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

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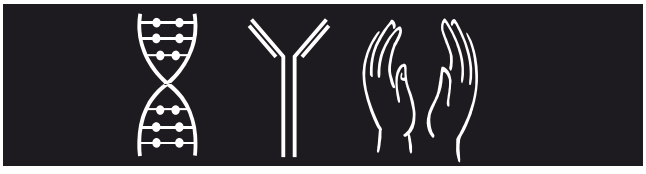
Chapter 1

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).

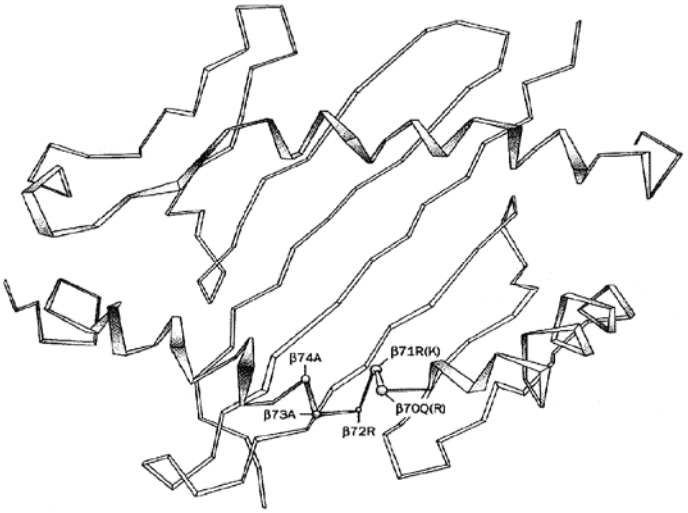


Figure 1. Structure of the HLA-DRB1 molecule. The HLA-DRB1 molecule is composed of an α-chain and a highly polymorphic β-chain. The antigen-presenting binding site is formed by a β sheet (floor) and two helices at both sides. The amino acid residues at position 70-74 are indicated. Based on Brown JH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

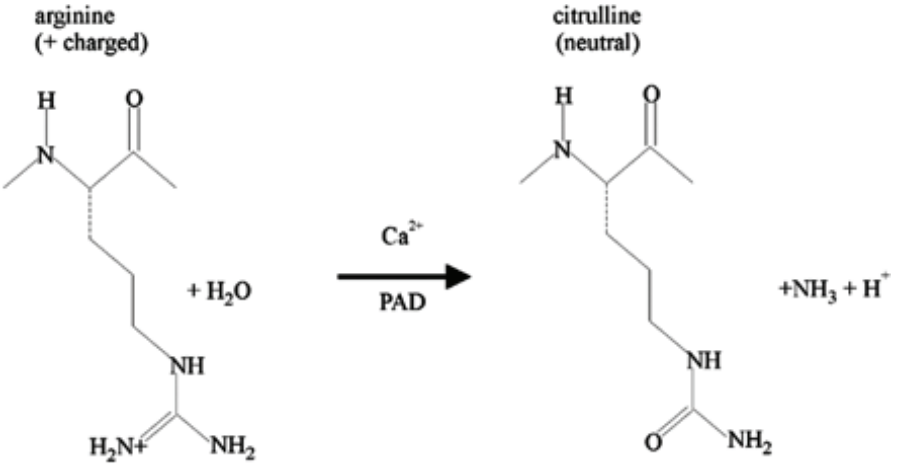


Figure 2. Conversion of arginine to citrulline, mediated by the enzym peptidylarginine deiminase (PAD).

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

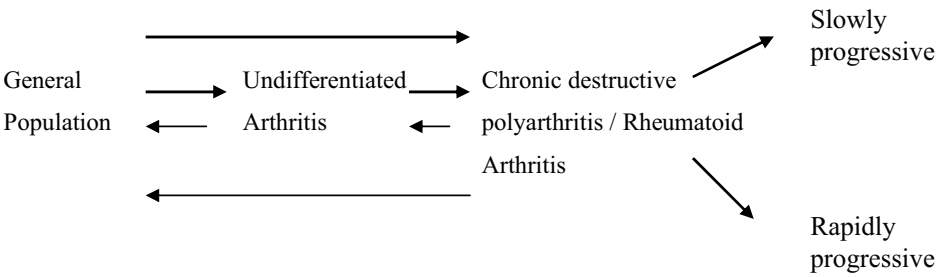


Figure 3. Variable disease course of patients with undifferentiated arthritis.

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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