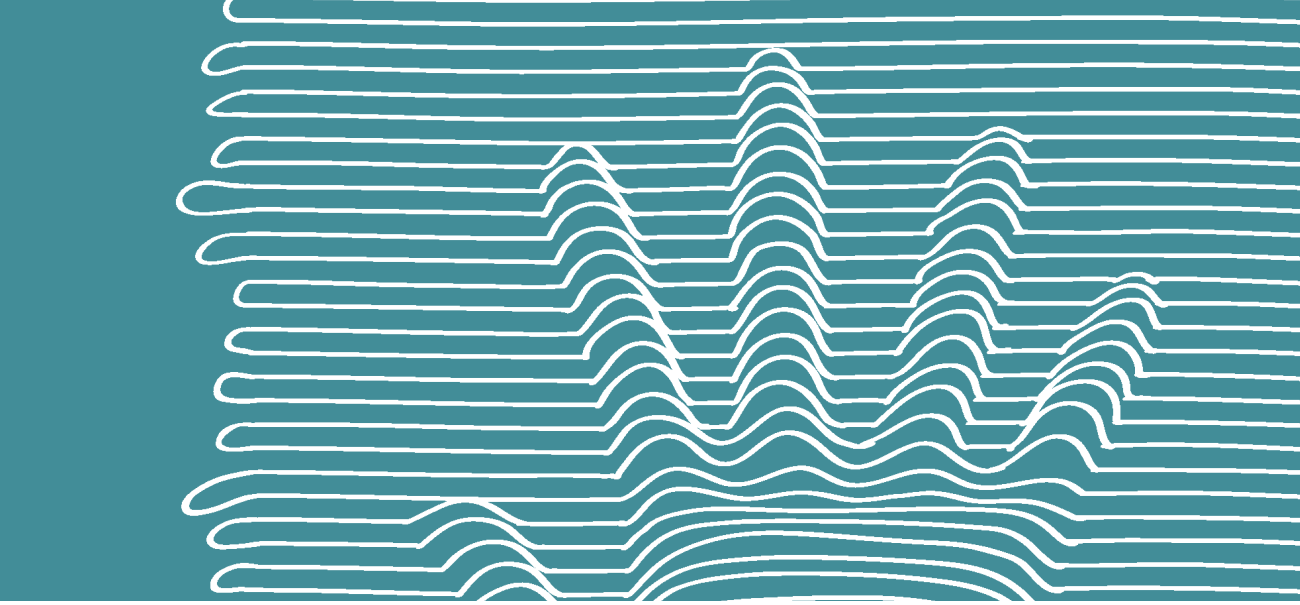

# CHAPTER 1

## Introduction

## INTRODUCTION

Rheumatoid arthritis (RA) is an auto-immune disorder characterized by chronic inflammation of synovial joints. Although the precise etiology of the disease has not yet been established, it is known that both environmental and genetic factors play a pivotal role in development of the disease. In the current thesis, studies to unravel the genetic basis of RA and the putative functional consequences of these genetic variances are described.

### Rheumatoid arthritis

RA is a chronic inflammatory disease characterized by persistent synovitis and destruction of synovial joints, leading to severe disability, decreased quality of life and premature mortality. In industrialized countries approximately 0.5-1.0% of the adult population is affected by the disease, with a dominance in females and elderly individuals. The disease reveals itself by joint swelling and joint tenderness, in which the small joints of the hands and feet are most commonly affected[1].

The definition of RA is phenotypic and is defined by use of classification criteria. These criteria were developed on a consensus procedure by clinical experts and resulted in the ACR 1987 criteria[2].

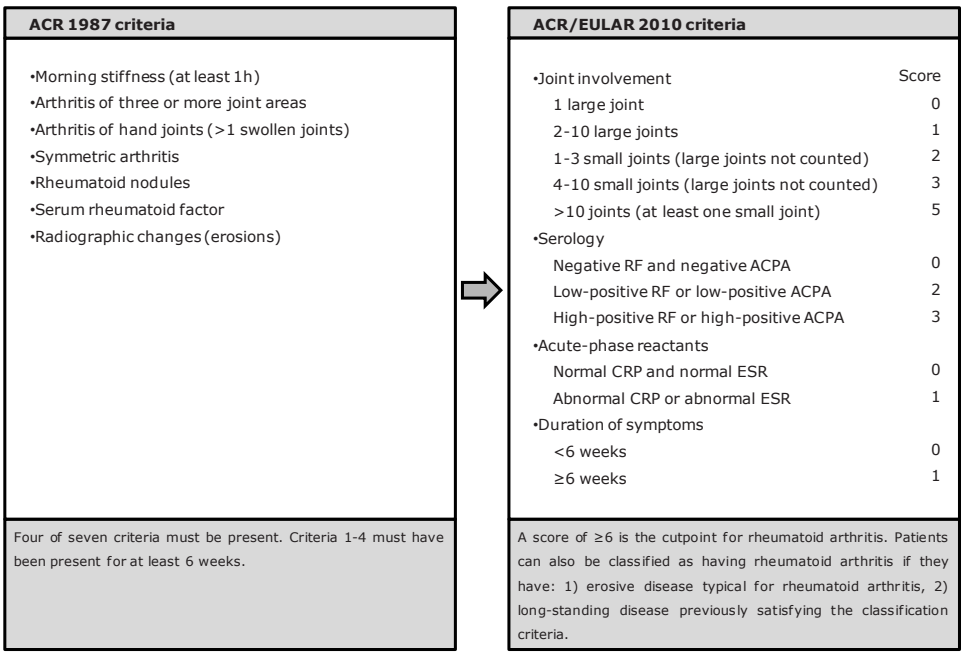


***Figure 1.*** *ACR 1987 criteria and ACR/EULAR 2010 criteria. Classification criteria for rheumatoid arthritis. ACR = American College of Rheumatology. EULAR = European League Against Rheumatism. RF = rheumatoid factor. ACPA = Anti Citrullinated Peptide Antibodies. CRP = C-reactive protein. ESR = erythrocyte sedimentation rate.*

These criteria are well accepted for disease definition, but have proven to be of limited value in defining the earliest forms of RA. Therefore, recently, a new set of criteria has been developed (Figure 1)[3].

The mainstay of treatment in RA, are the Disease Modifying Anti Rheumatic Drugs (DMARDs), which are a heterogeneous collection of therapeutic agents of which the mechanisms of action are, largely, not well understood. When arthritis stays uncontrolled despite these agents, or when toxic effects arise upon administration of these drugs, biologic agents, such as tumor necrosis factor inhibitors can be used and have proven to be highly effective[1].

**Pathophysiology**

The inflamed synovial tissue is expanded by recruitment and retention of inflammatory cells, like macrophages, T- and B-cells. This leads to the formation of villous projections and the generation of pannus tissue, which is a feature seen in joints of RA patients. This pannus tissue results from proliferating synovium and is thought to mediate tissue destruction. Chronic synovitis is maintained by interaction of native and recruited inflammatory cells with subsequent establishment of cytokine networks. The combination of chronic inflammation and formation of pannus tissue will eventually lead to joint space narrowing and joint erosions[4,5].

RA is considered to have an auto-immune nature, because of the presence of autoantibodies and autoreactive T-cells in peripheral blood and synovial fluid. These autoantibodies are already present in the earliest stages of disease and can precede disease onset by several years[4].

The classic autoantibody in RA is rheumatoid factor (RF) and was first described in 1940 by Erik Waaler. This antibody is directed to the Fc portion of IgG, which is important for complement fixation and interaction with Fc receptors. The etiology of RF and its precise role in the pathogenesis of RA is still incompletely understood. RF is not unique for RA and is also present in other autoimmune diseases, infectious diseases and in healthy elderly individuals. The sensitivity varies between 60 and 70% and the specificity between 50 and 90%[6].

An additional type of autoantibody is directed against citrullinated peptides and is called Anti Citrullinated Peptide Antibody (ACPA). Citrullination is the post translational modification of protein-bound arginine into the non-standard amino acid citrulline (Figure 2). This process is mediated by an enzyme called peptidylarginine deiminase (PAD)[7,8]. The role of citrullination remains to be determined, however, it is known that the process of citrullination typically occurs in apoptotic cells. This results in a small change of molecular mass and loss of a positive charge. It has been proposed that this process prepares intracellular proteins for degradation during apoptosis[9,10].

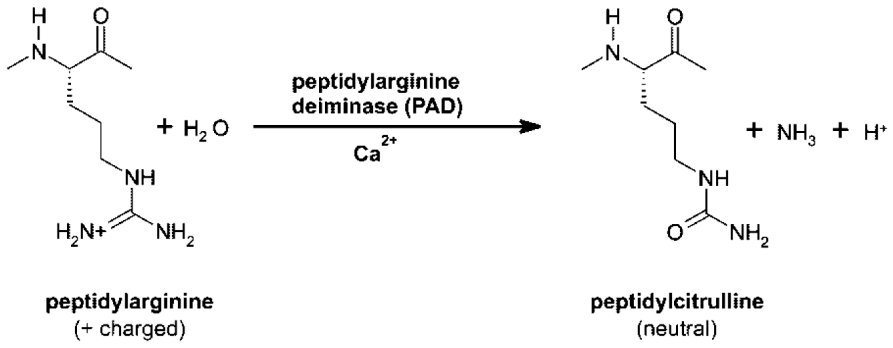


***Figure 2.*** *Citrullination of an aminoacid. Posttranslational modification of arginine into citrulline, mediated by peptidylarginine deiminase (PAD).*

Although the presence of these antibodies has first been described in the early 1970's, it was not until the last decade that it became clear that these antibodies are highly predictive and specific for RA. The observations that these antibodies precede disease onset and that citrullinated antigens can be found in the inflamed joint, lead to the hypothesis that these antibodies play a role in the pathophysiology of RA[11,12].

**Risk factors**

*Environmental factors*

The major environmental risk factor for RA is smoking, which doubles the risk of developing the disease. This risk factor, however, is restricted to the ACPA positive disease subset[13]. Other factors that might influence the development of the disease have been proposed, but supporting evidence is weak and should be investigated more thoroughly.

*Genetic factors*

The first evidence for a genetic component in the susceptibility of RA came from twin and family studies. These studies showed that among siblings, the prevalence of RA was increased to 2-4% and that the concordance rate for monozygotic twins was 12-15%, as compared to 2-4% for dizygotic twins[14,15]. Additionally, the heritability of RA, which estimates the extent to which variation in liability to disease in a population can be explained by genetic variation, was calculated to be about 50%[16]. These studies demonstrate that genetic factors have a substantial impact on RA susceptibility.

The most important genetic risk factor was first described over 30 years ago and is confined to the human leukocyte antigen (HLA) locus[17]. Studies have shown that several HLA-DRB1 alleles are associated with the disease and that different alleles associate in different ethnic populations[18,19]. Interestingly, the association is confined to the ACPA positive

subset of the disease[20].

The HLA-DRB1 alleles encode the variable region of the HLA class II molecule. This variable region constitutes the binding groove for the peptide that is presented by the HLA molecule to T-cells. The product of the associated HLA-DRB1 alleles appears to share the same amino acid sequence at position 70-74 in the third hypervariable region of the HLA class II molecule. Therefore, the collection of different associated alleles are called the Shared Epitope (SE) alleles[21,22]. The SE hypothesis postulates that the SE motif is directly involved in RA pathogenesis by allowing presentation of certain (arthritogenic) peptides to T-cells[23]. To date, no specific peptide that is presented in this binding groove and subsequently activates autoreactive T-cells has been identified.

The total genetic contribution of the SE alleles to RA has been quantified in twin studies and is estimated to be 50-60% of the genetic contribution[16]. Therefore the HLA locus is thus far the most powerful genetic factor and accounts for approximately 30% of the genetic burden to RA[25].

It required almost 30 years before evidence of genetic associations outside the HLA locus were convincingly demonstrated by the discovery of *PADI4* and *PTPN22*. *PADI4* was discovered in a Japanese RA population by fine mapping of a linkage region on chromosome 1p36[26]. Interestingly, this gene encodes the PAD enzyme, which is needed for the posttranslational modification of arginine into the, by ACPAs recognized, citrulline[8]. The association has been convincingly replicated in other Asian populations, but could not be established the in Caucasian population[27,28].

The discovery of *PADI4* was followed by the identification of the genetic association with *PTPN22*. This gene was initially identified in a multi-tiered, case control study of putative functional single nucleotide polymorphisms (SNPs) in a Caucasian population from North America. The identified risk allele encodes an amino acid change, which is thought to alter the proteins normal function[29]. Well powered studies have successfully replicated the association and it is thought of as the most reproducible genetic association outside the HLA region[30]. The associated variant shows a decreasing frequency going north to south in European populations, with a minor allele frequency of approximately 15% in Scandinavia to 2.5% in the Italian population[29]. The risk allele is virtually absent in Asian populations and attempts of identifying additional *PTPN22* alleles in this population have not been successful[31]. Contrasting, in the case of *PADI4*, the risk allele is present in the Caucasian population, but has not proven to be associated with the disease.

After 2004 the identification of new genetic risk factors accelerated, due to advances in genotyping technology, available at reasonable costs. By candidate gene approach the CTLA4 gene was identified, followed by the identification of the C5/TRAF region[32,33]. This region was detected concurrently in a genome wide association study (GWAS)[34]. Linkage data led to the identification of *STAT4* as a genetic risk loci[35]. Large GWAS in ACPA positive individuals allowed the identification of new genetic risk factors to take enormous steps forward. In 2008 additional risk loci were identified by a meta-analysis of various GWAS,

of which the most significant finding is located near the CD40 gene[36]. Currently, over 100 genetic loci are considered as established genetic risk factors for RA, all with relatively weak effects with moderate ORs[37].

**Aim and outline of this thesis**

The aim of this thesis is to get more insight into the genetic contribution of loci located outside the HLA region to RA susceptibility and the functional role the associated variants play in disease pathology.

In recent years many genetic risk loci, located outside the HLA region, have been identified. Some of these loci represent true associations, while others will prove to be false positive findings. In **chapter 2** and **chapter 3** replication studies to identify the true genetic contribution of *STAT4, IL2/IL21, CTLA4* and *IL2RA* to RA susceptibility was investigated in the Leiden RA population. Subsequently, to establish the true genetic effect of these loci, a meta-analysis combining all previously published data was performed.

In **Chapter 4** we investigated the genetic contribution of the, then known, non HLA genes in a subset of North American Natives, that confer a higher risk to develop RA. Subsequently, we investigated whether an interaction between the genes was present and would confer a higher risk obtaining the disease.

To identify additional genetic risk factors for RA, a candidate gene study to investigate the genetic contribution of the *VTCN1* region to RA susceptibility was performed in **chapter 5.** Based on previously published genetic association data of this region with juvenile idiopathic arthritis and indications in mouse models, we hypothesized that the *VTCN1* region might play a role in the development of RA.

In **chapter 6** the genetic contribution of the PTGES gene was explored in relation to disease susceptibility and various disease outcomes, like gender, DAS28 and age of onset of RA. Gene expression was investigated in RA synovium and related to genotype.

Additionally, a candidate gene study on the genetic susceptibility of the C1Q region with RA was investigated in **chapter 7**. We hypothesized that C1Q, which plays a pivotal role in activation of the classical pathway of the complement system, is a genetic risk factor for RA susceptibility. Subsequently, gene expression profiles and C1q serum levels were investigated in relation to genotypes.

Subsequently, in **Chapter 8**, the role of the complement system to activate autoantibodies was summarized in a review that discusses the different pathways of activation.

The results of the studies performed in this thesis were summarized and discussed in **chapter 9.**

## REFERENCES


1. Scott DL, Wolfe F, Huizinga TW Rheumatoid arthritis. Lancet 376; 1094-108.
2. Arnett FC, Edworthy SM, Bloch DA *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31; 315-24.
3. Aletaha D, Neogi T, Silman AJ *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 69; 1580-8.
4. Feldmann M, Brennan FM, Maini RN Rheumatoid arthritis. Cell 85; 307-10.
5. Shiozawa S, Tokuhisa T Contribution of synovial mesenchymal cells to the pathogenesis of rheumatoid arthritis. Semin Arthritis Rheum 21; 267-73.
6. Visser H, Gelinck LB, Kampfraath AH, Breedveld FC, Hazes JM Diagnostic and prognostic characteristics of the enzyme linked immunosorbent rheumatoid factor assays in rheumatoid arthritis. Ann Rheum Dis 55; 157-61.
7. Steiner G, Smolen J Autoantibodies in rheumatoid arthritis and their clinical significance. Arthritis Res 4 Suppl 2; S1-S5.
8. Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. Bioessays 25; 1106-18.
9. Tarcsa E, Marekov LN, Mei G, Melino G, Lee SC, Steinert PM Protein unfolding by peptidylarginine deiminase. Substrate specificity and structural relationships of the natural substrates trichohyalin and filaggrin. J Biol Chem 271; 30709-16.
10. Wang Y, Wysocka J, Sayegh J *et al*. Human PAD4 regulates histone arginine methylation levels via demethylimination. Science 306; 279-83.
11. Nielen MM, van SD, Reesink HW *et al*. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 50; 380-6.
12. Rantapaa-Dahlqvist S, de Jong BA, Berglin E *et al*. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 48; 2741-9.
13. Stolt P, Bengtsson C, Nordmark B *et al*. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. Ann Rheum Dis 62; 835-41.
14. Aho K, Koskenvuo M, Tuominen J, Kaprio J Occurrence of rheumatoid arthritis in a nationwide series of twins. J Rheumatol 13; 899-902.
15. Silman AJ, MacGregor AJ, Thomson W *et al*. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br J Rheumatol 32; 903-7.

16. MacGregor AJ, Snieder H, Rigby AS *et al*. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 43; 30-7.

17. Stastny P Mixed lymphocyte cultures in rheumatoid arthritis. J Clin Invest 57; 1148-57.

18. Wakitani S, Murata N, Toda Y *et al*. The relationship between HLA-DRB1 alleles and disease subsets of rheumatoid arthritis in Japanese. Br J Rheumatol 36; 630-6.

19. Wordsworth BP, Lanchbury JS, Sakkas LI, Welsh KI, Panayi GS, Bell JI HLA-DR4 subtype frequencies in rheumatoid arthritis indicate that DRB1 is the major susceptibility locus within the HLA class II region. Proc Natl Acad Sci U S A 86; 10049-53.

20. Huizinga TW, Amos CI, van der Helm-van Mil AH *et al*. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum 52; 3433-8.

21. Gregersen PK, Moriuchi T, Karr RW *et al*. Polymorphism of HLA-DR beta chains in DR4, -7, and -9 haplotypes: implications for the mechanisms of allelic variation. Proc Natl Acad Sci U S A 83; 9149-53.

22. Gregersen PK, Shen M, Song QL *et al*. Molecular diversity of HLA-DR4 haplotypes. Proc Natl Acad Sci U S A 83; 2642-6.

23. Gregersen PK, Silver J, Winchester RJ The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 30; 1205-13.

24. Ollier WE, Kennedy LJ, Thomson W *et al*. Dog MHC alleles containing the human RA shared epitope confer susceptibility to canine rheumatoid arthritis. Immunogenetics 53; 669-73.

25. Deighton CM, Walker DJ, Griffiths ID, Roberts DF The contribution of HLA to rheumatoid arthritis. Clin Genet 36; 178-82.

26. Suzuki A, Yamada R, Chang X *et al*. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 34; 395-402.

27. Burr ML, Naseem H, Hinks A *et al*. PADI4 genotype is not associated with rheumatoid arthritis in a large UK Caucasian population. Ann Rheum Dis 69; 666-70.

28. Lee YH, Rho YH, Choi SJ, Ji JD, Song GG PADI4 polymorphisms and rheumatoid arthritis susceptibility: a meta-analysis. Rheumatol Int 27; 827-33.

29. Begovich AB, Carlton VE, Honigberg LA *et al*. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 75; 330-7.

30. Lee YH, Bae SC, Choi SJ, Ji JD, Song GG The association between the PTPN22 C1858T polymorphism and rheumatoid arthritis: a meta-analysis update. Mol Biol Rep.

31. Ikari K, Momohara S, Inoue E *et al*. Haplotype analysis revealed no association between the PTPN22 gene and RA in a Japanese population. Rheumatology (Oxford) 45; 1345-8.

32. Kurreeman FA, Padyukov L, Marques RB *et al*. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. PLoS Med 4; e278.

33. Plenge RM, Padyukov L, Remmers EF *et al*. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. Am J Hum Genet 77; 1044-60.

34. Plenge RM, Seielstad M, Padyukov L *et al*. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. N Engl J Med 357; 1199-209.

35. Remmers EF, Plenge RM, Lee AT *et al*. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med 357; 977-86.

36. Raychaudhuri S, Remmers EF, Lee AT *et al*. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat Genet 40; 1216-23.

37. Okada Y, Wu D, Trynka G *et al*. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 506; 376-81.