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The Netherlands

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

Helm-van Mil, A.H.M. van der

### **Citation**

Helm-van Mil, A. H. M. van der. (2006, October 26). *Genetics, autoantibodies and clinical features in understanding and predicting rheumatoid arthritis*. Retrieved from <https://hdl.handle.net/1887/4929>

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**Note:** To cite this publication please use the final published version (if applicable).

## **Chapter 6**

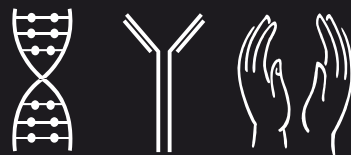
# **Understanding the genetic contribution to rheumatoid arthritis**

A.H.M. van der Helm - van Mil

J.Z. Wesoly

T.W.J. Huizinga

Curr Opin Rheumatol 2005;17(3):299-304.



## 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 multicaser 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  $\pm$  SD of about  $11 \pm 4$  false-positive peaks were expected, the number of true RA linkage peaks was estimated to be  $8 \pm 4$ .

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 citrullination caused by mutations in enzymes involved in citrullination. 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 citrullinated 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 citrullinated 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 RRRRAA) at position 70-74 in the third hypervariable region of the DR $\beta$ 1 chain. These residues constitute an  $\alpha$ -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 to 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 its 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 citrinnulated antigens is influenced by the better fit of antigens after citrinnulation 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 themselves 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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