suspect that considering the relatively mild medications used in the ‘80 and begin ‘90, the natural disease course of the patients is only limited affected.

In conclusion, the present study observed after correction for differences in disease duration and autoantibody status an increase in variation of radiological joint destruction comparing respectively monozygotic twins, dizygotic twins and unrelated pairs of RA patients. This indicates to an important contribution of genetic factors to radiological joint damage in RA. More extensive twin studies are needed to quantify the genetic contribution to disease severity.

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Chapter 14

Genetics and clinical characteristics to predict rheumatoid arthritis. Where are we now and what are the perspectives?

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ABSTRACT

In medicine, the perspective of prediction contains three major issues: the variability of the host, the characteristics of the disease-causing agent and the interaction between these factors (the disease process itself). The current review focuses on the prediction of RA in patients with undifferentiated arthritis. Data from inception cohorts have revealed that in about a third of the patients presenting with recent onset arthritis to a rheumatologist no diagnosis can be made, so-called undifferentiated arthritis. In a portion of these patients RA develops, whereas a substantial portion remits spontaneously. Current epidemiological data on RA incidence rates are compatible with host (genetic) factors being an independent predictor of susceptibility to RA. The majority of the genetic factors have still to be identified but HLA-alleles and PTPN22-alleles have now been identified as risk factors in a number of populations. Disease characteristics such as anti-CCP antibodies and erosions on X-rays are identified as being of high predictive value. In the future, models that take into account both genetic and clinical characteristics will have to be evaluated in patient groups with undifferentiated arthritis in order to establish the accuracy of these models in predicting with an 80% probability the chance on progression to RA. Such prediction models will be of help in treatment decisions in these patients.

INTRODUCTION

A number of different items are used in clinical prediction models. These items vary from variability of the host, the variability of the causing agent, and variability in the disease process and finally the dynamics of the interaction that takes time into account as well. Examples of the relevance of measuring pure host characteristics are e.g. the BRCA-1/2 carriership in families with a history of breast/ovarian cancer. Examples of the relevance of the characteristics of the disease-causing agent in sensu strictu are the causing micro-organisms in infectious diseases such as in community-acquired pneumonia. An example in rheumatology when both host and causing agent determine susceptibility is that certain micro-organisms (e.g. Chlamydia) particularly cause disease (reactive arthritis) in hosts that are HLA-B27 positive. Examples to detect differences in prognosis by studying the mode of interaction of host and disease-inducing process are the study of characteristics of diseased tissue such as micro-array studies in breast cancer. An example when the evolution of the disease over time is included in the determination of a response parameter is cervical abnormalities detected by a slightly abnormal cervical smear test. The value of such a test can be that the cervical smear test is advised to be repeated within three months to see whether natural regression of the abnormalities has occured or whether progression to abnormalities indicative of precancerous characteristics has occurred.

The outcome of a prediction model can be the development of a disease or disease severity. In rheumatology and particularly in rheumatoid arthritis (RA), physicians generally want to avoid morbidity and disability. Existing prediction models are therefore built to predict chronicity and erosiveness. Prediction models are of importance as they might help in treatment decisions. They may guide the choice in treatment options between wait and see, start with a relative mild treatment or initiate aggressive treatment directly. The current evidence on (early) treatment of RA is based on large trials with RA patients, in which RA is defined according to the ACR criteria. Inception cohorts include patients in who during the first visits with the current methodology a diagnosis can directly be made (about 60% of patients). About forty percent of patients in inception cohorts have a form of arthritis in which no definite diagnosis can be made; these patients are identified as undifferentiated arthritis (UA). These UA patients can go into remission, develop RA or develop other diagnosis (see Figure 1)(1). At present no data on the effects of treatment of UA patients are available. Early treatment of the patients with UA that will develop RA might be beneficial, whereas treatment of the group that will remit spontaneously is potentially harmful. The spontaneous remission rate of UA patients is about 40% (1). As current knowledge on the effects of treatment in RA is based on patients with RA classification according to the ACR-criteria it will be helpful to have a model to predict development of RA in UA patients. The prediction of RA is

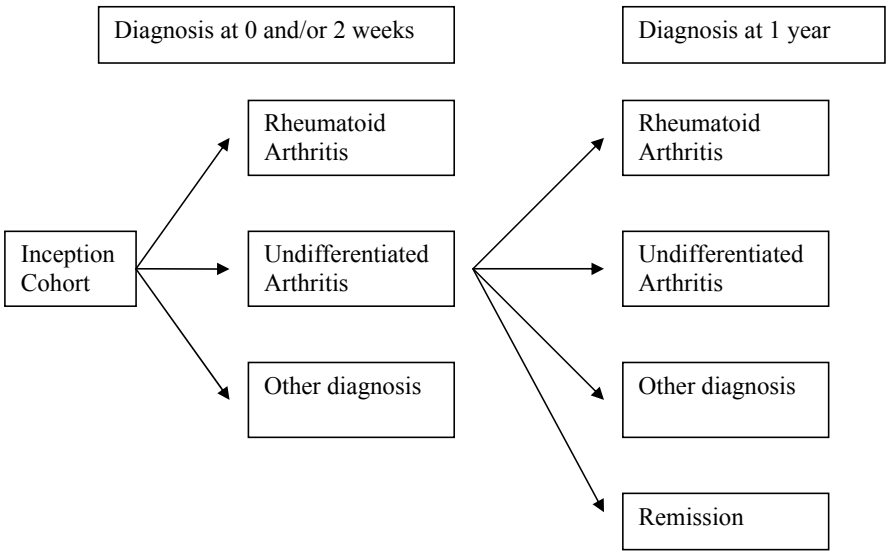

Figure 1. Flow diagram of inception cohort.

in most cases synonymous with prediction of disease persistency as the remission rate of RA is about 10-18% (2,3).

The current review is focussed on prediction of RA. The following sections will review the evidence that pure host characteristics are informative, pure disease characteristics are relevant and which evidence is available that a combination of disease characteristics, host characteristics and natural course allows prediction. The applicability of prediction models is also determined by basic epidemiological rules and the predictive value of a test/model is dependent on the prevalence of the disease in a given population. For the question on genetic testing "where we are now and what are the perspectives" this is relevant because most current data on genetic factors describe the comparison between cases and healthy controls. These are relevant data to undrape pathogenesis, but are difficult to be interpreted with regard to relevance in diagnostic testing in individual patients.

VARIABILITY IN THE HOST DETERMINES INCIDENCE OF RA

This hypothesis has a number of relevant implications for RA. First, it assumes that the trigger or triggering events for RA are common and therefore the opportunity to encounter these triggering events is not the limiting or determining factor. Second, it has implications on the thoughts whether RA is one disease or an assembly of truly different diseases and third it assumes that a number of DNA variants are associated with disease and that a DNA fingerprint might predict disease susceptibility.

Does RA have a common trigger?

The assumption that RA has a common or a combination of commonly available triggers imply that such triggers can be encountered in most populations. An argument in favour of this assumption is that RA is a worldwide condition indicating that the triggers leading to RA are worldwide available. The incidence of RA is age related, with a higher RA incidence with higher age. This age-related incidence of RA provides some evidence how many triggers are needed to get RA. Roberts-Thompson et al. studied population data obtained from the Australian Bureau of Statistics in order to assess the number of events necessary leading to RA (4). By computer modelling in which the age-specific incidence rates, the proportion of population at risk and the age at onset are included, the number of random events that must occur for the disease to manifest (given a stochastic model) was calculated. This number varied somewhere between 4 and 6 events. In inception cohorts, the patients with UA at inclusion that after 1 year of follow-up had persistent UA were significantly younger than the UA patients that during the first year developed RA (1). The difference in age between the groups that do and do not progress to RA might reveal a difference in time period and subsequent chance to acquire sufficient numbers of triggering events.

RA has a variable incidence in different populations. In Pima Indians the incidence rates in the same time periods were 10 times higher than in the Caucasian US population and 5 times higher than in the Japanese population (5). This implies that the frequency of the events leading to RA is different in the different populations and/or that the host factors in the different populations differ. The relative contribution of genetic or environmental factors is difficult to determine, but based on studies of populations that have migrated to different environments, it is likely that the majority of the difference in rates of RA in different populations can be explained by genetic factors (6). Moreover, the differences in the frequency of the identified genetic risk factor for RA, the HLA-alleles encoding the shared epitope, associate with the frequency of RA in the respective populations (7). An additional argument that RA is caused by commonly available triggers is the absence of geographical clustering of RA incident cases (8). In the aggregate, these data suggest that in modern lifestyle a combination of triggers is common in a variety of cultures but that host characteristics determine whether these triggers can lead to RA.

Further understanding can be achieved by studying populations among whom RA is not prevalent. A study in indigenous people (Aboriginals) in Australia found no paleopathological or ethnographical evidence to support the existence of RA before white settlement (9). Similarly in a rural Nigerian population RA was not observed (10) indicating that either the common trigger was not present at that time or that these populations are genetically protected. Arguments for this last statement are the much lower frequency of HLA-DR alleles encoding RA risk alleles in these populations. Careful studies have now identified RA in Aboriginals. However, in all the Aboriginal RA patients some evidence of

prior interracial marriage was found. This indicates that genetic admixture is necessary for development of RA. Yet, a contribution of changing lifestyles that is concomitant to racial admixtures cannot be easily excluded.

In a study from Minnesota that studied RA incidence rates from 1955 to 1995, the incidence rate fell progressively over the 4 decades of study, from 61.2/100,000 in 1955-1964, to 32.7/100,000 in 1985-1994 (11). A Japanese study showed that the incidence rates fell in Japan as well and the falling incidence rates over time have occurred in diverse populations such as Indians and Finnish (12). There are several possible explanations for the decrease in RA incidence. As this decrease is apparent in various populations, an explanation is likely a factor that has an identical effect in all populations throughout the world in the birth cohorts from 1890 to 1950. It is proposed that this is caused by a change in the population genome (13). The explanation for this genetic drift is that in previous times, human reproductive success was very unevenly distributed, with a minority of fertile women who gave birth to the majority of newborns. For example, in the 1912 Australian census, 50% of the children were the offspring of 1 in 7 of the women (14). However, in recent times this predominance steadily decreased since both fertile and less fertile women have equally contributed to the next generation. There are also other explanations for the decrease in RA incidence rates over time. Besides a real time dependent decline in RA, changing methodology in classification may be also important (15). In addition to a decrease in RA incidence, a decrease in RA severity over time is also reported. This decline seems to be contributable to earlier and more aggressive treatment (16).

In summary, the overview of the studies on incidence rates of RA are compatible with the notion that host characteristics are the major factors that drive whether a patient will develop RA. We suggest that most persons nowadays will encounter those 4-6 triggering events and that host factors are the driving force to explain differences in incidence rates.

What is the evidence in support of the assumption that RA is one disease versus an assembly of different diseases?

At present, RA is formally diagnosed when patients fulfil the criteria that were formulated by the ACR in 1987. Whether the patients that have RA according to these criteria, all have the same disease -characterised by an identical pathogenesis-, is questionable. Recently it was observed in a European and American population that RA patients carrying antibodies to citrullinated proteins (anti-CCP antibodies) have an association with different genetic risk factors than patients that lack these antibodies. The shared epitope encoding HLA-alleles only conferred risk to anti-CCP positive RA and not to anti-CCP negative RA (17). Anti-CCP antibodies are reported to have high disease specificity and are often present before the clinical presentation (18,19); anti-CCP antibodies are therefore thought to play a role in RA pathogenesis. The finding that the shared epitope alleles only correlate

with anti-CCP positive disease strongly suggests that RA patients that have anti-CCP antibodies have differences in the pathophysiological pathway compared to RA patients that are anti-CCP negative. This consequently induces the question whether anti-CCP positive and negative RA are different disease entities with distinct clinical characteristics. In a recent study RA patients with and without anti-CCP antibodies were extensively compared with regard to clinical characteristics. No differences were found in the characteristics on disease presentation between these two patient groups: among others the age of disease onset, the type of initial symptoms, the distribution of initial symptoms, the presence and duration of morning stiffness, and the number and distribution of painful or swollen joints was similar in anti-CCP positive and negative RA patients (20). From these data it can be concluded that different pathophysiological pathways end in one phenotypical presentation of the disease. Specific characteristics of the host such as the presence of anti-CCP antibodies subsequently associate with the course of the disease.

Which potential genetic risk factors for RA are known?

The HLA Class II molecules are the most powerful recognized genetic factors so far for RA, contributing to at least 30% of the total genetic effect. The HLA-DRB1 alleles *0101, *0102, *0401, *0404, *0405, *0408, *1001, *1402 share a conserved amino acid sequence at position 70-74 in the third hypervariable region of the DRβ1 chain. These residues constitute an α-helical domain forming one side of the antigen presenting binding site. The Shared Epitope hypothesis postulates that the shared epitope motif itself is directly involved in the pathogenesis of RA by allowing the presentation of an arthritogenic peptide. Extensive evidence exists showing associations between the shared epitope encoding alleles and susceptibility to RA. The presence of shared epitope encoding alleles is associated with odds of 3-4 to develop RA (21,22).

The second genetic risk factor is a risk allele of a haematopoietic-specific protein tyrosine phosphatase, PTPN22. This allele was identified in 17% of the North American white controls and 28% of the RA patients and confers odds of about 2 to develop RA (23-26). This allele changed the function of the protein that functions as negative regulator of T-cell activation, leading to T-cells with a lower threshold for T-cell activation. This mutation is apparently leading to several autoimmune diseases since this mutation also conferred risk for SLE, type 1 diabetes and Graves disease (27,28). The last years an increasing number of SNPs associated with RA have been identified. Some of them have not been replicated and some show different results in different populations. A genetic risk factor that is currently under investigation and seems to be associated with RA, diabetes and myocardial infarction, is MHC2TA. This SNP associates with a lower expression of MHC molecules and in a Swedish cohort of 1288 RA patients and 709 controls; this SNP conferred a 1.3 higher risk to develop RA (29). The findings on this SNP await replication. In Japanese patients and controls an association between haplotypes (combinations of SNPs on one chromosome

that tend to inherit together) of the gene encoding PADI4 with increased susceptibility to RA was observed (30). The RA-susceptible PADI4 variant was shown to produce a more stable transcript than the non-susceptible variant, implying an increased production of PADI4 and therefore higher level of citrullination by the RA-susceptible variant. Unless a higher level of citrullination, the described PADI4 haplotypes did not correlate with (the level of) anti-CCP antibodies (30). The association of PADI4 with RA is shown in the Japanese population. Data from Caucasians from France and the UK showed no association with PADI4 haplotypes and RA (31,32). Susceptibility genes can interact such that the resulting predisposition of carrying both genes is larger than the summed odds ratios of the individual genes. The presence of such interaction is important with regard to prediction. In 820 Japanese RA patients and 620 controls a risk for RA of 1.3 was identified for a risk allele in the organic cation transporter gene SLC22A4 9(33). Intriguingly, the identified SNP affects the transcriptional efficiency of SLC22A4 in vitro by altering the binding affinity of a haematopoietic transcription factor, called RUNX1. A small but significant association was observed with the minor allele in the RUNX1-gene. Importantly homozygosity for both susceptibility alleles (SLC22A4 and RUNX1) showed a high odds ratio of 9, indicative for a gene-gene interaction (33). Recently the effects of this RUNX-1 SNP was not found in a Caucasian population. (34). A SNP in the promoter region of FCRL3 is recently shown to be associated with susceptibility to RA (35). For a large number of other genes suggested to be relevant in the pathophysiology of RA, association was observed in only one study without replication. These studies concerned Beta-adrenergic receptor gene SNPs, RANKL, ICAM-1, VEGF, PDCD-1 and IL1-RA gene (36-40).

Besides genetic risk factors that confer a higher risk to develop RA, there also a genetic risk factors that protects to RA. This concerns particularly the HLA-DRB1 alleles that encodes for the amino acids DERAA (DRB1*0103, *0402, *1102, *1103, *1301, *1302, *1304). Interestingly, the HLA-DRB1 alleles can encode for different alleles with an opposite effect on disease susceptibility. The protective effect of the DERAA-encoding alleles is independent from the shared epitope encoding alleles with predisposing effects (22,41,42). Both in the presence and in the absence of shared epitope encoding alleles the DERAA-encoding alleles confer significant lower odds of 0.6 to develop RA (22).

In summary, the current knowledge on well-validated genetic risk factors to be included in a DNA fingerprint is limited to HLA-DRB1 and PTPN22. HLA-DRB1 is estimated to account for ~30% of the genetic component of this autoimmune disease (43) while the contribution of PTPN22 is much smaller. This implies that a significant part of the genetic contribution is still to be identified. In a number of whole genome scans (44) a considerable number of peaks of linkage have been identified. In a study to estimate the number of true RA gene regions that took into account both the heterogeneity of RA and the performance of a dense genome scan, it was found that 8 +/- 4 regions (mean +/- SD) were true-positives and evidence for 3 additional regions was provided from covariate-based

analysis (45). One of those regions is the HLA-DRB1 locus, meaning that at least 10 +/- 4 additional genes will be identified with each quite modest effect. Technical progress such as SNP-based linkage analysis has been demonstrated to allow loci to be defined more precisely (46). The chance that this will lead to the identification of the majority of the genetic risk factors is larger when RA is caused by a dozen common genetic variants than when RA is the result of many rare mutations. Given the fact that HLA and PTPN22 have already been identified, we speculate that RA is caused by a dozen common genetic variants. The statistical methods to evaluate many gene variants with disease status, as in candidate-gene case-control studies are still in its infancy especially for the low effect sizes of the individual disease loci and the sometimes low frequencies of the disease allele(s). The standard methods to evaluate the association of multiple markers with disease status are based on multimarker multivariate analyses. For such multimarker multivariate analyses, one typically uses logistic regression to test simultaneously the main effects (and possibly interactions) of multiple markers. For each marker, a covariate can be created, such as the number of rare alleles at each marker. When this type of coding is used in logistic regression, the resulting score statistic for each marker implies many degrees of freedom, implying that the overall model suffers from weak power. Moreover complex models tend to overfit the data stressing the necessity for replication in independent cohorts. Despite these difficulties the outlook is that the genetic contribution to RA is about 50-60% (47). This number is estimated by the comparison between the concordance rates in monozygotic twins and the prevalence in the respective populations (47). This high percentage implies that measurement of genetic host characteristics is likely to have a role in a predictive test.

Which environmental risk factors for RA are known?

So far smoking has been shown the only plausible environmental risk factor for RA. An association with smoking in RA is particularly found for rheumatoid factor positive RA compared to rheumatoid factor negative disease (48,49). Current or ex-smokers have a risk for autoantibody positive RA with an odds ratio of 1.7-1.9. The risk increases with cumulative dose of smoking (48). A recent report investigated whether smoking is primarily associated with the development of rheumatoid factor or anti-CCP antibodies. This study nicely revealed a gene-environment interaction by showing that in the presence of HLA-shared epitope alleles smoking significantly contributes to the development of anti-CCP antibodies (50).

A predictive effect of oral anticonceptives on RA has been claimed (51). This finding has however not been replicated in the Nurses' Health study (52).

Predictive value of a DNA fingerprint-test

A large problem in transferring the data on genetic risk factors to prediction models is that the most current studies compared patients and controls, revealing odds ratios that are determined on group levels. The value of these genetic risk factors for individual predictive testing may be limited. Compare for example the statistical models to predict the pre-test probability on BRCA-1/2 genes. BRCA1 or 2 carriers have a very strong risk for ovarian/breast cancer. The statistical models to predict the presence of a BRCA1/2 risk allele are only informative in a selected population with affected family members (53). This example underlines that findings for a whole group cannot be automatically used for prediction in subgroups of patients or for individuals. Genes may confer risk to subgroups of RA patients. For example, the well-known HLA-shared epitope alleles predispose particularly to anti-CCP positive RA (17). The BRCA- example also elucidates that the predictive value of a test depends on the prevalence of a disease in a population. For UA, a number of inception cohorts of patients with recent onset arthritis have identified patients with a form of arthritis that has the potential for a persistent course, without fulfilling the classification criteria of other rheumatic disorders (54). In nine cohorts, the proportion of patients with UA that evolved into RA within 1 year varied from 17% to 32%. Thus, in this group of UA patients the pre-test probability of developing RA varies between 17-32%. Given the dynamics of development of UA to either remission or progression to RA, the evaluation of predictive models for this patient group is highly relevant.

CHARACTERISTICS OF THE DISEASE PROCESS AND PREDICTION

The theoretical background of this section is the assumption that the expression of the disease in an initial phase allows prediction of the outcome. The genomic revolution has fuelled much optimism that gene expression profiles allow such outcome measures. Gene expression profiles are currently used in breast cancer to select the patients that would benefit from adjuvant therapy (55). However, others warned that the prognostic value of the published micro-array results in cancer studies should be considered with caution as the list of genes identified as predictors of prognosis was highly unstable and the molecular signatures depended strongly on the selection of patients (56). The prognostic value of the several micro-arrays in oncology therefore needs replication.

Disease characteristics present at the presentation of UA that predict progression to RA

The most important and best-validated disease characteristics with regard to prediction are auto-antibodies (anti-CCP and rheumatoid factor, RF) and the presence of erosions on the radiographs of hands and feet at initial presentation. In univariate analysis, the

presence of anti-CCP antibodies in patients with a UA conferred an odds ratio of 38 to develop RA, compared to anti-CCP negative patients with UA (57). A logistic regression model showed odds of 16 for anti-CCP antibodies in the prediction of RA (58). Raza et al. followed 124 patients with synovitis for less than 3 months for 72 weeks and assessed the prognostic value of anti-CCP antibodies and RF (59). In this study the combination of anti-CCP antibodies and RF had a positive predictive value of 100% and a negative predictive value of 88% for a diagnosis of RA (59).

Clinical disease characteristics of 329 UA patients that presented to the Leiden Early Arthritis Clinic differed between the UA patients that developed RA versus those who did not develop RA. Disease characteristics associated with RA development were a higher age (55 versus 46), female sex (66% versus 50%), duration of morning stiffness (60 minutes versus 15 minutes), longer duration of symptoms (131 versus 81 days) and a higher number of swollen joints (4 versus 2)(1).

Visser et al. developed a clinical model for the prediction of three forms of arthritis outcome: self-limiting disease, persistent non-erosive disease and persistent erosive disease (60). For the development of this model the first 524 consecutive patients referred to the Leiden Early Arthritis Clinic were studied and the arthritis outcome was recorded after two years of follow-up. The developed prediction model consisted of 7 variables: symptom duration at first visit, morning stiffness for ≥ 1 hour, arthritis in ≥ 3 joints, bilateral compression pain in the metatarsophalangeal joints, RF positivity, anti-CCP positivity and the presence of erosions at study entry. The ROC area under the curve for discrimination between self-limiting and persistent non-erosive arthritis was 0.84 and for discrimination between persistent nonerosive and erosive arthritis 0.91 (60). After the addition of predisposing HLA-Class II alleles, the discriminative ability of the model was not significantly improved (60). The model derived by Visser used all patients of the Leiden Early Arthritis Clinic instead of only the patients with UA. The advantage of the model of Visser is that it can be used for a "random" patient with arthritis that visited an outpatient clinic of a rheumatologist, the disadvantage is that it also predicts occurrence of RA in patients that already fulfilled the classification criteria for RA. Currently it is analyzed whether the clinical characteristics used in this model also have predictive value in patients with UA.

To what extent can clinical observation be used in prediction?

Clinical observation of the natural course is the best way of predicting what the subsequent course will be. From a retrospective viewpoint, the history of the patients can be used as illustrated in the model proposed by Visser in which a long duration of complaints was associated with higher odds for chronic and erosive disease (60). The policy to include a "wait and see" policy can only be taken in the context when the possible disadvantages are considered. The progress form UA to RA is characterized by the acquisition of certain

phenotypic characteristics that form the ACR classification criteria, including joint destruction with subsequent deformities, and extra-articular features such as nodules. Given the accumulating evidence that appropriate therapy might prevent the development of a detrimental RA phenotype, observation without treatment is in our view only justified when the patients doesnot fullfill the ACR criteria. Specific studies that compare the initiation of treatment as a function of disease duration are scarce. Valuable data were obtained in a 5-year follow-up study by Egsmose et al. in which early treatment with intramuscular gold was compared with a delayed treatment strategy (61). The early treatment group showed improvement with respect to signs and symptoms, physical function and radiographic progression, thus supporting the hypothesis of a therapeutic 'window of opportunity'. In another trial by van der Heide et al. immediate versus delayed introduction of disease-modifying anti-rheumatic drug (DMARD) therapy were compared in patients with recently diagnosed RA (62). Early introduction of DMARDs showed greater patient improvement with regards to signs and symptoms, physical function and radiographic progression. Van Aken et al. compared the conventional pyramid strategy, consisting of sequential institution of NSAIDs and subsequent DMARD therapy, with immediate initiation of DMARD therapy in an observational study: again, the early treatment group showed less radiographic progression (63). Finally, an observational study performed at the Norfolk Arthritis Register provided evidence that patients in whom DMARD therapy was initiated within 6 months of diagnosis of RA had a better 5-year radiographic outcome than patients starting DMARD therapy 6 months after RA diagnosis (64). All the aforementioned studies have investigated the importance of timing of treatment with regard to diagnosis.

In conclusion, a role for clinical observation in prediction for development of RA seems only justified in UA patients with a low probability to develop RA.

CONCLUSION

In the search to predict RA, current data indicate that host characteristics are relevant to predict RA. The identification of these host characteristics have yielded HLA alleles as risk and as protective alleles and identified PTPN22 as the second risk gene. Progress to identify genetic risk factors is slow due to the fact that each gene most likely has a very small effect and the lack of good statistical models to analyse combinations of genetic risk factors. Clinical factors to be included in a predictive model are most likely the duration of morning stiffness, the presence of an anti-CCP response and the presence of erosive abnormalities on X-rays of hands and feet.

SUMMARY AND FUTURE PERSPECTIVE

Prediction of the future is impossible but a model can provide a probability to an individual patient. Such a prediction model should guarantee a clinician and patient enough certainty (e.g. 80%) that a patient is assigned to the correct category. In the context of UA were about a third will develop RA and two third will not develop RA, it is not exactly known what the minimum value of R^2 has to be to get a valuable prediction model. The R^2, the fraction of explained variation, is a measure for the model's ability to predict. It compares the mean squared error of the prognostic model to the mean squared error of the model without any prognostic variables and does not have a dimension. Some indication for an acceptable R^2 can be obtained from a similar problem, the prediction of the severity of joint destruction in RA. De Vries et al. recently determined the adequacy of clinical parameters in the prediction of joint destruction (64). This model had a R^2 of 0.36 and correctly classified 62% of patients. Furthermore it was calculated that to get a correct classification of 80% of the patients, such a hypothetical model should have a R^2 of 0.9 (65). A model that predicts joint damage scores gives an estimate for a continuous variable and is therefore different from a model that predicts the absence or presence of development of RA. Nevertheless the data as presented by De Vries et al. indicate the requirements for a model to adequately predict disease development in patients with UA. Currently, to our knowledge there are no prediction models analysed that are able to determine with at least 80% certainty whether an individual patient will or will not develop RA. However, given that more genetic factors that associate with susceptibility to RA will most likely be identified in the next decade, we expect that the results of the genetic variants will be included in future prediction models. The predictive value of disease characteristics such as anti-CCP antibodies has already been identified and given its very large and very specific effect, this will be included in prediction models. Clinical characteristics have not yet been defined in great detail but we expect that with the current inclusion of many patients in different early arthritis initiatives, these data will become available in the next decade. Given the expectation that genetic, serological and clinical data each contain independent information, it should be possible to combine these datasets to gain more accurate prognostic information. Hopefully the R^2 of such a model will be sufficiently large to allow prediction at the patient level.

This review is focussed on prediction of the diagnosis of RA but not on prediction of the prognosis of RA. This is caused by the lack of epidemiological data whether severity of RA such as rate of joint destruction is caused by e.g. genetic factors. In the next decade we expect that these basic epidemiological data will become available leading to development of predictive tests for outcome of RA as well.

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Chapter 15

Arthritis of the large joints, in particular the knee, at first presentation is predictive for a high level of radiological destruction of the small joints in rheumatoid arthritis

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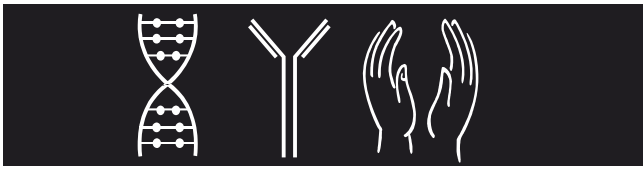

ABSTRACT

Objectives. To investigate the predictive value of the distribution of inflamed joints at first presentation for the severity of the disease course in RA.

Methods. From 1009 consecutive patients included in the Leiden Early Arthritis Clinic, 285 patients fulfilled the ACR-criteria for RA within 1-year of follow-up. Of these, 28 patients achieved remission. Radiographs of hands and feet were scored according to the Sharp-van der Heijde method and the 28 patients with the most destructive disease were selected. The distribution of inflamed joints of the patients with the extreme disease courses was compared. The association between the distribution of inflamed joints and the level of destruction of the joints of hands and feet in the whole group of RA-patients was assessed using regression analysis.

Results. Comparison of the patients with extreme disease courses using univariate and logistic regression analyses revealed that arthritis of the large joints, in particular the knee, was associated with severe RA. In the whole group of RA-patients, the total number of swollen joints and the presence of knee arthritis associated independently with the level of destruction of the small joints. RA-patients with knee arthritis had higher C-reactive protein levels than patients without knee arthritis and investigating the distribution of inflamed joints together with other variables yielded the number of swollen joints, C-reactive protein level, presence of anti-CCP antibodies and symptom duration as predictors for RA severity.

Conclusions. Arthritis of large joints, in particular the knee, at first presentation is associated with a destructive course of RA.

INTRODUCTION

The initial clinical presentation of rheumatoid arthritis (RA) is variable and the number as well as the distribution of inflamed joints may vary between a monoarthritis and an extensive polyarthritis. In general, RA is considered to be a chronic potentially destructive disease, but the severity of the disease course for an individual patient is difficult to predict at baseline. RA patients that present with an extensive polyarthritis may have a mild disease course or remit spontaneously, while patients that initially present with a monoarthritis may experience a severe destructive course of the disease. The implication of being able to predict the disease course in RA is obvious, given the value of early treatment and the common use of aggressive treatment strategies (1-3). Several studies have assessed associations between clinical variables and RA severity (4-17). In these studies the presence of morning stiffness, symptom duration >6 months, rheumatoid factor (RF), antibodies against cyclic-citrullinated peptides (CCP), early radiological erosions and an elevated C-reactive protein (CRP) were correlated with a more severe outcome of the disease (4-17). So far, it is not known whether the distribution of inflamed joints is associated with the disease outcome in RA. Therefore, the present study aimed to investigate the predictive value of the distribution of inflamed joints at first presentation for the severity of the disease course in RA. In order to identify the joints that are associated with a severe disease outcome, the distribution of swollen joints of RA-patients with extreme disease courses, sustained remission and progressive erosive disease, were compared. The comparison of the extremes of phenotypes may reduce the risk of missing risk factors caused by regression to the mean and this approach, in addition to studying the whole group of patients, may lead to the detection of additional prognostic factors. Subsequently, in the whole group of RA-patients the association between the distribution of inflamed joints at baseline and the level of radiological destruction of the small joints of the hands and feet during follow-up was determined and the ability of the identified joints to predict RA severity in relation with other clinical parameters was assessed.

PATIENTS AND METHODS

Patients

For this study, patients from the Leiden Early Arthritis Clinic, a population-based inception cohort of patients with newly diagnosed early arthritis were selected This cohort started in 1993 at the department of Rheumatology of the Leiden University Medical Center, the only referral center for rheumatology in a health care region of ~400,000 inhabitants in the Netherlands. General practitioners were encouraged to refer patients directly when arthritis was suspected; patients were included if physical examination revealed

arthritis (for further reading see 18). In the study period (1993-1999), 1009 patients with early arthritis were included. After one year follow-up, 285 patients had fulfilled the 1987 ACR-criteria for RA (19). From these RA patients, two categories of patients with extreme disease courses were selected: those who entered sustained remission (remitting RA) and those had progressed to the most destructive disease (severe RA). Remission was defined as satisfying the proposed American Rheumatism Association (ARA) criteria for clinical remission (20) after having discontinued the use of Disease Modifying Antirheumatic Drugs (DMARDs) for at least one year. After one additional year of follow-up the presence of sustained remission was confirmed. 28 patients had achieved sustained remission after mean disease duration of 3.7 years (range 210-3159 days SD 852 days). For more details on these patients see (21). The remitting patients are further referred to as "remitting RA". Radiographs of the hands and feet were taken at baseline and yearly thereafter and the level of radiological joint destruction was scored according to the Sharp-van der Heijde method (22-23). The rheumatologist that scored the radiographs was unaware of the study question. The patients with the most destructive disease course were identified by selecting the 28 patients that had the highest Sharp-van der Heijde scores after one year of follow-up. This corresponded with a Sharp-van der Heijde score >34 (mean 59). These patients are further referred to as "most severe RA".

Methods

At inclusion the rheumatologist questioned the patient about the initial symptoms as well as the presence of morning stiffness and the symptom duration. Physical examination at baseline included a swollen joint count, scaling each joint on a 0-1 scale (0 denotes no swelling and 1 indicates the presence of swelling). Subsequently, the joints or joint groups were categorized (e.g. all metacarpophalangeal joints on one side were counted as one joint). The following joint(groups) were studied: shoulders, elbows, wrists, metacarpophalangeal joints, proximal and distal interphalangeal joints, knees, ankles and metatarsophalangeal joints. For the analysis a dichotomous approach was used that indicated the presence or absence of swelling per joint group.

Baseline laboratory parameters included ESR, CRP, IgM rheumatoid factor ELISA as previously described (24) and anti-CCP2 antibodies (ELISA, Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands). According to the instruction of the manufacturer a level > 25 arbitrary units was considered positive.

Statistical analysis

The baseline parameters of the patients with remitting and severe RA were compared using the Mann-Whitney test for continuous variables and the chi-square test for nominal variables. Subsequently, a logistic regression analysis with backward selection procedure ($p>0.10$ as removal criteria, using likelihood ratio test) was performed with the presence

of remitting RA or most severe RA as dependent variable and the individual joint groups that were significantly associated with the disease outcome in the univariate analysis and the total number of swollen joints as possible explanatory variables.

To validate the findings, the association between the joint groups and radiological joint destruction was assessed in the total group of RA patients. The Sharp-van der Heijde scores at 1 year follow-up were entered as a dependent variable in a linear regression analysis (backward selection procedure, $p>0.10$ as removal criteria) with the total number of swollen joints and the joint count for the individual joint groups as possible explanatory variables. To assess the association between the distribution of swollen joints and the level of radiological joint destruction in relation to other clinical variables that might be associated with RA severity, an other linear regression analysis (backward selection procedure, $p>0.10$ as removal criteria) was performed. In this analysis the Sharp-van der Heijde score at one year follow-up was entered as a dependent variable and the total number of swollen joints, the swollen joint count for the individual joint groups, gender, age, the presence of anti-CCP antibodies, RF, morning stiffness, the CRP-level and symptom duration and as possible explanatory variables.

The statistical package for the social sciences (SPSS) version 11.0 (SPSS, Chicago, IL) was used to analyse the data. A p-value ≤ 0.05 was considered significant.

RESULTS

Univariate comparison of patient characteristics at first presentation of RA patients with extreme disease courses

The patients in the remitting and most severe RA group had no significant differences in the distribution of gender, age and morning stiffness. The patients with the most severe disease course harboured more frequently RF and anti-CCP antibodies, had higher levels of CRP and had higher Sharp-van der Heijde scores at inclusion compared to patients with remitting RA (Table 1). The patients with the most severe disease course also had a significantly higher number of swollen joints at first presentation than the patients with remitting RA. The distribution of swollen joints was different between the two groups: the patients with the most severe RA had significantly more often arthritis of the shoulders, elbows, proximal interphalangeal joints, knees and ankles (Table 2). There were no differences in the prevalences of swollen metacarpophalangeal and metatarsophalangeal joints between the two groups of RA-patients.

Regression analysis in the RA patients with extreme disease courses

To investigate which joint (groups) were independently associated with the disease outcome, a logistic regression analysis with backward selection procedure was performed

Table 1. Baseline patient characteristics of the RA patients with the extreme disease courses

	Remitting RA N=28	Most severe RA N=28	P
Female, n (%)	18 (64)	17 (61)	0.7
Age, years, median (IQR)	59 (48-71)	59 (50-72)	1.0
Morning stiffness, minutes, median (IQR)	138 (30-180)	137 (30-180)	1.0
Symptom duration, days median (IQR)	127 (57-207)	152 (87-281)	0.1
C-reactive protein mg/l, median (IQR)	29 (7-46)	56 (26-75)	0.01
IgM RF positive, n (%)	6 (21)	23 (82)	<0.05
Anti-CCP 2 positive, n (%)	3 (11)	21 (75)	<0.05
Total number of swollen joints, median (IQR)	5.3 (3-7)	7.3 (5-9)	<0.05
Sharp-van der Heijde score, median (IQR)	0 (0-2)	10 (1-17)	<0.05

Table 2. Distribution of swollen joints of the RA patients with the extreme disease courses

Joint group n (%)*	Remitting RA (N=28)	Most severe RA (N=28)	Odds ratio	95% CI	P
Shoulder	1 (3%)	6 (21%)	6.4	2.0-26.4	<0.05
Elbow	4 (14%)	8 (29%)	2.5	1.2-5.5	<0.05
Wrist	19 (68%)	21 (75%)	1.4	0.7-2.8	0.3
Metacarpophalangeal	23 (82%)	23 (82%)	1.0	0.5-2.2	1.0
Proximal interphalangeal	17 (61%)	23 (82%)	2.9	1.5-5.9	<0.05
Distal interphalangeal	1 (4%)	1(4%)	1.0	0.2-4.1	1.0
Knee	7 (25%)	17 (61%)	4.7	2.5-9.0	<0.05
Ankle	4 (14%)	9 (32%)	2.9	1.4-6.3	<0.05
Metatarsophalangeal	8 (29%)	9 (32%)	1.2	0.6-2.7	0.6

* The numbers (percentages) indicate the number (percentage) of patients with swelling of (at least one of) the joints of the specific joint groups.

with the presence of remitting RA or most severe RA as dependent variable and the individual joint groups that were significantly associated with the disease outcome in the univariate analysis (shoulder, elbow, proximal interphalangeal, knee, ankle) and the total number of swollen joints as possible explanatory variables. In this analysis only the presence of a swollen knee (odds ratio 7.0, P=0.004) was significantly associated with the disease outcome (Table 3).

Table 3. Results of logistic regression analysis with backward selection procedure and remitting or severe RA as dependent variable and the total number of swollen joints and the joints with a significant result in univariate analysis as possible explanatory variables

Joint group	P	Odds ratio	95% CI
Shoulder	0.07	8.7	0.8-90.1
Elbow	0.08	4.1	0.9-19.4
Knee	0.004	7.0	1.9-25.9

Regression analysis in all 285 RA patients

To validate the findings, the association between the distribution of swollen joints and radiological joint destruction was assessed in the total group of RA patients. The Sharp-van der Heijde score at 1 year follow-up was entered as a dependent variable in a linear regression analysis with a backward selection procedure and the total number of swollen joints and all evaluated joint groups as possible explanatory variables. This analysis revealed that in the total group of RA-patients the total number of swollen joints (p=0.004) and swelling of the knee (p=0.03) were independently associated with the level of radiological joint destruction of hands and feet (Table 4). A similar analysis was performed with the Sharp-van der Heijde scores at 2 and 3 year follow-up as dependent variable (X-rays were available of respectively 202 and 162 patients). In these analyses only swelling of the knee associated independently with the level of radiological joint destruction (B=7.2, SE=3.9, P=0.05 for the 2-year time point and B=3.5, SE=4.7, P=0.005 for the 3-year time point).

Table 4. Results of linear regression analysis with backward selection procedure in the 285 RA-patients with Sharp-van der Heijde score at 1-year follow-up as dependent variable and distribution and total number of swollen joints as possible explanatory variables.

Joint group	B	SE	P
Knee	1.4	0.5	0.004
Total number of swollen joints	6.1	2.9	0.03

B indicates regression coefficient

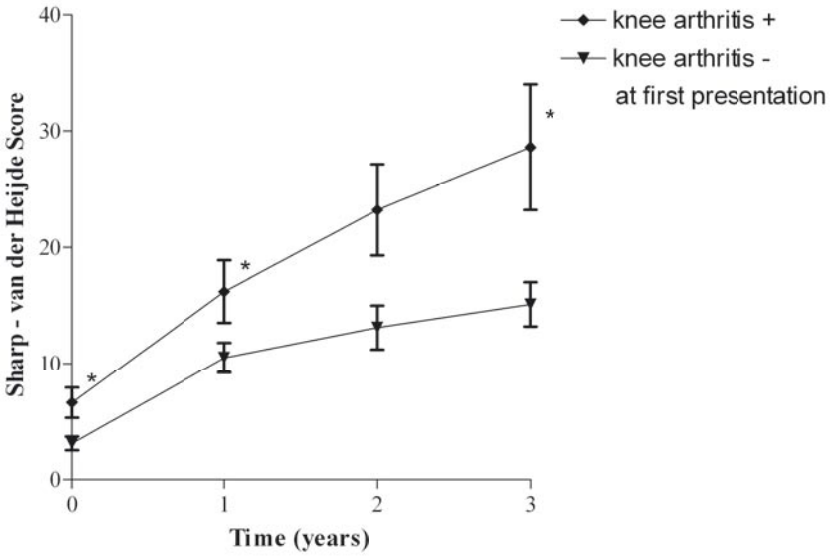

Figure 1. **The level of radiological destruction of the small joints of hands and feet during three years of follow-up of RA-patients with and without arthritis of the knee at first presentation.** *P<0.05 (Mann-Whitney test). At the 2 and 3 year time point radiographs of hands and feet were available of 202 and 162 patients respectively.

Figure 1 displays the level of radiological destruction of the small joints of the hands and feet during three years of follow-up of the RA-patients that at first presentation did have and did not have arthritis of the knee (Figure 1).

In conclusion, regression analyses in both the whole group of RA-patients and using the approach of the extremes of the disease courses yielded the total number of swollen joints and particularly the presence of arthritis of the knee as independently associated with the level of joint destruction of the small joints of hands and feet during follow-up.

Predictive ability of distribution of swollen joints in relation with other clinical characteristics for RA severity

Next, we investigated the predictive ability of the presence of arthritis of the knee and the total number of swollen joints in relation to other clinical variables that are observed or described to be associated with a severe disease outcome: CRP, RF, anti-CCP antibodies, morning stiffness and symptom duration (4-17, Table 1). A linear regression analysis with a backward selection procedure revealed that the total number of swollen joints (B=0.9, SE=0.4, P=0.03), the presence of anti-CCP antibodies (B=8.4, SE=2.4, P<0.001), the CRP-level (B=0.02, SE=0.007, P<0.01) and the symptom duration (B=0.2, SE=0.04, P<0.001) were independently associated with the level of joint destruction after 1 year of follow-up. In this analysis the presence of arthritis of the knee was not an independent predictor for disease severity. The fraction of explained variation of this model was 0.2. As after correction for clinical variables in a regression analysis the presence of arthritis of the knee was not independently associated with RA severity, in contrast to the CRP-level, the presence of anti-CCP antibodies and the symptom duration, we hypothesized that these variables might be distributed differently among the RA-patients with and without arthritis of the knee. Arthritis of the knee was present in 106 (39%) patients at first presentation. The presence of anti-CCP antibodies and the symptom duration, but also the variables age, gender, RF and morning stiffness were not significantly different between the RA patients with and without arthritis of the knee. However, there was a significant difference in the level of CRP: RA-patients with arthritis of the knee at first presentation had higher CRP-levels (mean 48 mg/l, SD 35 mg/l) compared to RA-patients without involvement of the knee (mean 22 mg/l, SD 24 mg/l, P<0.001).

DISCUSSION

The present study aimed to investigate the predictive value of the distribution of inflamed joints at first presentation for the severity of the disease course in RA and demonstrated using two different approaches that the presence of arthritis of large joints, and arthritis of the knee in particular, is independently associated with the level of joint destruction.

In regression analysis both arthritis of the knee and the total number of swollen joints at baseline were significantly associated with a severe disease outcome. Since all RA-patients fulfilled the ACR-criteria and per definition had involvement of the small joints and as the presence/absence of arthritis was evaluated as a dichotomous variable per joint group, in the present study a difference in the total number of swollen joints reflects a difference in the number of swollen large joint groups. From all joints the presence of arthritis of the knee at baseline was the strongest predictor for a higher level of radiological destruction of the small joints of the hands and feet.

After the identification of the knee and the total number of swollen joints as predictors for severe radiological destruction of small joints, we were interested whether these parameters were of additional value to established parameters for disease severity (anti-CCP antibodies, RF, C-reactive protein, morning stiffness, symptom duration). Therefore, a linear regression analysis was performed that included not only the distribution and number of swollen joints but also other clinical variables. This analysis revealed a significant association between with the level of radiological joint destruction and the total number of swollen joints, C-reactive protein, anti-CCP antibodies and symptom duration. The presence of knee arthritis was not significantly associated with RA severity in this analysis. A post-hoc analysis demonstrated that patients with arthritis of the knee had higher levels of CRP compared to the patients without arthritis of the knee. Apparently the CRP-level is a stronger predictor for the level of joint destruction than the presence of an inflamed knee. Interestingly, the presence of anti-CCP antibodies was not different between patients with and without knee arthritis. These data suggest that inflammation of large synovial joints, like the knee, induces a higher amount of pro-inflammatory cytokines (among others Il-6) that subsequently triggers an increased production of CRP by the liver. Holt et al previously showed that in patients with inflammatory arthritis the concentration of synovial Il-6 in knee joints was associated with the plasma level of Il-6 and also with plasma level of CRP (25). Although having arthritis of the knee was also correlated with a high level of CRP in the present study and the CRP-level appeared to be a better predictor for disease severity compared to knee arthritis, in clinical practice a physician establishes the presence of arthritis of large joints as the knee directly during the first visit, whereas laboratory results are not immediately available. Furthermore, the presence of an elevated CRP-level can also be caused by non-rheumatologic factors such as a transient infection. Since the present study aimed to investigate the predictive value of the distribution of inflamed joints for the disease outcome in RA, the finding that the presence of arthritis of large joints and the knee in particular is associated with a more destructive disease might be of help in clinical practice.

Studies that investigate the natural disease course in RA are nowadays hampered by the fact that aggressive and effective disease modifying antirheumatic drugs including biologicals are used. The effective reduction of the disease activity diminishes the level of joint destruction. Despite the indisputable benefit for RA patients, the natural course of the disease is altered and the patients that are currently treated for RA are less suitable for studies that aim to identify variables that predict the disease outcome. The patients included in the present study were treated for RA between 1993 and 1999. In this time era, therapy with DMARDs was started in a relatively late stage and medications of choice were among others antimalarials, of which is known that their ability to halt disease progression is limited. None of the included patients were treated with biologicals. From the 28 patients that achieved sustained remission 16 patients had received no DMARD therapy, 6 were treated with antimalarials and the remaining 7 were prescribed methotrexate or sulphasalazine. The patients included in the present study are more suitable for a study assessing risk factors for RA severity, than the patients that are nowadays included in our Early Arthritis Clinic.

In conclusion, early recognition of patients with RA with a potentially severe disease course is important since these patients in particular may benefit from the therapeutic options that are currently available. It is already known that the presence of anti-CCP antibodies, symptom duration and CRP are associated with RA severity. This study reveals that also the presence of arthritis of large joints and particularly arthritis of the knee are predictive for a destructive disease course.

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Chapter 16

A rule to predict disease outcome in patients with recent-onset undifferentiated arthritis to guide individual treatment decisions

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ABSTRACT

Objectives. In patients with undifferentiated arthritis (UA) methotrexate is an effective drug to inhibit symptoms, structural damage, and progression towards rheumatoid arthritis (RA). However 40-50% of UA-patients remit spontaneously. Thus adequate treatment decision-making in early undifferentiated arthritis necessitates identification of the UA-patients that will develop RA.

Methods. A prediction rule was developed using data from the Leiden Early Arthritis Clinic, an inception cohort of patients with recent onset arthritis (n=1700). The patients that presented with UA were selected (n=570); progression to RA or other diagnosis was monitored after one-year follow-up. The clinical characteristics with independent predictive value for RA development were selected using logistic regression analysis. The diagnostic performance of the prediction rule was evaluated using the area under receiver operating characteristic curve (AUC). Cross-validation controlled for over-fitting of the data (internal validation). For external validation, an independent cohort of UA-patients was used.

Results. The prediction rule consists of nine clinical variables: gender, age, localization of symptoms, morning stiffness, tender and swollen joint count, C- reactive protein, rheumatoid factor and anti-CCP antibodies. Each prediction score varies between 0 and 14 and corresponds to a chance (percentage) RA development. For several cut-off values the positive and negative predictive values were determined. The AUC of the prediction rule, the prediction model after cross-validation and the external validation cohort were 0.89, 0.87 and 0.97 respectively.

Conclusions. In early undifferentiated arthritis the risk to develop RA can be predicted, thereby allowing individualized treatment decisions to initiate disease-modifying antirheumatic drugs in patients who present with UA.

INTRODUCTION

Individualized treatment decision-making is one of the most important challenges of medicine. To this end a number of studies have appeared that associated clinical variables or gene-expression profiles with disease outcome, thereby providing help for clinicians in treatment decisions in several diseases (e.g. breast cancer, Hodgkin disease, lymphoma 1-4). Treatment in rheumatoid arthritis (RA) is since the last decennium characterized by earlier and more aggressive treatment with disease-modifying antirheumatic drugs (DMARDs), as this treatment strategy prevents joint damage and functional disability (5-7). In rheumatological practice, the majority of patients that present with a recent onset arthritis have an undifferentiated arthritis (UA), arthritis in whom with the available classification criteria no diagnosis can be made. From several inception cohort studies it is known that about 40-50% of these UA-patients remit spontaneously, whereas one-third develops RA (8-10). Recent evidence indicates that treatment with methotrexate in patients with early UA hampers progression to RA and progression of joint damage (11), underscoring the need for guidance to start a clinically beneficial but potential harmful drug in UA. Ideally, only the UA-patients that develop RA are treated with DMARDs in contrast to those that remit spontaneously. At present, although several risk factors for the development of RA have been identified (8,12), a model that predicts the disease course specifically in patients with recent-onset UA is lacking. The present study aimed to develop a model that predicts the progression from UA to RA, using clinical variables that are easily assessed in daily clinical practice. The derived prediction rule was internally validated controlling for over-fitting of the data, and subsequently externally validated in an independent cohort of UA-patients.

METHODS

Patients

The prediction rule is derived using the Leiden Early Arthritis Clinic, an inception cohort containing more than 1900 patients with recent-onset arthritis of whom about 1700 have completed at least one-year follow-up. This cohort started in 1993 at the department of Rheumatology of the Leiden University Medical Center, the only referral center for rheumatology in a health care region of ~400,000 inhabitants in the Netherlands (13). General practitioners were encouraged to refer patients directly when arthritis was suspected; patients were included if physical examination revealed arthritis. At first visit various variables were collected. The rheumatologist answered a questionnaire inquiring about the initial symptoms as reported by the patient: type, localization and distribution of initial joint symptoms, symptom duration and course of start complaints. The smoking and family history were assessed. Patients rated the morning stiffness on a visual analogue

scale (0-100). For the present study, severity of morning stiffness was used instead of duration of morning stiffness as the first is proven to be a better discriminator (14,15). The Health Assessment Questionnaire (HAQ) yielded an index of disability. A 44-joint count for tender and swollen joint was performed, scoring each joint on a 0-1 scale (16). Compression pain of metacarpophalangeal and metatalohalangeal joints was recorded. Baseline blood samples were taken for determination of ESR, C-Reactive protein (CRP), IgM rheumatoid factor (RF, ELISA), and antibodies to cyclic-citrullinated peptide 2 (CCP; ELISA, Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands). The cut-off level for anti-CCP positivity was 25 arbitrary units. Radiographs of hands and feet were made and scored according to Sharp-van der Heijde (17). Patients gave their informed consent and the local Ethical Committee approved the protocol.

Disease Outcome

570 patients had two weeks after inclusion (when results on laboratory and radiological investigations were known) an arthritis that could not be classified according to the ACR-criteria and were documented as undifferentiated arthritis (UA). After 1-year follow-up the disease status of all UA-patients was examined to determine whether they had developed RA or other diagnosis according to the ACR-criteria. Inherent to the design of an inception cohort the duration of follow-up differed within the study population and at the moment of analysis (July 2005) the majority of UA-patients (94%) had been followed for more than one year (mean follow-up 8 years, SD 3 years).

External validation cohort

Patients included in the placebo-arm of the PROMPT-trial, a double-blind placebo-controlled randomized trial in which patients with recent onset UA were treated with either methotrexate or placebo, were used for validation (n=55) (11). Exclusion of the UA-patients that were also included in the EAC cohort resulted in 36 independent UA-patients. Two of these were lost to follow-up. For each patient the progression score at baseline was calculated and the development of RA after 1-year follow-up was assessed (11).

Statistical analysis

The UA-patients that did or did not develop RA were compared using the Chi-square test for nominal variables and the student's t-test for continuous variables. Symptom duration was categorized. Subsequently all clinical variables were entered as possible explanatory variables in a logistic regression analysis with the disease outcome (RA or non-RA) at one-year follow-up as dependent variable. Using a backward selection procedure, the most significant independent variables were identified, using $p>0.10$ as removal criteria. In the logistic regression model the predicted probability on RA is related to the covariates via the prognostic index: $B_1*x_1+B_2*x_2+B_3*x_3\ldots B_k*x_k$. The B (regression coefficient) of the covariate indicates an estimate

of the relative magnitude of the prognostic power of the concerning variable. Using the prognostic index, for every subject the predicted probability on RA development was calculated. For continuous variables (age, VAS-score, tender and swollen joint count, CRP) the effect was studied both as continuous variable and categorized. Categories were made using clinically applied cut-off levels and percentiles. Categories were pooled if corresponding regression coefficients were similar. Data on VAS morning stiffness were missing in 160 subjects, data on anti-CCP antibodies in 64 subjects and data on disease duration in 22 subjects. To prevent that these subjects were excluded from the logistic regression analysis, the median value was imputed. The multivariate regression analysis was performed using 562 UA patients as in 8 patients one or more of the following variables were missing: rheumatoid factor (n=1), CRP (n=1), tender joint count (n=5), swollen joint count (n=4). To get a simplified prediction rule, the regression coefficients of the predictive variables were rounded to the nearest number ending in .5 or .0 resulting in a weighted score; subsequently the independent predictive variables were summed. The calculated prediction scores were compared with the observed percentage progression to RA. The positive and negative predictive values were determined for several cut-off values of the prediction scores. To evaluate the diagnostic performance, a receiver-operating characteristic (ROC) curve was constructed. The area under the ROC curve (AUC) provided a measure of the overall discriminative ability of a model. For internal validation, cross-validation was performed to control for over-fitting (18). Cross-validation mimics the prediction situation and yields for each observation a prediction score based on the other (n-1) observations (18). To validate the model a ROC-curve was made using the cross-validated predictions as well as the external validation cohort. The Statistical Package for Social Sciences (SPSS), version 10.0 (SPSS, Chicago, IL) was used.

RESULTS

Disease outcome

Of 570 UA-patients, 177 developed RA during the first year of follow-up, 94 patients developed other rheumatological diseases, 149 patients remained unclassified and 150 patients achieved clinical remission defined as discharge from the outpatient clinic because of absence of arthritis without DMARDs. For further analysis, the patients with other rheumatological diagnosis, unclassified arthritis and remission were assembled as the non-RA group (n= 393).

Univariate analyses

Characteristics of UA-patients that did and did not develop RA are compared in Table 1. In univariate analysis, all variables except smoking were significantly associated with progression to RA.

Table 1. Characteristics at inclusion of UA-patient that did not and did progress to RA.

Patient characteristic	Non-RA N=393	RA N=177	P
Age, mean (SD)	48.6 (17.0)	56.3 (15.3)	<0.001
Female, n (%)	208 (53)	121 (68)	0.001
Positive family history for RA, n (%)	81 (21)	54 (31)	0.01
Course start complaints, n (%)			
acute <24 hr	116 (30)	36 (20)	
subacute > 24 hr	123 (31)	51 (29)	
creping	141 (36)	86 (49)	
intermittent	13 (3)	4 (2)	0.02
Symptom duration at inclusion, n(%)			
< 6 weeks	103 (27)	18 (11)	
6 weeks – 3 months	80 (21)	43 (25)	
3- 6 months	89 (23)	47 (28)	
> 6 months	107 (28)	61 (36)	<0.001
Localisation affected joints, n(%)			
small hand/feet	171 (44)	95 (54)	
big joints	165 (42)	32 (18)	
both	57 (15)	50 (28)	<0.001
Localisation affected joints, n(%)			
symmetric	147 (37)	118 (67)	<0.001
Localisation affected joints, n(%)			
upper extremities	177 (45)	71 (40)	
lower extremities	139 (35)	22 (12)	
both	77 (20)	84 (47)	<0.001
Morning stiffness (VAS), mean (SD)	35.5 (30.0)	53.3 (30.1)	<0.001
Compression pain MCP joints, n(%)	159 (40)	116 (66)	<0.001
Compression pain MTP joints, n(%)	134 (34)	103 (58)	<0.001
Number tender joints, median (IQR)	3 (2-7)	8 (4-12)	<0.001
Number swollen joints, median (IQR)	2 (1-4)	4 (2-7)	<0.001
CRP level (mg/L), median (IQR)	8 (3-21)	14 (7-43)	<0.001
ESR level (mm1st hr), median (IQR)	17 (8-38)	32 (19-53)	<0.001
Rheumatoid factor positive, n (%)	56 (14)	84 (47)	<0.001
Anti-CCP positive, n(%)	38 (11)	83 (51)	<0.001
HAQ score, mean (SD)	0.7 (0.6)	1.0 (0.7)	<0.001
Smoking, n(%)	187 (48)	84 (47)	1.0
Erosiveness, n(%)	29 (7)	29 (16)	0.001

Multivariate analyses, derivation of prediction rule

In a logistic regression analysis the independent predictive variables for RA development were: age, gender, localization of joint complaints (small/big joints, symmetric/asymmetric, upper/lower extremities), morning stiffness, tender and swollen joint count, CRP-level, RF and anti-CCP antibodies (Table 2). Age as continuous variable was more predictive than categorized; the other continuous variables were categorized. The resulting model had a fraction of explained variation (Nagelkerke R^2) of 0.57 and, when taking a predicted

probability of 0.5 as cut off value, predicted 83% of patients correctly. The coefficients for the simplified prediction score are listed in Table 2. Figure 1a presents a form to easily calculate the prediction score. The prediction score ranges between 0 and 14; a higher score indicates a higher risk to develop RA. For every UA-patient the prediction score was calculated. Figure 1b shows the predicted risk on RA as function of the prediction score (obtained from a logistic model with score as independent variable). Table 3 presents the observed percentage with progression to RA in relation to the calculated score. All UA-patients with a prediction score ≤3 did not progress to RA during the one-year follow-up, and all UA-patients with a score ≥11 had progressed to RA. The patients with intermediate scores (4-10) had progressed to RA in increasing frequency at rising scores. Table 3 also shows the percentage of the patients that progressed to RA for several cut-off values of the prediction score. For example if the scores 5.0 and 9.0 were chosen as cut-off values, 97% of UA-patients with a score a score ≤5.0 did not develop RA and a score ≥9.0 was associated with progression to RA in 84% of patients. If the cut-off values were 6.0 and 8.0,

Table 2. Independent predictive variables for RA development resulting from multivariate regression analysis

Variable	B *	OR	95%CI	P	Points #
Gender	0.8	2.1	1.3-3.6	0.003	1
Age	0.02	1.02	1.01-1.04	0.011	0.02/yr
Localisation small joints hand/feet	0.6	1.8	1.1-3.1	0.024	0.5
Localisation symmetric	0.5	1.6	1.0-2.8	0.075	0.5
Localisation upper extremities	0.8	2.1	1.1-4.4	0.04	1
upper and lower extremities	1.3	3.5	1.7-7.5	0.001	1.5
VAS morning stiffness					
0-25	-	-	-	-	-
26-50	0.9	2.4	1.2-4.5	0.009	1
51-90	1.0	2.7	1.3-5.6	0.006	1
>90	2.2	9.3	3.0-28.7	<0.001	2
Number tender joints					
0-3	-	-	-	-	-
4-10	0.6	1.8	0.9-3.3	0.082	0.5
>10	1.2	3.3	1.5-7.0	0.003	1
Number swollen joints					
0-3	-	-	-	-	-
4-10	0.4	1.5	0.8-2.7	0.18	0.5
> 10	1.0	2.8	1.1-7.6	0.038	1
CRP level					
0-4	-	-	-	-	-
5-50	0.6	1.6	0.9-3.0	0.13	0.5
>50	1.6	5.0	2.0-12.1	0.00	1.5
RF positive	0.8	2.3	1.2-4.2	0.009	1
Anti-CCP positive	2.1	8.1	4.2-15.8	<0.001	2

***** B means regression coefficient

Points for the simplified prediction rule derived from the regression coefficient

1. What is the age in years?	Multiply with 0.02		_____
2. What is the gender?	In case female:	1 point	_____
3. How is the distribution of involved joints?			
	In case small joints hands / feet:	0.5 point	_____
	In case symmetric:	0.5 point	_____
	In case upper extremities	1 point	
	or in case upper & lower extremities:	1.5 points	_____
4. What is the length of the VAS morning stiffness (range 0-100 mm)?			
	In case 26-90 mm:	1 point	
	In case > 90 mm:	2 points	_____
5. What is the number of tender joints?			
	In case 4-10:	0.5 point	
	In case 11 or higher:	1 point	_____
6. What is the number of swollen joints?			
	In case 4-10:	0.5 point	
	In case 11 or more:	1 point	_____
7. What is the C-reactive protein level (mg/L)?			
	In case 5-50:	0.5 point	
	In case 51 or higher:	1.5 points	_____
8. Is the Rheumatoid factor positive?	If yes:	1 point	_____
9. Are the anti-CCP antibodies positive?	If yes:	2 points	_____
		Total score	_____

Figure 1a. Form to calculate a patient's prediction score

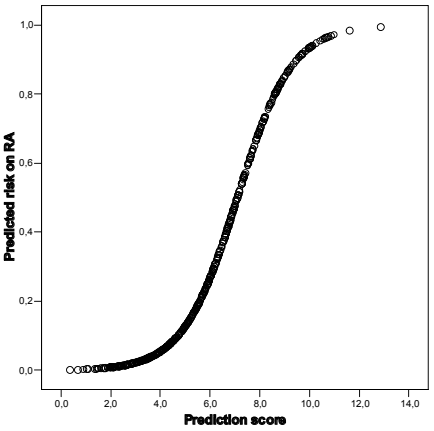

Figure 1b. The subjects' prediction scores plotted versus the predicted risk to develop RA.

91% of UA-patients with a score ≤ 6.0 did not develop RA (negative predictive value 91%, 95%CI 88-94%) and a score ≥8.0 corresponded with progression to RA in 84% (positive predictive value 84%, 95%CI 75-91%). With these cut-off values 145 UA-patients (25%) had a score between 6.0 and 8.0, indicating that for these patients no adequate prediction could be made. Twenty-five UA-patients did not fulfill the 1987 ACR-criteria for RA after one-year follow-up, but developed RA later in the disease course. These patients had a median prediction score of 5.7 (IQR 4.8-6.2); this value is in-between the scores of the UA-patients that did and did not develop RA during the first year of follow-up (median score 7.7, IQR 6.6-8.8 and median score 4.6, IQR 3.3-5.9 respectively).

Discriminative ability

The discriminative ability of the logistic regression model and the prediction rule were evaluated with a ROC curve (Figure 2). Both had an AUC of 0.89 (SE 0.014). The finding that the AUC of the logistic regression model and the prediction rule were equal, indicates that the derivation of the prediction rule from the logistic regression model had not introduced a loss in discriminative ability.

Internal validation

Cross-validation was used to control for over-fitting. This procedure yielded for every patient a predicted probability on RA, based on the model fitted on the other patients

Table 3. Prediction score and number (%) of patients that did not or did progress to RA, as well as several cut-off values for prediction scores with corresponding chances on RA development

Score*	Non-RA n (%)	RA n (%)
0	1 (100)	0 (0)
1	8 (100)	0 (0)
2	42 (100)	0 (0)
3	58 (100)	0 (0)
4	78 (93)	6 (7)
5	73 (85)	13 (15)
6	63 (74)	22 (26)
7	37 (49)	38 (51)
8	16 (33)	33 (67)
9	6 (14)	36 (86)
10	5 (23)	17 (77)
11	0 (0)	8 (100)
12	0 (0)	1 (100)
13	0 (0)	1 (100)
14	0	0
Total	387	175
Score ≤ 4.0	145 (99)	1 (1)
4.0 -10.0	240 (60)	159 (40)
≥10.0	2 (12)	15 (88)
Score ≤ 5.0	223 (97)	8 (3)
5.0-9.0	157 (55)	131 (46)
≥ 9.0	7 (16)	36 (84)
Score ≤ 6.0	296 (91)	28 (9)
6.0-8.0	76 (52)	69 (48)
≥8.0	15 (16)	78 (84)

* Prediction scores were rounded to the nearest number ending in .5 or.0. (i.e. scores ≤0.5 are in the category 0, >0.5 and ≤1.5 in the category 1, etc)

(18). The AUC of the cross-validated predictions nearly equaled the AUC of the prediction score: 0.87 (SE 0.015, Figure 2), indicating that over-fitting is not a major problem.

External validation

In the validation cohort 47% of UA-patients had progressed to RA after one-year follow-up. The prediction scores of the UA-patients that did not and did develop RA are presented in Figure 3. The UA-patients who had progressed to RA had a median prediction score of 8.0 (IQR 6.1-9.1) and the patients who did not develop RA had a median prediction score of 4.6 (IQR 3.5-5.5). 94% of the patients with a prediction score ≤6.0 had not progressed to RA and RA-development was observed in 83% of patients with a score >6. All patients with a score ≥8.0 had progressed to RA and 78% of patients with a score <8 did not develop RA. 17% of the UA-patients in the validation cohort had a prediction score

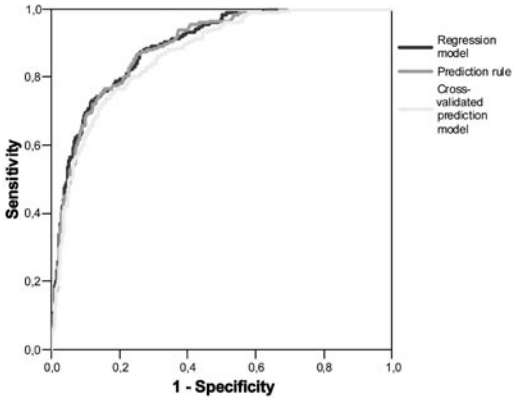

Figure 2. Receiver operator curve of logistic regression model, prediction rule and cross-validated prediction model. The AUC of the logistic regression model, prediction rule and cross-validated prediction model were respectively 0.89, 0.89 and 0.87.

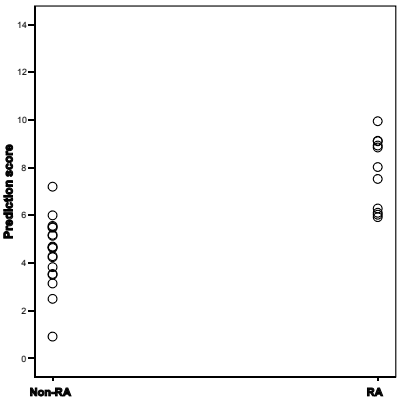

Figure 3. The prediction scores of the patients included in the external validation cohort that did not and did develop RA.

between 6 and 8; two-third of them had not developed RA and one-third had developed RA. When treatment decisions were based on the prediction rule using the cutoff level ≥8 for initiating treatment and ≤6 for withholding treatment, only 6% of the patients should have been inaccurately withheld from treatment and no patients should have been inaccurately treated. The AUC of the validation cohort was 0.97 (SE 0.024).

DISCUSSION

The currently developed rule predicts in UA-patients the risk to develop RA using nine clinical variables that are all commonly assessed during the first visit: gender, age, localization of joint symptoms, morning stiffness, counts for tender and swollen joints, CRP, rheumatoid factor and anti-CCP antibodies. The resulting prediction score corresponds with a chance on progression to RA. The positive and negative predictive values of the prediction score depend on the chosen cut-off values. The discriminative ability was excellent with an AUC of 0.89, and 0.87 after internal validation correcting for over-fitting. The subsequent validation in a small independent cohort revealed an AUC of 0.97. As the developed prediction rule is accurate and easily assessed in daily clinical practice, the present model is an important step forward in achieving individualized treatment in patients with recent-onset UA.

As current evidence on treatment of RA is based on large trials using patients fulfilling the 1987 ACR-criteria for RA, fulfilment of these criteria was used as outcome. Alternative

outcome measurements such as disease persistence or remission can be considered, but no generally accepted definitions for these disease states are present and there are no trials of patients with these disease states providing guidance in treatment decisions. Nevertheless, the use of fulfilment of the ACR-criteria as outcome may lead to circularity as the items of the ACR-criteria are expected to result as predictive variables. However, several studies have shown that the ACR-criteria themselves have low discriminative value in patients with UA (12, 19-23) and only part of the variables of the present prediction rule are items of the ACR-criteria. In the end it will most likely not make a large difference whether the outcome of a prediction rule is the diagnosis RA or disease persistence, as the ACR-criteria are formulated based on RA-patients with longstanding/persistent disease (mean disease duration 8 years) and the reported remission rate in these patients is low: 10-15% (24,25).

Misclassification may have occurred when patients who presented with UA were treated with any drug that has hampered the progression to RA. In case of misclassification, patients that normally had progressed to RA are now classified as non-RA. Exclusion of these eventual misclassified patients, with supposedly high prediction scores as they were prone to develop RA, will result in an increased discriminative ability of the current prediction rule.

The presence of erosions on radiographs of hands and/or feet is reported to have a high specificity (but low sensitivity) for discriminating between self-limiting and persistent disease (23). Although in univariate analysis erosions were significantly more present in the UA-patients that developed RA compared to the UA-patients that did not (16% vs. 7%), multivariate regression analysis revealed that the presence of erosions was not an independent prognostic variable. The presence of erosions appeared to be associated with a higher age (median 64 years in erosive versus 49 years in non-erosive disease), number of swollen joints (median 5 joints in erosive versus 2 joints in non-erosive disease) and presence of rheumatoid factor (46% in erosive versus 23% in non-erosive disease). As the presence of erosions was not identified as a variable with an independent predictive value, data on erosions were not included in the prediction rule.

A model for the prediction of a self-limiting, persisting or erosive arthritis exists (23). For this model's development all consecutive patients referred with arthritis were incorporated, including the patients in whom during the first weeks a definite diagnosis was made. Decisions on initiation of DMARDS are seldom problematic in these patients. At present, support in treatment decisions is needed in patients with recent onset UA (26), as the disease outcome in these patients is variable. The present study therefore selected the patients with UA from a total number of 1700 consecutive patients and developed a prediction rule specifically for UA.

The positive and negative predictive values of the prediction score depend on the chosen cut-off level. If the upper and lower cut-off values were 8.0 and 6.0, the corresponding positive predictive value and negative predictive value were respectively 84% and 91%. In the original cohort 25% of patients had a prediction score between 6.0 and 8.0; these patients had an equal chance to develop RA or not. Apparently, clinical characteristics are in these patients insufficient to predict the disease outcome. In the validation cohort, the prediction score discriminated even better: a hundred percent of patients with a score of 8.0 or higher had progressed to RA and 94% of patients with a score of 6.0 or lower did not develop RA. This indicates that when treatment decisions were based on the prediction rule using the cutoff level ≥8 for initiating treatment and ≤6 for withholding treatment, only 6% of the patients should have been inaccurately withheld from treatment and no patients should have been inaccurately treated. In the validation cohort 17% of patients had a prediction score between 6.0 and 8.0; for treatment decisions in these patients the observed risk to progress to RA can be weighted against the individual risk profile for treatment toxicity. Although the validation cohort is relatively small and the current prediction rule should be evaluated in other early arthritis cohorts, we feel that the current model allows physicians and patients an evidence-based choice whether or not to initiate DMARDs in the majority of patients presenting with UA.

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Chapter 17

SUMMARY AND DISCUSSION

Rheumatoid arthritis is a chronic and self-amplifying autoimmune process that ultimately leads to the destruction of cartilage and bone. The pathophysiologic development of inflammation in RA is complex and involves both the adaptive and the innate arms of the immune system. The events triggering the autoimmune mechanisms are thought to be a combination of genetic and environmental factors and the process is best described by a multiple hit model.

The most important genetic risk factor for RA, the HLA-DRB1 alleles encoding for the shared epitope motif, was recognized three decades ago (1). In this thesis the shared epitope alleles are investigated in the perspective of the presence and absence of another important risk factor, the autoantibodies. The shared epitope alleles appeared to predispose only to RA-patients that carry anti-CCP antibodies and not to anti-CCP negative RA (**chapter 7**). This finding led to the hypothesis that the shared epitope alleles confer risk to anti-CCP antibodies, rather than to (anti-CCP positive) RA. To investigate this assumption, the progression from recent-onset UA to RA was studied in relation with the HLA-DRB1 alleles and autoantibodies (**chapter 10**). In patients that presented with UA, the shared epitope alleles were associated with anti-CCP antibodies, but after correction for the presence of anti-CCP antibodies the share epitope alleles were not an independent risk factor for progression to RA. Furthermore, the shared epitope alleles were primarily associated with the anti-CCP antibodies and not with RF. Although no formal conclusions on causality can be drawn from this association study, the findings presented in this chapter suggest that the shared epitope alleles confer risk to anti-CCP antibodies and that these antibodies mediate the association between the shared epitope alleles and RA.

Next, the best-known environmental risk factor for RA, smoking, was studied. The presence of both shared-epitope alleles and smoking resulted in a gene-environmental interaction for the presence of anti-CCP antibodies (**chapter 9**). The data of this study indicate that the gene-environmental interaction is more pronounced for the development of anti-CCP antibodies than for the development of RF. Further analysis revealed that tobacco exposure in the presence of shared epitope alleles did not significantly increase the risk for anti-CCP antibodies among patients with after one-year follow-up persistent undifferentiated arthritis (UA). This might indicate that the gene-environmental interaction is specific for RA. On the other hand, the gene-environmental interaction seems specific for anti-CCP antibodies and, in that case, the interaction should not be depended on a clinical diagnosis. Considering the small number of (anti-CCP positive) patients with persistent undifferentiated arthritis in this study, the power of the present study was probably inadequate to conclude definitely on the presence/absence of a gene-environmental interaction in UA.

The finding that the shared epitope alleles primarily associate with RA in the presence of anti-CCP antibodies led us to address the question whether anti-CCP negative RA is

associated with other, non-shared epitope encoding, HLA-DRB1 alleles. Analysis revealed that HLA-DR3 was more frequently present in the anti-CCP-negative RA and anti-CCP negative UA patients compared to controls, indicating that HLA-DR3 is associated with anti-CCP negative arthritis (**Chapter 8**).

The observation that anti-CCP positive and negative RA have different genetic risk factors indicates that different pathogenetic mechanisms underlie anti-CCP-positive and anti-CCP-negative disease. Therefore, it is conceivable that anti-CCP positive and negative RA are different disease entities and thus have different phenotypical properties. An extensive comparison of clinical characteristics at first presentation between anti-CCP positive and negative RA patients (evaluating among others morning stiffness, type and distribution of early symptoms, C-reactive protein level, tender and swollen joint count) revealed no significant differences. Nevertheless, during the disease course RA patients with anti-CCP antibodies had more swollen joints and more radiological joint destruction compared to RA patients without anti-CCP antibodies (**chapter 11**). Thus, although different risk factors are associated with anti-CCP positive and negative RA, the presence or absence of anti-CCP antibodies is not associated with a distinguishable clinical phenotype at the first presentation of the disease.

Considering the results of the above mentioned association studies, and the findings that citrullinated antigens are found in inflamed joints, anti-CCP antibodies are specific for RA and anti-CCP antibodies are generally present before the onset of clinical symptoms, it seems likely that the anti-CCP antibodies are important in the pathogenesis of RA. So far, there little evidence demonstrating that anti-CCP antibodies induce or mediate the development of chronic joint inflammation. Kuhn and collegues injected monoclonal antibodies reactive to citrullinated fibrinogen in mice and observed an exacerbation of arthritis. They also showed in a collagen-induced arthritis model that tolerization of CCP-specific immune responses resulted in a strong inhibition of arthritis severity (24). As these results are not yet replicated, the issue whether anti-CCP antibodies are involved in RA pathogenesis or are a byproduct of a more general immunological reaction is not clarified.

The Shared Epitope hypothesis postulates that the shared epitope motif is directly involved in the pathogenesis of RA. Data providing evidence that this hypothesis is correct is lacking. Since its initial description, the Shared Epitope hypothesis has been proven robust because of its consistent association with RA susceptibility and severity among varied ethnic populations, but so far a specific athritogenic peptide that binds to the HLA-DR proteins and induces a T cell response has not been found.

Since the identification of citrullinated proteins and anti-CCP antibodies it has been proposed that in genetically predisposed (shared epitope positive) subjects and an inflammatory environment citrullinated proteins break immunologic tolerance and initiate an

HLA-class II restricted T cell response (2,3). Subsequently, the help provided by T cells might allow maturation and switching of B cells, resulting in further maturation of the anti-CCP immunoglobulin response (2). With this hypothesis in mind, it is interesting to note that the data presented in chapter 7 show that carrying two copies of shared epitope alleles confers a significant higher risk on anti-CCP positive RA than the presence of one shared epitope allele (odds ratio 12 and 4 respectively). Compare these findings with the data presented in chapter 10. This chapter reveals that the presence of two shared epitope alleles is not associated with higher anti-CCP antibody levels compared to the presence of one shared epitope allele (the shared epitope alleles act like the previously described immune response genes). At first sight these observations seem contradicting, but this might be explained as follows. In the presence of more shared epitope alleles/ more HLA molecules, more antigen can be presented and immune tolerance is broken more easily, leading to a dose-response relation between the shared epitope alleles and the presence of anti-CCP antibodies. Conversely, antigen presentation might not be the limiting factor that determines the antibody level and the level of autoantibodies might be influenced by other factors. Nonetheless, it must be realized that at present no convincing experimental data exist showing that in shared epitope positive humans the presence of citrullinated peptides induces T cell proliferation. Currently, there are two studies that assess the effect of citrullination on T cell activation in relation with MHC molecules. Hill et al showed in mice transgenic for MHC-DRB1*0401 a vimentin peptide citrullinated in a region with contact to SE-binding site harboured a higher affinity for the MHC-DRB1*0401 molecule resulting in a better T cell acitivation compared to its noncitrullinated counterpart (4). Ireland et al used hen egg-white lysozyme as a model antigen and demonstrated the occurrence of a specific T cell response after citrullination (5). Both studies were performed in mice and the immunopathologic role of citrullinated peptides in humans needs further investigation.

This thesis considered not only the shared epitope alleles but also four other genetic risk factors for RA. The first is also located on the HLA class II alleles and is positioned on the same locus. It concerns the alleles encoding for the amino acids D^{70}ERAA. From previous (smaller) studies it was not clear whether the presence of DERAA was really protective or whether the observed effect was the result of the absence of predisposing shared epitope alleles. By comparing subgroups of RA-patients and controls with an equal amount of shared epitope alleles, we were able to show that the presence of the DERAA-encoding alleles had a protective effect on RA that was independent from the presence/absence of shared epitope alleles (**Chapter 2**). The presence of DERAA was associated with both a lower risk to develop RA and a less severe disease course. The protective effect of DERAA on susceptibility was not confined to anti-CCP positive RA, but seemed stronger for anti-CCP positive RA than for anti-CCP negative RA (odds ratio's of 0.3 and 0.7 respectively).

Considering the severity of RA, the protective effect of DERAA was most pronounced in the patients that were shared epitope positive, smoked or had anti-CCP antibodies. We realize now that these risk factors all influence the same pathway. Thus, the protective influence of DERAA can be detected best in patients that are prone to severe disease (Chapter 2). It is remarkable that although the presence of DERAA was evidently associated with less severe joint destruction and the (small group of) RA patients that were homozygous for DERAA had a non-destructive disease course, the presence of DERAA was not associated with a higher rate of clinical remission. Speculatively, the pathways associated with the expression of DERAA-encoding HLA-alleles are able to dampen the pathways underlying bone and cartilage destruction, but do not affect the principal pathway that drives chronicity. It has been demonstrated that peptides carrying the DERAA motif are naturally processed by human antigen-presenting cells (6). As discussed in chapter 2 the protective effect of DERAA might be due to the activation of regulatory T cells. These T cells are believed to play a key role in mediating transplantation tolerance and inhibiting the induction of tumor immunity; and might likewise suppress the immune response in RA. There are however other presumptions on the pathways underlying the effects of DERAA. The amino acid sequence DERAA is present in several microorganisms, such as mycobacteria, Haemophilus influenzae, Salmonella, and is also present in vinculin, a protein localized in the cell's cytoplasma. It can be speculated that patients that carry DERAA-encoding alleles are immunotolerant for DERAA when encountered during infections, but that DERAA-negative individuals develop an immune reaction against DERAA. During apoptosis vinculin, a part of the cytoskeleton, will be degraded, possibly leading to its presentation in the context of MHC-molecules. Indeed, it has been described that apoptotic cells overexpress vinculin and after ingestion by dendritic cells can be presented toward T cells under certain pathophysiological conditions (8). It is conceivable that DERAA-negative patients harbouring a DERAA-directed T cell response against all sorts of pathogens experience an accelerated disease progression as a consequence of T cell cross reactivity with vinculin-DERAA that is perhaps chronically presented in the inflamed joint in RA. According to this hypothesis, the presence of DERAA does not result in immunosuppression/protection but the absence of DERAA enforces the immune reaction and thereby disease progression. Currently, laboratory experiments are being undertaken to investigate this hypothesis.

The findings presented in chapter 2 lead to the question whether the presence of DERAA is helpful in predicting the development of RA in patients with UA. Analysis (not described in this thesis) revealed that in univariate analysis the presence of DERAA-encoding alleles in patients with recent-onset UA was significantly associated with a lower rate of progression towards RA. In multivariate analysis the absence of DERAA-encoding alleles appeared not to be an independent risk factor for the development of RA. Further analysis showed

that the presence of DERAA was associated with a lower number of tender and swollen joints, a lower level of C-reactive protein and a lower frequency of anti-CCP and RF positivity. This implies that the DERAA-encoding alleles associate with a milder phenotype and that the clinical and serological factors are stronger predictors for the disease course in UA-patients than the DERAA-encoding alleles. The determination of the DERAA-encoding alleles therefore does not add to the discriminative ability of the prediction model described in chapter 16.

Non-HLA genetic risk factors that are investigated in this thesis are single nucleotide polymophisms in the genes encoding for PTPN22, TNFR2 and RAGE.

The C1858T SNP in the *PTPN22* gene encoding for a lymphoid tyrosine phosphatase (Lyp) is associated with RA susceptibility, which was first identified by Begovich et al (9). This intracellular Lyp binds a Csk kinase and Csk-Lyp inhibits T-cell-receptor signaling. *In vitro* experiments have shown that in T-allele carriers Lyp binds less efficiently to Csk suggesting that T cells expressing the T-allele might be hyperresponsive (9). Knocking out the murine homologue of *PTPN22* resulted in lower thresholds for T-cell-receptor signaling (25). A recent study revealed a more active phosphatase in T-allele carriers and the authors suggested that the increased efficacy to inhibit T-cell-receptor signaling may lead to insufficient activity of regulatory T cells (26). The study described in **chapter 4** replicates the association between *PTPN22* and RA, but shows that compared to healthy controls the 1858 T-allele confers risk to only anti-CCP antibody positive RA and not to anti-CCP negative RA. A similar finding has been described for rheumatoid factor (10, chapter 4). In addition, the present study observed no correlation between *PTPN22* and the degree of radiological joint destruction in RA. This seems unsuspected regarding the finding that *PTPN22* associates with anti-CCP positive RA and anti-CCP antibodies are associated with severe disease. The most likely clarification for this finding is mathematical: 48 of 76 patients (63%) carrying the genotype CT or TT had anti-CCP antibodies compared to 149 of 274 (54%) patients with genotype CC. Thus, the percentages anti-CCP positivity were in the same range (and not significantly different), making it comprehensible that no significant difference in the degree of joint destruction was observed between these two groups of RA-patients. It is not excluded that, considering the small ORs of the effect of *PTPN22,* the number of RA-patients was too small to discern an effect of *PTPN22* on RA severity. Finally, this study showed that *PTPN22* is not only a risk factor for RA but also for (persistent) UA. This is in line with reports showing that the 1858 T-allele is associated with multiple autoimmune diseases as SLE, type 1 diabetes and Graves disease (11-14).

The investigated SNPs in the *TNFR2* and *RAGE* genes were not associated with RA. In **chapter 3** the *TNFR2 196M/R* genotypes of the patients with the most severe joint destruction and with clinical remission were similar. An effect of this SNP on RA severity, if present, was likely to be found by the comparison of the extremes of the phenotypes.

The absence of an association between *TNFR2 196 M/R* and RA severity is replicated by the French (15). Interestingly, one of the two reports that initially observed an association between this *TNFR2* SNP and susceptibility to familial RA was recently corrected, as the described finding appeared to be due to an error in genotyping (16).

There are findings that suggest a role for receptor for advanced glycosylation end products (RAGE) signaling in the pathogenesis of RA. RAGE is upregulated in synovial tissue macrophages in active RA (17) and activation of RAGE may lead to upregulation of adhesion molecules and expression of pro-inflammatory cytokines (18). Although initial findings suggested an association between the G82S SNP and RA susceptibility, this association is depended on the strong linkage disequilibrium between RAGE and the HLA-DR4 allele (**chapter 5**).

In conclusion, the genetic risk factors that associate with RA susceptibility and are described in this thesis are the shared epitope HLA-DRB1 alleles and the C1858T *PTPN22* SNP (predisposing effects) and the DERAA encoding HLA-DRB1 alleles (protective effects). Considering that the heritability of RA is estimated to be 50-60% (19) and that the identified genetic factors account for at most half of this percentage, more genetic risk factors for RA susceptibility are likely to be identified.

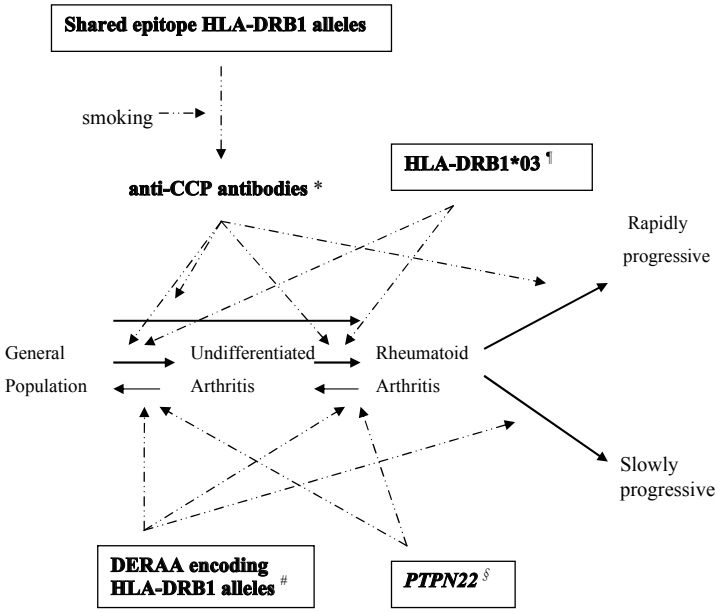
The genetic factors that associate with RA severity and are identified so far are the HLA-DRB1 alleles that encode for the shared epitope motif (effect via anti-CCP antibodies) or encode for DERAA. The heritability of RA severity is not known. The observation of an increase in variation of radiological joint destruction comparing respectively monozygotic twins, dizygotic twins and unrelated pairs of RA patients (after correction for differences in disease duration and autoantibody status) indicates that genetic factors are important for RA severity (**chapter 13**). More extensive twin studies are needed to quantify the genetic contribution to RA severity.

The level of joint destruction in RA patients correlated with the degree of *in vitro* measured invasiveness of fibroblast-like synoviocytes in a matrigel matrix (**chapter 12**). This study provides the first evidence that the *in vitro* measured invasiveness of fibroblast-like synoviocytes might be of relevance for the pathophysiology of RA.

The described genetic and environmental risk factors for the development of both UA and RA and for RA severity can be positioned in a multiple hit model, see Figure 1.

From the patients' and clinicians' perspective, an increased understanding of the pathogenesis of RA is particularly relevant when it has implications for (the treatment of) individual patients. Both UA and RA are diseases with a heterogeneous course. Achieving individualized treatment decisions in these patients is an important challenge for the nearby future. This signifies that for individual patients the predicted prognosis is

weighted against the possible desired and toxic effects of various treatments. For early UA, recent evidence indicates that treatment with methotrexate hampers progression to RA (20). However, from several inception cohorts it is known that only about one third of the patients that present with UA progress to RA and 40-50% remits spontaneously (20-23); these patients are preferably not treated with methotrexate. This underscores the need for a model that identifies the UA-patients with a high risk to progress to RA. The development of such a model is described in **chapter 16**. The derived model predicts in UA-patients the risk to develop RA using nine clinical variables that are all commonly assessed during daily clinical practice (gender, age, localisation of joints symptoms, morning stiffness, counts for tender and swollen joints, CRP, RF and anti-CCP antibodies). The resulting prediction score (a value between 0 and 14) corresponds with a chance on progression to RA. The positive and negative predictive values depended on the chosen cut-off values. If the upper and lower cut-off value were 8.0 and 6.0, the corresponding positive and negative predictive values were 84% and 91%. Twenty-five percent of the patients had a score between 6.0 and 8.0; in these patients no adequate prediction was made. Clinical characteristics are apparently insufficient to predict the disease outcome for these patients and it would be of interest to assess whether the addition of genetic factors increases the predictive ability. The known genetic risk factors for RA susceptibility, HLA-DRB1 alleles and *PTPN22*, do not have additive predictive value in this model, as the shared epitope encoding alleles correspond with the presence of anti-CCP antibodies that are already included in the prediction model and *PTPN22* confers risk both to UA and RA. Overall, the discriminative ability of the model was excellent with an area under the receiver operator curve of 0.87 after internal validation and 0.97 for external validation. As the cohort used for external validation was relatively small, the developed prediction rule needs to be validated in other cohorts of patients with UA. Since the developed prediction rule is accurate and easily assessed in clinical practice, the present model might prove to be an important step forward in achieving individualised decision-making in patients with UA. For the future it is hoped and expected that early treatment of the UA-patients with a high risk to develop RA will hamper the progression to RA. As the social impact of RA is nowadays still considerable (see introduction), the goal of such a therapeutic strategy is to diminish the impact of arthritis on patients' daily life.



* Predisposing effect on susceptibility to RA (chapter 7) and UA and development from UA to RA (chapter 10,16). Association with destructive disease (chapter 11). Interaction with smoking (chapter 9).

¶ Predisposing effect on anti-CCP negative RA (chapter 8).

Protective effect on susceptibility to UA (discussion), susceptibility to RA (chapter 2) and severity of RA (chapter 2).

§ Predisposing effect on both UA and RA (chapter 4).

Figure 1. Multiple hit model for the development of RA.

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Chapter 18

NEDERLANDSE SAMENVATTING

Het onderwerp van dit proefschrift is reumatoïde artritis. Bij deze ziekte staat ontsteking centraal. Het meest frequent zijn de gewrichten van de handen en voeten betrokken, maar alle gewrichten kunnen bij deze ziekte aangedaan zijn. De ontsteking richt zich tegen de bekleding van het gewrichtskapsel, het synovium. De ontstekingsreactie van het synovium zorgt voor een gezwollen, pijnlijk gewricht. De ontsteking van het synovium tast ook het kraakbeen en het bot aan; deze botaantasting is op röntgenfoto's te zien. Het beloop van reumatoïde artritis is wisselend: sommige patiënten hebben weinig gezwollen of pijnlijke gewrichten en nauwelijks of geen beschadigingen, terwijl andere patiënten vaak gezwollen gewrichten hebben met veel botaantasting op de röntgenfoto. De gevolgen van reumatoïde artritis zijn enorm. Een onderzoek uitgevoerd onder Nederlandse patiënten met reumatoïde artritis liet zien dat 42% van de patiënten problemen had met werken, 40% overdag extra rust moest nemen en 50-60% van de patiënten belemmerd was in de vrije tijdsbesteding. Volgens een recent overzicht van TNO komt reumatoïde artritis voor bij 1 op de 1000 mannen en 2 op de 1000 vrouwen in Nederland. Het grote aantal patiënten gecombineerd met de forse beperkingen ten gevolge van de ziekte heeft ook financiële consequenties. De directe kosten die uit de ziekte voortvloeien worden in Nederland op €5250 per patiënt per jaar geschat. Het laatste decennium is de behandeling van reumatoïde artritis veranderd. Er wordt in een eerder stadium gestart met krachtiger medicijnen, wat leidt tot een betere controle van de ziekte. Er is reeds bewijs dat deze manier van behandelen gunstige effecten heeft op zowel de mate van botbeschadiging als op het functioneren van patiënten.

Voordat de diagnose reumatoïde artritis gesteld mag worden, moet aan 4 van 7 opgestelde criteria voldaan zijn. Deze criteria zijn: 1) het hebben van ochtendstijfheid, 2) het hebben van ontsteking van 3 of meer gewrichten, 3) betrokkenheid van handgewrichten, 4) symmetrische gewrichtsontsteking, 5) het hebben van reumanoduli, 6) het hebben van reumafactor in het bloed en 7) het hebben van botaantasting op de röntgenfoto. Ten tijde van het eerste bezoek bij de reumatoloog hebben sommige patiënten direct voldoende kenmerken om de diagnose reumatoïde artritis te stellen (22%). In 40% van de patiënten zijn er wel gewrichtsontstekingen maar kan geen diagnose worden gesteld. Het ziektebeeld bij deze patiënten wordt ongedifferentieerde artritis genoemd. De uitkomst van ongedifferentieerde artritis is divers: ongeveer eenderde van de patiënten ontwikkelt reumatoïde artritis en in 40-50% verdwijnt de gewrichtsontsteking spontaan. Hoewel de behandeling van reumatoïde artritis momenteel effectiever is dan een decennium geleden, is het streven in toekomst om de gewrichtsontsteking op een vroeg moment te remmen, bijvoorbeeld in de fase van ongedifferentieerde artritis, zodat verdere progressie tot reumatoïde artritis voorkomen wordt. Voor het ontwikkelen van een dergelijke behandeling is het van belang dat het ontstaan van reumatoïde artritis beter begrepen wordt. Daarnaast is het van belang om te kunnen voorspellen welke patiënten met ongedifferentieerde artritis een

hoge kans hebben om reumatoïde artritis te krijgen. Dit voorkomt dat patiënten met een ongedifferentieerde artritis waarbij de kans groot is dat de gewrichtsontsteking spontaan verdwijnt onnodig behandeld worden. Dit proefschrift probeert in de eerste plaats een bijdrage te leveren aan het begrip over de ontstaanswijze van en risicofactoren voor het ontwikkelen van reumatoïde artritis. Ten tweede beschrijft dit proefschrift een model dat bij individuele patiënten met een ongedifferentieerde artritis gebruikt kan worden om de kans op progressie tot reumatoïde artritis te voorspellen.

In het eerste deel van het proefschrift wordt het verband van 4 genetische factoren en reumatoïde artritis beschreven. Hoofdstuk 2 laat zien dat mensen die bepaalde genen hebben, HLA-genen die coderen voor een bepaalde aminozuurvolgorde (DERAA), een verlaagd risico hebben om reumatoïde artritis te krijgen. Indien mensen met deze genen reumatoïde artritis krijgen verloopt de ziekte ook milder en treedt er minder botbeschadiging op. In hoofdstuk 4 wordt een tweede genetische risicofactor voor reumatoïde artritis beschreven, genaamd PTPN22. Het blijkt dat mensen die een bepaalde variant van deze genetische factor hebben vaker reumatoïde artritis en ongedifferentieerde artritis krijgen dan mensen zonder deze genetische variant. In de hoofdstukken 3 en 5 worden twee genetische factoren (het betreft de genen coderend voor TNFR2 en RAGE) beschreven die niet geassocieerd bleken te zijn met respectievelijk de ernst en het optreden van reumatoïde artritis.

De belangrijkste genetische risicofactor voor reumatoïde artritis is zo'n 30 jaar bekend en bestaat uit bepaalde HLA-genen die coderen voor een gemeenschappelijke structuur die 'shared epitope' genoemd wordt (hierna kortweg HLA-shared epitope). Reumatoïde artritis wordt beschouwd als een auto-immuun ziekte omdat het kenmerken heeft van een afweer die tegen het eigen lichaam is gericht. Een voorbeeld hiervan is de aanwezigheid van reumafactor, een antistof die bij ongeveer 60% van de patiënten met reumatoïde artritis voorkomt en gericht is tegen een onderdeel van bepaalde andere antistoffen. Reumafactor kan echter ook in het bloed aanwezig zijn bij andere ziekten of bij gezonde mensen. De laatste jaren is er veel belangstelling voor een tweede antistof, de anti-CCP antistoffen, die gericht tegen bepaalde (gecitrullineerde) eiwitten. Deze antistoffen lijken veel specifieker voor reumatoïde artritis te zijn dan reumafactor is. Het tweede deel van dit proefschrift onderzoekt de relatie tussen de HLA-shared epitope genen en de anti-CCP antistoffen. De analyse beschreven in hoofdstuk 7 laat zien dat de HLA-shared epitope genen alleen een risicofactor zijn voor reumatoïde artritis patiënten die anti-CCP antistoffen hebben en niet voor reumatoïde artritis patiënten zonder deze antistoffen. Een verdere analyse beschreven in hoofdstuk 10 geeft aanleiding te veronderstellen dat de HLA-shared epitope genen niet een directe risicofactor zijn voor het ontwikkelen van reumatoïde artritis maar primair geassocieerd zijn met de aanwezigheid van anti-CCP antistoffen, die vervolgens

gecorreleerd zijn met reumatoïde artritis. Het onderzoek beschreven in hoofdstuk 9 laat zien dat roken door mensen die HLA-shared epitope genen hebben een extra groot risico geeft op het ontwikkelen van anti-CCP antistoffen. Gezien de bevindingen van hoofdstuk 7-10 lijkt het erop dat de risicofactoren voor reumatoïde artritis verschillend zijn voor patiënten met en zonder anti-CCP antistoffen. Om die reden is in hoofdstuk 11 vergeleken of de aard van de klachten en de kenmerken bij het lichamelijk onderzoek bij de eerste ziektepresentatie tussen beide groepen patiënten verschillend is. Dit bleek niet zo te zijn. Wel bleken de mensen met anti-CCP antistoffen een ernstiger ziektebeloop te hebben met meer gezwollen gewrichten en meer botbeschadigingen op de röntgenfoto's.

In het derde deel van dit proefschrift worden twee aspecten die samenhangen met de ernst van reumatoïde artritis beschreven. In hoofdstuk 12 wordt bekeken of de in het laboratorium gemeten ingroei van bepaalde cellen uit de gewrichtsbekleding (fibroblast-like synoviocytes) in een kraakbeenmodel correleert met de mate van kraakbeendestructie en botbeschadiging bij patiënten gemeten op röntgenfoto's. Deze correlatie bleek inderdaad aanwezig te zijn, wat suggereert dat deze fibroblast-like synoviocytes betrokken zijn bij het ontstaan van gewrichtsaantasting in reumatoïde artritis. In hoofdstuk 13 wordt gekeken of er aanwijzingen zijn dat genetische factoren invloed hebben op de ernst van de ziekte. Hiertoe is de mate van gewrichtsaantasting van eeneiige tweelingen, twee-eiige tweelingen en niet-verwante patiëntparen met reumatoïde artritis vergeleken. De achtergrond is dat eeneiige tweelingen 100% identieke genen hebben, twee-eiige tweelingen gemiddeld 50% identieke genen en niet-verwante patiënten minder dan 50% identieke genen. Het bleek dat de variatie in gewrichtsaantasting het grootst was in de niet-verwante patiëntparen en het kleinst in de eeneiige tweelingen. Deze bevinding geeft aanleiding te veronderstellen dat genetische factoren van belang zijn voor de mate van ernst van reumatoïde artritis.

Het vierde deel van dit proefschrift gaat over het voorspellen van het beloop van de ziekte. Hoofdstuk 14 geeft een overzicht van de beschreven risicofactoren voor reumatoïde artritis en beschrijft voorwaarden waaraan een voorspelmodel moet voldoen. In hoofdstuk 15 is onderzocht of de verdeling van ontstoken gewrichten een voorspellende waarde heeft. Het blijkt dat het hebben van een gewrichtsontsteking van de knie bij de eerste ziektepresentatie gepaard gaat met een grotere kans op botbeschadigingen tijdens het verloop van reumatoïde artritis. In hoofdstuk 16 is de ontwikkeling van een voorspelmodel beschreven. Dit model voorspelt bij patiënten met een ongedifferentieerde artritis de kans op progressie tot reumatoïde artritis. Het model lijkt een adequaat voorspellend vermogen te hebben. Een voordeel van dit model is dat de benodigde gegevens om de kans op progressie tot RA te berekenen gebruikelijk verzameld worden tijdens het gesprek, lichamelijk onderzoek en laboratorium onderzoek op de polikliniek en het model daardoor makkelijk toepasbaar lijkt in de dagelijkse praktijk. Het model is reeds gevalideerd in een kleine,

onafhankelijke groep patienten met ongedifferentieerde artritis en het voornemen is om dit model ook in andere groepen patiënten met ongedifferentieerde artritis te toetsen. Er vanuit gaande dat ook in deze patiënten het ontwikkelde model het ziektebeloop goed zal voorspellen, biedt het in hoofdstuk 16 beschreven model een belangrijke hulp voor zowel artsen als patiënten bij het nemen van behandelbeslissingen in geval van een vroege ongedifferentieerde artritis. De hoop en verwachting voor de toekomst is dat een vroege behandeling van patiënten die een hoge kans hebben om reumatoïde artritis te ontwikkelen, bijvoorbeeld in het stadium van een ongedifferentieerde artritis, daadwerkelijk progressie naar reumatoïde artritis voorkomt. Het doel van een dergelijke behandeling is om de impact van een chronische gewrichtsziekte als reumatoïde artritis op het werkzame en sociale leven te verminderen.

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CURRICULUM VITAE

De auteur werd op 10 maart 1974 geboren te Delft. In 1992 behaalde zij het diploma Voorbereidend Wetenschappelijk Onderzoek (cum laude) aan het Westland college te Naaldwijk, waarna zij startte met de studie Biomedische Wetenschappen aan de Rijksuniversiteit Leiden. In 1993 werd het propedeutisch examen cum laude afgelegd en startte zij met de studie Geneeskunde, ook aan de Rijksuniversiteit Leiden. In 1996 werd het doctoraal examen in die studie en in 1998 het artsexamen succesvol afgelegd (beide cum laude). Van december 1998 tot en met april 1999 deed ze wetenschappelijk onderzoek bij de afdeling algemeen interne geneeskunde, sectie gerontologie en geriatrie van het Leids Universitair Medisch Centrum onder begeleiding van Dr. G.J. Blauw en Prof. Dr. R.G.J. Westendorp. In mei 1999 startte de auteur haar opleiding tot internist; de eerste twee jaar in het Rijnland ziekenhuis te Leiderdorp (opleider dr. F.C. Cluitmans) en vanaf mei 2001 in het Leids Universitair Medisch Centrum (opleider Prof. Dr. A.E. Meinders). Op 1 mei 2005 volgde registratie als internist. Op 1 september 2003 startte de auteur een stage op de kliniek reumatologie, waarna ze de opleiding tot reumatoloog vanaf 1 januari 2004 voorzette met een poliklinische stage (opleider Prof. Dr. F.C. Breedveld). Op het moment van afronden van dit proefschrift (februari 2006) hoopt zij in in het najaar van 2006 de opleiding tot reumatoloog af te ronden en aansluitend werkzaam te blijven binnen de afdeling reumatologie van het LUMC.

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