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## Genetic studies in rheumatoid arthritis

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# Chapter 1

## General Introduction



DeoxyriboNucleic Acid, DNA, a substance of high molecular weight, was identified in 1871 by a young Swiss scientist, Friedrich Miescher<sup>1</sup>. Decades later, in one of the culminating points in biology of all time, James Watson and Francis Crick cracked the DNA code<sup>2</sup>. The two strands of the double helix are anti-parallel, with a sugar phosphate backbone on the outside while bases on the inside form the rung of the ladder. Each rung is composed of two base pairs. Either an adenine-thymine (A-T) pair that form a two-hydrogen bond together, or a cytosine-guanine (C-G) pair that form a three-hydrogen bond.

Nearly forty years later, a new defining moment was attained when the Human Genome Project was initiated in 1990. It was successfully completed in 2003, a year which marks the 50th anniversary of the discovery of the double helix structure of DNA by Nobel Prize winners, James Watson and Francis Crick. The overall result was the generation of a high-quality reference DNA sequence for the human genome's 3.2 billion base pairs.

Available to researchers worldwide, the human genome reference sequence provides a magnificent and unprecedented biological resource that serves as a basis for research and discovery and, ultimately, a number of practical applications. In 2002, it inspired a consortium of researchers to embark on the HapMap project to characterize all single nucleotide polymorphisms (SNPs) which are single base differences between individuals. By the end of 2005, researchers had created a map of these patterns across the genome by determining the genotypes of one million or more sequence variants, their frequencies and the degree of association between them, in DNA samples from populations with ancestry from parts of Africa, Asia and Europe<sup>3</sup>. In 2007, this consortium reported the completion of over 3 million SNPs, which represents a third of the estimated 10 million SNPs in the human genome<sup>4</sup>. Together, these research milestones have successfully formed the foundation of current genetic and genomic concepts.

The central goal of human genetics is to understand the inherited basis of human variation, not only in determining differences in phenotypes between individuals but also in elucidating predisposition to disease. With these tools in hand, we can now discover sequence variants that affect common disease, facilitate the development of diagnostic tools and enhance our ability to choose targets for therapeutic intervention.

This thesis focuses on investigating genetic risk factors in rheumatoid arthritis (RA), an autoimmune disease with unknown etiology. While there is a clear genetic component to the development of RA<sup>5</sup>, unraveling the genes predisposing to this complex disease, as well as other autoimmune diseases, has been rather elusive. In this chapter, I will provide an overview on rheumatoid arthritis, its genetic basis and the genes identified prior to the start of our research.

## **Rheumatoid Arthritis**

Rheumatoid Arthritis (RA) (MIM 180300 [OMIM] ) is a chronic autoimmune disease that affects approximately 0.5-1% of the adult population worldwide and is associated with significant disability and early mortality<sup>5</sup>. Patients suffer from inflammation of the synovial membrane that covers the joint. Joints become red, swollen and tender, and stiffness prevents their use. By definition, RA affects multiple joints. Most commonly, small joints of the hands and feet are affected, although larger joints like the shoulder and knee can also be involved, differing per individual. Eventually, synovitis leads to erosion of the joint surface, causing deformity and loss of function<sup>6</sup>. While the causes of early mortality in RA are not well-known, it may be explained by several factors, e.g chronic exposure to inflammation and cumulative toxicity of immunosuppressive drugs resulting in an increased risk to infectious, cardiovascular, gastrointestinal and respiratory disease<sup>7</sup>.

Besides these deleterious consequences for the individual patient, there is a considerable socio-economic impact leading to direct and indirect costs of many billion Euros per year. The total cost of the disease in 2006 was estimated at €45 billion in Europe and €42 billion in the United States<sup>8</sup>. It is therefore of the utmost importance to understand the basis of this disease so as to prevent exorbitant socio-economic costs, but primarily to alleviate patient suffering.

### ***Diagnosis and Clinical phenotypes of RA***

The diagnosis of RA is based upon a set of clinical and laboratory measures as defined by the American College of Rheumatology (Table 1). Once a diagnosis is established, the disease course of RA patients remains highly variable, ranging from mild symptoms to chronic inflammation and extensive joint damage.

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints
3. Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

**Table 1.** American College of Rheumatology (ACR) 1987 revised criteria for the classification of Rheumatoid Arthritis. A patient is diagnosed with rheumatoid arthritis if he/she has satisfied at least 4 of the 7 criteria described below. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded.

RA patients who fulfill the ACR criteria can be divided into two main subsets; those who possess circulating autoantibodies “autoantibody positive” and those who do not “autoantibody negative”. In the context of this thesis, the term autoantibody positive or negative will refer to the presence or absence of either of the two autoantibodies that play a major role in RA, namely, Rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). The classical autoantibody associated with RA is RF, an autoantibody directed against the Fc part of immunoglobulin G. RF is not unique to RA, and is present in other autoimmune diseases, infectious diseases and healthy (elderly) individuals. RF is found in 60-70% of RA patients<sup>9</sup>. In contrast, ACPAs are antibodies directed against citrullinated proteins. Citrullination is the post-translational modification of protein-bound arginine into the non-standard amino acid citrulline. This process results in a small change in molecular mass and the loss of a positive charge<sup>10</sup>. These autoantibodies appear early in RA and can be detected years before disease onset<sup>11,12</sup>. While being found in 50–70% of patients, ACPAs display a unique specificity for RA and are rarely detected in other diseases or in healthy controls<sup>10,12,13</sup>. The relevance of these autoantibodies in disease is exemplified by the fact that patients harboring these autoantibodies generally have a more severe disease course<sup>14,15</sup>. However, whether either of these two autoantibodies are part of a mechanism of disease initiation is still unclear. In contrast, no doubt exists that these autoantibodies represent a very useful tool in both diagnostic and prognostic terms<sup>16</sup>, as well as in defining a more homogeneous subset of patients to enhance the discovery of risk factors involved<sup>17</sup>.

### ***The role of environment and genetics in RA***

RA is considered to be a complex disease, and although the full etiology remains unclear, it is widely accepted that interrelated contributions from environmental and genetic factors play a major role<sup>18</sup>. Interestingly, environmental risk factors so far encompass age, gender, smoking, pregnancy, infections, diet and weather<sup>19</sup> (reviewed in Kobayashi et al). While there is an elevated incidence with an increase in age<sup>20</sup>, the female to male ratio of RA patients is ~3:1<sup>5</sup>, and generally smokers have a higher propensity to develop RA<sup>21</sup>.

There is however a rather large genetic component to RA. Evidence from twin studies demonstrates excess disease concordance between monozygotic (15%) when compared with dizygotic (3.6%) twins<sup>22</sup>. From such studies, the genetic contribution to RA has been estimated between 50% and 60%<sup>23</sup>. The increased risk of disease in siblings of patients with RA compared with that of the general population ( $\lambda_s$ ) has been estimated to be between 2 and 17 fold<sup>24</sup>. These data altogether provide compelling evidence of the role of genetics in the development of RA.

### **HLA, the most prominent genetic risk factor in RA**

The most prominent genetic association is confined to the human leukocyte antigen (HLA) locus on the short arm of chromosome 6. HLA-DR gene variants have been consistently associated with RA across several populations and in microsatellite-based whole genome screens on affected sibling pair families in Europe, US, UK and Japan<sup>25-31</sup>. This method involves a search for increased sharing of particular genetic regions among affected siblings. The association of HLA with RA has been mapped to the third hypervariable region of DR $\beta$ -chains, especially aa 70–74, encoding a conserved amino acid motif (QKRAA, QRRAA, or RRRRAA). This susceptibility epitope, called the shared epitope (SE), is found in multiple RA-associated DR molecules, including *DR1*, *DR4*, *DR10* and *DR14* (i.e. *DRB\*0101*, *DRB\*0102*, *DRB\*0401*, *DRB\*0404*, *DRB\*0405*, *DRB\*0408*, *DRB\*1001* and *DRB\*1402*)(32). However, amino acids encoding the DERRA motif (i.e. *DRB\*0103*, *DRB\*0402*, *DRB\*1102*, *DRB\*1103*, *DRB\*1301*, *DRB\*1302* and *DRB\*1304*) at the same position have a protective effect on the development of RA<sup>33-36</sup>.

The HLA region is gene-rich consisting of over 100 immune-related genes that could be potentially relevant to the pathogenesis of RA<sup>37-39</sup>. Therefore, understanding of the biological role of this region in RA remains to be discovered. However, since the association with the HLA region only accounts for approximately 30% of the genetic burden to RA, it implies that additional genetic risk factors play a role in RA<sup>40</sup>.

### **Non-HLA genes in RA**

A number of markers outside the HLA region did emerge from these four microsatellite-based whole genome studies which were suggestive of linkage with RA, although the effect of the HLA region is by far the strongest. One problem is that such studies have weak power to detect modest effects. In contrast, because such studies are simultaneously testing a large number of regions which may be linked with disease, the likelihood of a false-positive result is very high. Regions of linkage also tend to encompass large genetic regions containing a large number of genes making it difficult for researchers to progress from the linkage region to the causal gene. It is therefore not surprising that studies often failed to replicate their results.

One of the few success stories came from a Japanese group. Following indications of linkage from the overlapping locus on chromosome 1p36 among certain genome-wide scans, Yamamoto and colleagues observed that this genomic region contains a cluster of enzymes that is functionally associated with the production of rheumatoid arthritis-specific autoantibodies. These enzymes are the peptidylarginine deiminases (PADIs), which posttranslationally convert arginine residues to citrulline. Fine-mapping of this region containing four of these enzymes (PADI 1-4) located next to one another revealed that polymorphisms in the *PADI4* gene are strongly associated with RA in the Japanese<sup>41</sup>. However, whether this gene is associated with RA in Caucasians remains a question of debate and so far, no conclusive evidence has been obtained<sup>42,43</sup>.

Another major breakthrough, employing a large-scale approach did not come from classical linkage studies but association studies between patients and healthy individuals in 2004. Begovich and colleagues performed a large-scale screen utilizing putative functional SNPs and identified a non-synonymous SNP (R620W) in the gene, protein tyrosine phosphatase non-

receptor type 22 (*PTPN22*) more frequently in patients than in healthy individuals<sup>44</sup>. Well powered studies have successfully replicated the same association of rheumatoid arthritis and the R620W polymorphism, in populations of European descent from the UK, Finland, Sweden, Germany, Netherlands, Spain and Canada<sup>45</sup> (reviewed by Bowes *et al*, 2008). Such consensus was previously unprecedented. Intriguingly, both HLA-SE alleles and *PTPN22* are associated with the development of ACPA positive disease(17). It is of note that the *PTPN22* R620W SNP is not polymorphic in the Japanese population and a haplotype analysis of the region reveals no association<sup>46</sup>.

The same *PTPN22* polymorphism has also been associated with several autoimmune diseases in Caucasians including among others Juvenile Idiopathic Arthritis and Systemic Lupus Erythematosus, Graves disease and Addison's disease<sup>47-49</sup>, indicating the genetic risk factors may not be unique to a specific disease but can be promiscuously associated with other autoimmune diseases.

An alternative approach to genome-wide strategies is to use a candidate gene screen which takes a hypothesis-driven approach. While this strategy has generated a huge amount of literature, it has not been very fruitful in the identification of consistent and replicable risk factors for RA outside the HLA region. With the advent of new technologies in genotyping a large number of variants and the availability of SNP data from the HapMap consortium, considerable progress has been made in this field, enabling researchers to perform highly improved association studies.

### Outline of this thesis

The identification of RA-associated genes outside of the HLA region has been a challenge. Although the expected effect of genetic factors outside the HLA region are modest, the identification of risk loci through human genetic studies offers *prima facie* evidence that a biological pathway is critical in disease pathogenesis. Therefore, the aim of this thesis was to take a candidate gene approach to identify risk factors involved in rheumatoid arthritis. It is divided into three parts in which **part one** is dedicated towards the investigation of a region of the genome encompassing genes highly involved in the immune system, namely *Tumour Necrosis Factor (TNF) Receptor associated factor 1/Complement component 5 (TRAF1/C5)* on chromosome 9q33. In the **second part**, we have investigated the role of an immunoregulatory cytokine interleukin 10 (*IL10*) located on chromosome 1q32 and in **part three** we have investigated the role of additional genetic risk factors in RA.



In the **first part** of the thesis, we have investigated the role of the *TRAF1/C5* region in RA as well as other autoimmune diseases. In **chapter 2**, we have described the *TRAF1/C5* region as one of the few widely-replicated genetic risk factors for RA. Based on available information in mouse models<sup>50-52</sup> and indications from human studies<sup>53</sup>, we hypothesized that the *C5* region may play a role in the development and/or exacerbation of arthritis. By genetic fine-mapping studies, we identified the haplotype associating with disease and replicated our findings in 4 different cohorts derived from three different populations from the Netherlands, US and Sweden. Intriguingly, a genome wide association study (GWAS) on SNPs performed by Plenge *et al* identified the same *TRAF1/C5* region, in addition to the previously known *HLA* and *PTPN22*, as genetic risk factors for RA<sup>54</sup>.

To further establish this risk factor, we have reproduced this association in trio families in which both parents are unaffected and one offspring affected with RA (**Chapter 3**). This locus represents the third genetic risk factor for which association is found in family-based studies as well. Together, these findings firmly establish the *TRAF1/C5* region as the one of the confirmed genetic risk factors for RA.

In the recent years, it has been suggested that there may be a considerable heritable component to autoimmune diseases<sup>55</sup>. While certain diseases such as RA tend to occur among several members of the same family indicating a genetic component to that specific disease, it is also common to observe different autoimmune diseases in various family members as well as in a particular individual, suggesting that certain individuals may have inherited a set of genetic risk factors predisposing them to the development of an autoimmune disease. Our work has now shown that the *TRAF1/C5* region is not only relevant for RA but is also relevant in patients with a polyarticular form of juvenile arthritis (JIA) (**Chapter 4**). Behrens and colleagues have now also independently reported an association of a perfect proxy to JIA further supporting our findings<sup>56</sup>.

To test whether this region predisposes to other diseases we also investigated its relevance in a well-powered study including four additional autoimmune diseases including Type I Diabetes (T1D), Celiac Disease (CD), Systemic Sclerosis (SSc), Systemic Lupus Erythematosus (SLE) patients and a common set of controls consisting of healthy unrelated individuals that were geographically and ethnically matched. We observe and replicate modest associations to both T1D and SLE and did not observe any evidence of association to CD and SSc (**Chapter 5**).

With these studies, we have provided considerable evidence that the *TRAF1/C5* region is not only relevant to RA but that the frequency of the same allele is increased in JIA, T1D and SLE. It is therefore highly likely that the *TRAF1/C5* region is a genetic risk factor involved in a shared pathway underlying multiple autoimmune diseases.

The **second part** of this thesis addresses the role of *interleukin 10* (*IL10*) genetic variants in regulating expression levels and their role in disease. IL10 is a cytokine with key regulatory, anti-inflammatory and immuno-stimulatory functions involved in the pathogenesis of various diseases<sup>57-60</sup>. Interindividual differences in the production of IL10 have been extensively associated with polymorphisms and haplotypes of the *IL10* gene<sup>61</sup>. The A allele of IL10-2849, a polymorphism located in the promoter region, is associated with decreased IL10 production as measured by lipopolysaccharide (LPS) stimulated whole blood cultures<sup>62</sup>. A low innate production of IL10 using the same assay is associated with an increased risk of familial osteoarthritis (OA)<sup>63</sup>. Therefore, in **chapter 6**, we investigate the role of *IL10* in osteoarthritis and observe no association of 7 promoter SNPs with disease.

While there is a direct correlation between IL10 mRNA and protein levels and high and low IL10 producers have similar mRNA half-life<sup>(64)</sup>, little evidence existed that this variation in clinically relevant levels of IL10 is actually dictated by *IL10* haplotypes. In **chapter 7**, by using the technique of allele-specific transcript quantification (ASTQ), the ratio between two alleles (A and G) of the *IL10* gene was characterized in 15 healthy heterozygous individuals. We show that *IL10* alleles are indeed differentially transcribed in cells from heterozygous individuals and that *IL10* haplotypes likely dictate the production of IL10. These findings show, for the first time, that interindividual differences in IL10 protein levels could be partially explained at the allele-specific transcriptional level.

In RA, the IL10-A2849 G allele has been shown to be associated with differences in titres of autoantibodies (RF and ACPA). Moreover the rate of joint destruction in RA patients from the early arthritis cohort was twice as high in patients that were -2849G carrier to those who were not (median rate per year 8 versus 4 SHS units on X-rays of hand and feet)<sup>(65)</sup>. As the length of the haplotype block around *IL10* is highly relevant to the search for the functional polymorphism(s), we characterized the level of linkage disequilibrium in a region of 217 kb, encompassing *IL10* as well as its neighbouring homologues (*IL19*, *IL20* and *IL24*). We showed that the neighboring genes are unlikely to harbor functional cis-acting variants (**chapter 8**). In this chapter we also report an association of IL10 polymorphisms with restenosis. Stenosis occurs when a coronary artery constricts or narrows. One way to widen a coronary artery is by using percutaneous coronary intervention (PCI, or balloon angioplasty). Some patients who undergo PCI have restenosis (renarrowing) of the widened segment within about six months of the procedure. Restenosed arteries may have to undergo another angioplasty. Inflammation is thought to play a key role in the development of restenosis and concordantly, we observed that patients with a lower innate ability to make IL10, favouring a pro-inflammatory environment, are at a high risk of undergoing restenosis.

We further fine-mapped the immediate *IL10* region. Six tagging SNPs have been genotyped in our extensive and clinically well-defined RA cohorts to determine their relevance to clinical remission and severity of RA (**Chapter 9**). While there is conflicting evidence that the *IL10* gene is associated with the development of RA, there is no indication in our cohort of the involvement of *IL10* in either clinical remission or the progression of joint destruction in RA.

In the **third part** of this thesis, the role of other genetic risk factors (besides *IL10* and *TRAF1/C5*) is described. In **chapter 10**, the role of *TNF $\alpha$*  in predisposing patients towards a more severe disease course is investigated. While increased levels of *TNF $\alpha$*  are found in patients with RA<sup>18</sup> and the treatment of patients with anti-TNF agents do provide beneficial effects<sup>66</sup>, very little evidence exists that variations in the gene predispose individuals to the development or progression of rheumatoid arthritis, implying that increased TNF production in RA patients is most likely due to other molecules in the signaling cascade leading to the enhanced production of *TNF $\alpha$*  protein. One such molecule *TNF $\alpha$ -induced protein 3 (TNFAIP3)* on chromosome 6q23 has recently been associated with development of RA(67;68). *TNFAIP3* is a negative regulator of *NF $\kappa$ B* and as such is involved in inhibiting TNF-Receptor mediated signaling effects<sup>69</sup>. Interestingly, the initial association was detected in cohorts of patients with long-standing RA. However, no association was found in a Swedish early arthritis cohort. We therefore hypothesized that the 6q23 locus containing *TNFAIP3* may be predominantly associated with a phenotype consistent with more severe disease. To determine whether this is the case, we set out in **chapter 11** to analyze the effect of the 6q23 region on the rate of joint destruction in our large and well-described early RA cohort.

One of the rare success stories for RA from the classical microsatellite-based linkage approach came in 2007, when candidate genes were investigated under a linkage peak on chromosome 2q in the US study. The 13 candidate genes investigated revealed strong association at four strongly linked SNPs in an intron of the gene *signal transducer and activator of transcription 4 (STAT4)*<sup>67</sup>. The data from *STAT4* (Chr2q33) has already been consistently replicated in not only Caucasians but also in East Asians<sup>68-71</sup>. This is however not the case for the other signal observed under the same linkage peak in this study, namely cytotoxic T lymphocyte associated 4 gene (*CTLA4*). Originally identified as a determinant of susceptibility to autoimmune diseases including Grave's disease, Type 1 Diabetes and autoimmune hypothyroidism<sup>72</sup>, this locus has been a constant debate in RA<sup>42</sup>. In **chapter 12**, we perform independent replication and a meta-analysis of three loci including *STAT4*, *CTLA4* and the recently described 4q27 region containing the *IL2* and *IL21* genes<sup>73</sup>. We show a strong association with *STAT4* and as previously described, no preferential association was observed with ACPA status. More importantly, we confirm the role of *CTLA4* in RA, resolving a longstanding debate of whether does or does not predispose to RA. We additionally show for the first time that the association is restricted to ACPA positive individuals only. For the 4q27 locus, we provide independent replication of the data and indicate that for this locus, in contrast to previous findings, no differences in effects are seen in ACPA positive and ACPA negative individuals. However, these data have to be interpreted with caution due to a possible lack of power. Interestingly, we also observe an association between the 4q27 locus and juvenile arthritis as described in **chapter 13**.

While most described genetic risk factors in RA either predispose to the autoantibody positive subset of patients or both, data is extremely scarce when it comes to patients who harbor none. One genetic risk factor in the HLA region, DRB\*0301, has been consistently associated with ACPA negative disease<sup>74,75</sup>. In **chapter 14**, we describe the identification of the only non-HLA genetic factor, *Interferon Regulatory factor 5 (IRF5)*, showing a predominant association with ACPA negative disease. A recent report confirmed these findings but also show a small effect in ACPA positive disease<sup>76</sup>.

**Chapter 15** provides an overview of novel genetic risk factors in ACPA positive RA, identified with the use of a meta-analysis of three well-powered GWAS studies from the US, Sweden and the UK. Simultaneously, the UK group confirmed 3 of the loci identified in the meta-analysis including MMEL1, PCRKQ and KIF5A<sup>80</sup>. Two additional loci surfaced in their study providing compelling evidence for IL2RB and suggestive evidence for IL2RA. In **Chapter 16**, we provide the first independent study replicating these two risk factors in a non-UK population, underlining the relevance of the IL2 pathway in RA. Finally in **Chapter 17**, I discuss the biological relevance and potential implications of all these identified RA loci, summarizing the recent explosion of genetic findings in RA.

## References

- (1) Pollock MR. The discovery of DNA: An ironic tale of chance, prejudice and insight. *J Gen Microbiol* 1970; 63(1):1-20.
- (2) WATSON JD, CRICK FH. Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature* 1953; 171(4356):737-8.
- (3) A haplotype map of the human genome. *Nature* 2005; 437(7063):1299-320.
- (4) Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA et al. A second generation human haplotype map of over 3.1 million SNPs. *Nature* 2007; 449(7164):851-61.
- (5) Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res* 2002; 4 Suppl 3:S265-S272.
- (6) Majithia V, Geraci SA. Rheumatoid arthritis: diagnosis and management. *Am J Med* 2007; 120(11):936-9.
- (7) Gabriel SE, Crowson CS, Kremers HM, Doran MF, Turesson C, O'Fallon WM et al. Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years. *Arthritis Rheum* 2003; 48(1):54-8.
- (8) Lundkvist J, Kastang F, Kobelt G. The burden of rheumatoid arthritis and access to treatment: health burden and costs. *Eur J Health Econ* 2008; 8 Suppl 2:S49-S60.
- (9) 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* 1996; 55(3):157-61.
- (10) van Gaalen F, Ioan-Facsinay A, Huizinga TW, Toes RE. The devil in the details: the emerging role of anticitrulline autoimmunity in rheumatoid arthritis. *J Immunol* 2005; 175(9):5575-80.
- (11) Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004; 50(2):380-6.
- (12) Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003; 48(10):2741-9.
- (13) van Gaalen FA, Visser H, Huizinga TW. A comparison of the diagnostic accuracy and prognostic value of the first and second anti-cyclic citrullinated peptides (CCP1 and CCP2) autoantibody tests for rheumatoid arthritis. *Ann Rheum Dis* 2005; 64(10):1510-2.
- (14) De Rycke L, Peene I, Hoffman IE, Kruihof E, Union A, Meheus L et al. Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. *Ann Rheum Dis* 2004; 63(12):1587-93.

- (15) van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Toes RE, Huizinga TW. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005; 7(5):R949-R958.
- (16) Bugatti S, Codullo V, Caporali R, Montecucco C. B cells in rheumatoid arthritis. *Autoimmun Rev* 2007; 7(2):137-42.
- (17) van der Helm-van Mil AH, Huizinga TW, de Vries RR, Toes RE. Emerging patterns of risk factor make-up enable subclassification of rheumatoid arthritis. *Arthritis Rheum* 2007; 56(6):1728-35.
- (18) Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003; 423(6937):356-61.
- (19) Kobayashi S, Momohara S, Kamatani N, Okamoto H. Molecular aspects of rheumatoid arthritis: role of environmental factors. *FEBS J* 2008.
- (20) Goronzy JJ, Weyand CM. Rheumatoid arthritis. *Immunol Rev* 2005; 204:55-73.
- (21) Klareskog L, Padyukov L, Ronnelid J, Alfredsson L. Genes, environment and immunity in the development of rheumatoid arthritis. *Curr Opin Immunol* 2006; 18(6):650-5.
- (22) Silman AJ, MacGregor AJ, Thomson W, Holligan S, Carthy D, Farhan A et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br J Rheumatol* 1993; 32(10):903-7.
- (23) MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum* 2000; 43(1):30-7.
- (24) Seldin MF, Amos CI, Ward R, Gregersen PK. The genetics revolution and the assault on rheumatoid arthritis. *Arthritis Rheum* 1999; 42(6):1071-9.
- (25) Cornelis F, Faure S, Martinez M, Prud'homme JF, Fritz P, Dib C et al. New susceptibility locus for rheumatoid arthritis suggested by a genome-wide linkage study. *Proc Natl Acad Sci U S A* 1998; 95(18):10746-50.
- (26) Jawaheer D, Seldin MF, Amos CI, Chen WV, Shigeta R, Monteiro J et al. A genomewide screen in multiplex rheumatoid arthritis families suggests genetic overlap with other autoimmune diseases. *Am J Hum Genet* 2001; 68(4):927-36.
- (27) MacKay K, Eyre S, Myerscough A, Milicic A, Barton A, Laval S et al. Whole-genome linkage analysis of rheumatoid arthritis susceptibility loci in 252 affected sibling pairs in the United Kingdom. *Arthritis Rheum* 2002; 46(3):632-9.
- (28) Shiozawa S, Hayashi S, Tsukamoto Y, Goko H, Kawasaki H, Wada T et al. Identification of the gene loci that predispose to rheumatoid arthritis. *Int Immunol* 1998; 10(12):1891-5.
- (29) Eyre S, Barton A, Shephard N, Hinks A, Brintnell W, MacKay K et al. Investigation of susceptibility loci identified in the UK rheumatoid arthritis whole-genome scan in a further series of 217 UK affected sibling pairs. *Arthritis Rheum* 2004; 50(3):729-35.
- (30) Etzel CJ, Chen WV, Shepard N, Jawaheer D, Cornelis F, Seldin MF et al. Genome-wide meta-analysis for rheumatoid arthritis. *Hum Genet* 2006; 119(6):634-41.

- (31) Jawaheer D, Seldin MF, Amos CI, Chen WV, Shigeta R, Etzel C et al. Screening the genome for rheumatoid arthritis susceptibility genes: a replication study and combined analysis of 512 multicase families. *Arthritis Rheum* 2003; 48(4):906-16.
- (32) Zanelli E, Breedveld FC, de Vries RR. HLA class II association with rheumatoid arthritis: facts and interpretations. *Hum Immunol* 2000; 61(12):1254-61.
- (33) van der Helm-van Mil AH, Huizinga TW, Schreuder GM, Breedveld FC, de Vries RR, Toes RE. An independent role of protective HLA class II alleles in rheumatoid arthritis severity and susceptibility. *Arthritis Rheum* 2005; 52(9):2637-44.
- (34) de Vries N, Tijssen H, van Riel PL, van de Putte LB. Reshaping the shared epitope hypothesis: HLA-associated risk for rheumatoid arthritis is encoded by amino acid substitutions at positions 67-74 of the HLA-DRB1 molecule. *Arthritis Rheum* 2002; 46(4):921-8.
- (35) Shadick NA, Heller JE, Weinblatt ME, Maher NE, Cui J, Ginsburg G et al. Opposing effects of the D70 mutation and the shared epitope in HLA-DR4 on disease activity and certain disease phenotypes in rheumatoid arthritis. *Ann Rheum Dis* 2007; 66(11):1497-502.
- (36) Matthey DL, Dawes PT, Gonzalez-Gay MA, Garcia-Porrúa C, Thomson W, Hajeer AH et al. HLA-DRB1 alleles encoding an aspartic acid at position 70 protect against development of rheumatoid arthritis. *J Rheumatol* 2001; 28(2):232-9.
- (37) Horton R, Wilming L, Rand V, Lovering RC, Bruford EA, Khodiyar VK et al. Gene map of the extended human MHC. *Nat Rev Genet* 2004; 5(12):889-99.
- (38) Horton R, Gibson R, Coggill P, Miretti M, Allcock RJ, Almeida J et al. Variation analysis and gene annotation of eight MHC haplotypes: the MHC Haplotype Project. *Immunogenetics* 2008; 60(1):17-18.
- (39) Stewart CA, Horton R, Allcock RJ, Ashurst JL, Atrazhev AM, Coggill P et al. Complete MHC haplotype sequencing for common disease gene mapping. *Genome Res* 2004; 14(6):1176-87.
- (40) Deighton CM, Walker DJ, Griffiths ID, Roberts DF. The contribution of HLA to rheumatoid arthritis. *Clin Genet* 1989; 36(3):178-82.
- (41) Suzuki A, Yamada R, Chang X, Tokuhira S, Sawada T, Suzuki M et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003; 34(4):395-402.
- (42) Oliver JE, Worthington J, Silman AJ. Genetic epidemiology of rheumatoid arthritis. *Curr Opin Rheumatol* 2006; 18(2):141-6.
- (43) Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW 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* 2005; 77(6):1044-60.
- (44) Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC 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* 2004; 75(2):330-7.

- (45) Bowes J, Barton A. Recent advances in the genetics of RA susceptibility. *Rheumatology (Oxford)* 2008; 47(4):399-402.
- (46) Ikari K, Momohara S, Inoue E, Tomatsu T, Hara M, Yamanaka H et al. Haplotype analysis revealed no association between the PTPN22 gene and RA in a Japanese population. *Rheumatology (Oxford)* 2006; 45(11):1345-8.
- (47) Gregersen PK. Gaining insight into PTPN22 and autoimmunity. *Nat Genet* 2005; 37(12):1300-2.
- (48) Gregersen PK, Lee HS, Batliwalla F, Begovich AB. PTPN22: setting thresholds for autoimmunity. *Semin Immunol* 2006; 18(4):214-23.
- (49) Lee YH, Rho YH, Choi SJ, Ji JD, Song GG, Nath SK et al. The PTPN22 C1858T functional polymorphism and autoimmune diseases--a meta-analysis. *Rheumatology (Oxford)* 2007; 46(1):49-56.
- (50) Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003; 423(6937):356-61.
- (51) Ji H, Gauguier D, Ohmura K, Gonzalez A, Duchatelle V, Danoy P et al. Genetic influences on the end-stage effector phase of arthritis. *J Exp Med* 2001; 194(3):321-30.
- (52) Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FMA, Boackle SA et al. Arthritis Critically Dependent on Innate Immune System Players. *Immunity* 2002; 16(2):157-68.
- (53) Hogasen K, Mollnes TE, Harboe M, Gotze O, Hammer HB, Oppermann M. Terminal complement pathway activation and low lysis inhibitors in rheumatoid arthritis synovial fluid. *J Rheumatol* 1995; 22(1):24-8.
- (54) Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B et al. TRAF1-C5 as a risk locus for rheumatoid arthritis - A genome-wide study. *New England Journal of Medicine* 2007; 357(12):1199-209.
- (55) Gregersen PK, Behrens TW. Genetics of autoimmune diseases--disorders of immune homeostasis. *Nat Rev Genet* 2006; 7(12):917-28.
- (56) Behrens EM, Finkel TH, Bradfield JP, Kim CE, Linton L, Casalunovo T et al. Association of the TRAF1-C5 locus on chromosome 9 with juvenile idiopathic arthritis. *Arthritis Rheum* 2008; 58(7):2206-7.
- (57) de Jong BA, Westendorp RG, Eskdale J, Uitdehaag BM, Huizinga TW. Frequency of functional interleukin-10 promoter polymorphism is different between relapse-onset and primary progressive multiple sclerosis. *Hum Immunol* 2002; 63(4):281-5.
- (58) Lin MT, Storer B, Martin PJ, Tseng LH, Gooley T, Chen PJ et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med* 2003; 349(23):2201-10.
- (59) O'Garra A, Vieira P. T(H)1 cells control themselves by producing interleukin-10. *Nat Rev Immunol* 2007; 7(6):425-8.



- (60) Eskdale J, McNicholl J, Wordsworth P, Jonas B, Huizinga T, Field M et al. Interleukin-10 microsatellite polymorphisms and IL-10 locus alleles in rheumatoid arthritis susceptibility. *Lancet* 1998; 352(9136):1282-3.
- (61) Eskdale J, Keijsers V, Huizinga T, Gallagher G. Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. *Genes Immun* 1999; 1(2):151-5.
- (62) Westendorp RG, Langermans JA, Huizinga TW, Verweij CL, Sturk A. Genetic influence on cytokine production in meningococcal disease. *Lancet* 1997; 349(9069):1912-3.
- (63) Riyazi N, Slagboom E, de Craen AJ, Meulenbelt I, Houwing-Duistermaat JJ, Kroon HM et al. Association of the risk of osteoarthritis with high innate production of interleukin-1beta and low innate production of interleukin-10 ex vivo, upon lipopolysaccharide stimulation. *Arthritis Rheum* 2005; 52(5):1443-50.
- (64) Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. *Proc Natl Acad Sci U S A* 1998; 95(16):9465-70.
- (65) Lard LR, van Gaalen FA, Schonkeren JJ, Pieterman EJ, Stoeken G, Vos K et al. Association of the -2849 interleukin-10 promoter polymorphism with autoantibody production and joint destruction in rheumatoid arthritis. *Arthritis Rheum* 2003; 48(7):1841-8.
- (66) Maini RN, Elliott MJ, Brennan FM, Feldmann M. Beneficial effects of tumour necrosis factor-alpha (TNF-alpha) blockade in rheumatoid arthritis (RA). *Clin Exp Immunol* 1995; 101(2):207-12.
- (67) Plenge RM, Cotsapas C, Davies L, Price AL, Bakker PIW, Maller J et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. *Nature Genetics* 2007; 39(12):1477-82.
- (68) Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J et al. Rheumatoid arthritis association at 6q23. *Nat Genet* 2007; 39(12):1431-3.
- (69) Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* 2004; 430(7000):694-9.
- (70) Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med* 2007; 357(10):977-86.
- (71) Barton A, Thomson W, Ke X, Eyre S, Hinks A, Bowes J et al. Re-evaluation of putative rheumatoid arthritis susceptibility genes in the post-genome wide association study era and hypothesis of a key pathway underlying susceptibility. *Hum Mol Genet* 2008; 17(15):2274-9.
- (72) Kobayashi S, Ikari K, Kaneko H, Kochi Y, Yamamoto K, Shimane K et al. Association of STAT4 with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in the Japanese population. *Arthritis Rheum* 2008; 58(7):1940-6.
- (73) Orozco G, Alizadeh BZ, Delgado-Vega AM, Gonzalez-Gay MA, Balsa A, Pascual-Salcedo D et al. Association of STAT4 with rheumatoid arthritis: a replication study in three European populations. *Arthritis Rheum* 2008; 58(7):1974-80.

- (74) Lee HS, Remmers EF, Le JM, Kastner DL, Bae SC, Gregersen PK. Association of STAT4 with rheumatoid arthritis in the Korean population. *Mol Med* 2007; 13(9-10):455-60.
- (75) Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003; 423(6939):506-11.
- (76) Zhernakova A, Alizadeh BZ, Bevova M, van Leeuwen MA, Coenen MJ, Franke B et al. Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. *Am J Hum Genet* 2007; 81(6):1284-8.
- (77) Verpoort KN, van Gaalen FA, van der Helm-van Mil AH, Schreuder GM, Breedveld FC, Huizinga TW et al. Association of HLA-DR3 with anti-cyclic citrullinated peptide antibody-negative rheumatoid arthritis. *Arthritis Rheum* 2005; 52(10):3058-62.
- (78) Irigoyen P, Lee AT, Wener MH, Li W, Kern M, Batliwalla F et al. Regulation of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis: contrasting effects of HLA-DR3 and the shared epitope alleles. *Arthritis Rheum* 2005; 52(12):3813-8.
- (79) Dieguez-Gonzalez R, Calaza M, Perez-Pampin E, de la Serna AR, Fernandez-Gutierrez B, Castaneda S et al. Association of interferon regulatory factor 5 haplotypes, similar to that found in systemic lupus erythematosus, in a large subgroup of patients with rheumatoid arthritis. *Arthritis Rheum* 2008; 58(5):1264-74.
- (80) Barton A, Thomson W, Ke X, Eyre S, Hinks A, Bowes J et al. Rheumatoid arthritis susceptibility loci at chromosomes 10p15, 12q13 and 22q13. *Nat Genet* 2008; 40(10):1156-9.

