CHAPTER 9

Summary and discussion
RA has a complex multifactorial etiology, of which many elements remain unknown. Genetic and environmental risk factors play a major role in disease development. An important characteristic of RA is the production of autoantibodies. It was shown that ACPA-positive differs from ACPA-negative RA in phenotype (having more severe and persistent disease) as well as having different genetic and environmental risk factors [1, 2]. The most prominent example being the HLA shared epitope (SE) alleles, which accounts for 36% of overall genetic susceptibility, specifically predispose to ACPA-positive disease [3-5].

**Part I**

Several genetic risk factors associate differently with RA susceptibility in various populations. In Chapter 2, 3 and 4 of this thesis, part of the genetic variants that were reported to differentially associate with RA risk were investigated in a Dutch Caucasian population. Furthermore, these data were analysed within the context of autoantibody status besides considering the interaction with various environmental factors and gender in a trial to, hopefully, explain the ethnic disparity in those risk factors.

The functional polymorphism in the PTPN22 gene associated with RA susceptibility in Caucasians is extremely rare in Asian populations, and other polymorphisms within the gene show no evidence for association [6, 7]. In Chapter 2, we confirmed the association of C1858T variant of PTPN22 with RA susceptibility in Dutch Caucasians. This association was more evident and only statistically significant in autoantibody positive (rheumatoid factor (RF) positive) patients when compared to RF negative patients. Additionally we showed that C1858T is the sole PTPN22 variant predisposing to RA in Dutch Caucasians in contrast to other reports finding other associated PTPN22 variants [8].

FCRL3 promoter SNP (rs7528684) was shown to associate with RA risk in Japanese [9, 10] but not in Americans [11] and Spanish [12]. We investigated the FCRL3 promoter SNP association with RA in a Dutch Caucasian population in Chapter 3. Carrier analysis revealed that the recessive CC genotype carriers are at higher risk of developing RA compared to TT+TC genotype carriers. Additionally, stratification for autoantibodies (ACPA) didn’t reveal association with ACPA+ve or ACPA-ve disease. Similarly, no association of FCRL3
Summary and discussion

genotypes with RA severity was found. Meta-analysis of the previous studies in Japanese [9, 10], Americans [11], Spanish [12] and with the our data included confirmed that CC carriers are RA susceptible across various populations. Consequently, our findings suggest that the association of FCRL3 with RA susceptibility might not be specific to the Japanese population, and that FCRL3 is possibly involved in the pathogenesis of RA in a recessive trait fashion.

PADI4 gene polymorphisms found associated with RA susceptibility in multiple Asian populations but lack the association in Caucasians [13]. In Chapter 4, we tried to elucidate the genetic heterogeneity and differential role of PADI4 in RA observed between Asian and European populations. Additionally, we examined possible gene-environmental interactions between PADI4 polymorphism, sex and smoking status in 2 Japanese and 1 Dutch patients-controls sample sets. In the first Japanese sample set, PADI4 polymorphism (rs1748033) showed a greater risk for RA in men than in women and in those who ever smoked compared to never-smokers. Moreover, the highest risk was seen in males who ever-smoked. Similar trends were observed in the second Japanese as well as the Dutch sample sets. Those data suggest that PADI4 polymorphism highly predisposes male smokers to RA. Furthermore, the genetic heterogeneity observed between Asian and European populations may be partly explained by differences in smoking prevalence among men and gender of patients and controls studied for PADI4 genotypes.

Genetic susceptibility loci are usually identified via three main approaches: candidate gene, linkage, and GWAS. GWAS have become the most powerful and extensively employed approach in discovering susceptibility variants for complex disease traits. Advances in technology, reduced costs, and the ascertainment of large well-characterized case cohorts and panels of controls have made GWAS widely available. Despite its high-throughput and feasibility, GWAS has important limitations; the potential for false-positive results, lack of information on gene function, insensitivity to rare variants and structural variants (therefore, potential for false-negative results), requirement for large sample sizes, and possible biases due to case and control selection and genotyping errors [14]. Structural variants can be in the form of insertion deletion or copy number variation.
Variation in the copy number (CN) of genes within the human genome is an important source of genetic variation, and is defined as a sequence of DNA > 1 kb present in altered CN when compared with a reference genome [15]. In the diploid human genome, autosomal genes are normally present in two copies (one on each chromosome). However, when CNV is present the number of copies can vary from zero to greater than two. Studies have demonstrated a large number of genes within the human genome that display CNV in a relatively high frequency. CNV may be a major source of quantitative variation in expression, and has been proposed to contribute to phenotypic diversity and disease susceptibility [16-18].

FcγRs are found on most effector cells of the immune system where they are frequently co-expressed [19]. They interact with the Fc portion of the IgG molecule, therefore, link the IgG antibody mediated responses with cellular effector and regulatory functions [20]. They can be either stimulatory or inhibitory and play an integral role in the identification and destruction of both endogenous and foreign opsonised material [21]. The balance between the stimulating and inhibiting signal is crucial to maintaining immune homeostasis [19]. Functionally relevant polymorphisms have been identified in low-affinity FcγRs which affect the binding with IgG subclasses and have been shown to play an important role in immune complex mediated autoimmune diseases such as SLE [22, 23], RA [24], and immunocytopenic purpura [25]. The initial suggestion that there might be CNV at the FcγRs locus came from an isolated case report of serological surveys revealing rare individuals with complete FcγRIIIb deficiency and either neonatal autoimmune neutropenia or lupus nephritis. The deletion of FcγR3B was previously associated with SLE and with neonatal autoimmune neutropenia in offspring from FcγRIIIb-negative mothers [26, 27].

In Chapter 5 and 6 of this thesis we studied FcγR gene polymorphisms. The technical difficulties arising from the high homology of FcγRs and the presence of structural variations as CNV warranted the use of a different approach to investigate FcγRs polymorphisms. Therefore, besides genotyping SNPs, we used MLPA to test for CNV and combined the analyses to explore whether the presence or absence of CNV has any effect on SNPs genotyping and its association with RA risk.
Summary and discussion

A functional polymorphism in FcγR3A results in an amino acid substitution at position 158 of phenylalanine for valine which reduces the affinity of IgG1, IgG3 and IgG4 binding to the receptor. These variants, which may lead to a reduced clearance of immune complexes, have been shown in previous studies to contribute to the pathogenesis of autoimmune disorders [28]. This FcγRIIIA 158V/F polymorphism has been extensively studied in RA but revealed remarkably contradictory results. Some studies showed the 158V allele was associated with RA susceptibility [29-32], in another study the 158F allele was associated with RA [33], while in other studies no association with RA was observed [24, 28, 34-37]. Those, strikingly, contradicting results in the same ethnic group might be explained with the genetic complexity of the FcγR region combined with the heterogeneity of RA. Therefore, in Chapter 5 we investigated whether FcγRIIIA 158V/F SNP associates differently with ACPA-positive and ACPA-negative RA and assessed if the FcγRIIIA gene CNV affects the association of the FcγRIIIA 158V/F SNP with RA and whether the FcγRIIIA gene CNV confers risk for RA. In our cohort we found that FcγRIIIA 158VV genotype only confers risk for ACPA-positive RA. After stratifying for the presence of CNV, this association became slightly more evident. Though, FcγRIIIA CNV by itself is not associated with RA risk. Our data suggest that ACPA status and the presence of CNV can affect the FcγRIIIA association with RA and must be taken into consideration when studying FcγRIIIA polymorphisms.

FcγRIIIB is most likely involved in the uptake of immune complexes; it has been found to express two isoforms, NA1 and NA2, which differ by four amino acids in the membrane-distal Ig-like domain. Differential glycosylation of these isoforms affects their interaction with IgGs. The NA2 isoform has been shown to have a reduced power in the stimulation of phagocytosis and this has functional consequences in development of blood transfusion reactions, autoimmune neutropenia and renal disease in systemic vasculitis [38]. Chapter 6 focuses on investigating the CNV of FcγRIIIB gene and if it has an association with RA risk. CNV was studied with 3 different MLPA probes. Probes 1 and 2 showed CNV with copy numbers ranging from 0 to 5 but with different frequencies. Probe 3 showed almost no evidence of CNV. Quantitative-PCR was done for confirmation and its results correlated with those of probe 2. Thus, CNV was observed both in healthy controls as well RA patients but not significantly different between them. In healthy controls low copy number (1 copy) was
observed in 6.7% compared to 9.4% of individuals with high copy number (≥3 copies). Furthermore, sequencing of the FCGR3B promoter region revealed an insertion/deletion (indel) that explained the disparate CNV results of MLPA probe 1. This novel -256A.TG indel was found in 40.7% of healthy controls versus 35.9% of RA patients but not significantly different. Those data highlight the complexity and poor characterization of the FCGR3B gene sequence and indicate that design and interpretation of genotyping assays in this region must be performed with caution. The possible protective effect of the -256A.TG indel polymorphism must be addressed in larger studies.

Data from Chapter 5 and 6 denote that analysis of the associations of CNV results and disease susceptibility or severity is rather complicated. First, because of its low frequency and second, because of the existing SNPs within those gene copies with subsequent functionality and expression effects which might result in obscuring the real effect of CNV.

**Part II**

Although autoantibody formation is characteristic for RA and can precede RA manifestations by several years [39], the range of antibodies formed in RA are not entirely specific for RA and can be detected in other forms of inflammatory arthritis as well. Additionally, the majority of studies exploring the test characteristics of RF, anti-CCP and anti-MCV compare RA patients to healthy controls while in clinical practice those tests are used to differentiate early RA from other forms of early inflammatory arthritis. In chapter 7, we evaluated the diagnostic performance of the anti-MCV test in differentiating of RA from other forms of inflammatory arthritis in a large inception cohort of patients with early arthritis. Additionally, the anti-MCV test characteristics were compared with those of the anti-CCP2 and anti-CCP3.1 tests. In our cohort, anti-MCV antibodies were present in 13.9-19.4% of non-RA arthritides and it showed lower specificity to differentiate RA from other arthritides (82.9%) compared to anti-CCP2 (93.4%) and anti-CCP3.1 (90.0%), although it had a higher sensitivity (62% vs 56.9% and 58.1%, respectively). Therefore, our data suggest that the diagnostic performance of anti-MCV test is lower than anti-CCP tests in the differential diagnosis of early arthritis.
Part III

Albeit the 1987 ACR classification criteria for RA had been widely used for many years to identify RA patients, they lack the sensitivity to identify patients with early RA and fail to identify individuals with very early inflammatory arthritis who subsequently develop RA [40, 41]. Because the focus now is shifted towards early therapy, a new tool to identify early RA was warranted. Recently, the ACR and EULAR developed the 2010 new classification criteria for RA aiming at identification of patients with very early stages of RA [42]. Before the new classification criteria were developed, we wanted to explore if the 1987 ACR criteria can be improved. Even though bone erosions were part of the 1987 ACR criteria [43] no clear description of the quantity and location of erosions was given. Additionally, the prognostic value of bone erosions in UA patients was unknown while in RA patients it is predictive of a severe destructive disease course. The focus of Chapter 8 is to explore the predictive value of erosions for RA development in UA patients, define optimal erosions cut-off to predict RA, differential value of erosions in hands and feet and investigate whether information on erosions improves the discriminative ability of a recently developed prediction rule of RA-development [44, 45]. In Chapter 8, the presence of erosions was assessed using two methods. First method is erosive joint count. An erosive joint is defined as a joint with at least one erosion which in turn is defined as a lesion with an interrupted cortex. As such, this definition is the same as the erosion score in the Simplified Erosion Narrowing Score (SENS) and is in line with common clinical practice [46]. Second method is the erosion score of the Sharp/van der Heijde scoring (SHS) method in which the size and number of erosions per joint are weighted [47].

At baseline, 28.6% of UA patients had bone erosions. Presence of ≥2 erosive joints showed a positive predictive value of 53% for RA development and 68% for persistent disease. Additionally, the presence of feet erosions was equally predictive compared to erosions at hands. Incorporating the erosions data (SENS method) into the prediction rule of RA-development [44, 45] using all the different cut-off values, did not improve the predictive ability of the model. Similarly, using data on joint erosions in UA patients who cannot be accurately predicted to develop RA by the rule (scoring between 6.0 and 8.0) does not significantly add to the predictive ability of the known clinical and serological factors. Those
data indicate that the presence of erosions in UA patients is not always predictive for unfavourable disease outcomes.

In conclusion, over the past few years, understanding of the genetic basis of the susceptibility to RA has increased dramatically with new susceptibility genes now confirmed. Recently, Eyre et al. [5] confirmed the identified risk loci to account for 51% of the total genetic effect of which 36% explained by HLA in addition to 15% non-HLA genetic factors. The challenge remains now to identify the rest of those genetic effects and explore how these variants interact with each other as well as environmental factors to induce the development of RA. Translating the genetic information to help clinically defining subgroups of patients and aid clinical management is an interesting target, hopefully, in the near future. The ultimate goal of personalized treatment decision making incorporates genetic, serological as well as clinical factors.
Summary and discussion

References

Summary and discussion


