Preclinical rheumatoid arthritis

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Chapter 8

HLA-DR4 and antibodies against cyclic citrullinated peptides are associated in preclinical rheumatoid arthritis

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Abstract

Objective: The association between genetic markers (HLA-DR4 and SE) and the presence of auto antibodies (IgM-RF and anti-CCP) was determined in healthy persons before the start of symptoms.

Methods: In 56 pre-clinical RA patients HLA-DRB1 SE-alleles were determined by HLA-DQ-typing and associated to the onset of anti-CCP antibodies and rheumatoid factor in the years before RA symptoms started. Blood samples donated before symptoms commenced were obtained from the Sanquin Blood Bank.

Results: Of the 56 pre-clinical RA patients, 26 were positive for anti-CCP (46%), 13 for IgM-RF (23%), 32 for HLA-DR4 (57%) and 47 for SE (84%). Anti-CCP was significantly associated with the presence of HLA-DR4 (p=0.03, OR: 3.5; 95% CI 1.1-11.0). However, the association between anti-CCP and SE did not reach significance (p=0.11, OR: 3.7; 95% CI 0.7-19.4) probably due to the small number of patients. IgM-RF was not statistically significantly associated with HLA-DR4 (p=0.31, OR: 2.0; 95% CI 0.5-7.3) and the association with SE did just not reach significance (p=0.07, OR could not be calculated due to the absence of patients with the combination IgM-RF+ and SE-).

Conclusion: In preclinical RA, anti-CCP and carriership of HLA-DR4 are positively associated whereas a trend is observed for IgM-RF and SE alleles.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown origin, characterised by chronic joint inflammation leading to destruction of bone and cartilage, reduction of functional capacity and increased mortality (1, 2). Since structural joint damage is irreversible, early recognition and treatment of the disease is very important (3, 4) and tests to predict future RA in healthy persons at risk are needed. In a recent study it was found that one half of RA patients had autoantibodies, IgM Rheumatoid Factor (IgM-RF) and antibodies against cyclic citrullinated peptide (anti-CCP), a median of 4.5 years before the onset of symptoms (5). Particularly with the anti-CCP test it is possible to predict the development of RA in healthy individuals with an increased risk of developing RA, such as first degree family members of RA patients or persons with arthralgia without joint swelling or other signs of inflammation (5).
The cause of RA is multifactorial, most likely a combination of genetic and environmental factors. The main genetic factor is the presence of specific HLA-class II alleles (HLA-DR4 and DR1) encoding the shared epitope (SE) which are present more often among RA patients (SE carrier ship frequency 60-70%) compared with the healthy population (SE carrier ship frequency 42%) (6, 7). Therefore, genetic factors could be useful for the detection of RA in a healthy population. This study was designed to associate genetic markers (HLA-DR4 and SE) with the presence of auto antibodies (IgM-RF and anti-CCP) in healthy individuals before the start of symptoms of RA.

Patients and methods

From 5000 patients registered with RA at the Jan van Breemen Institute, a large outpatient rheumatology clinic, 79 patients were identified who had donated blood before the onset of RA, as described previously (5). IgM-RF and the first generation anti-CCP have been determined previously (5). Patients were considered as being positive for IgM-RF and/or anti-CCP if they were positive at least once for these tests before the start of symptoms. DNA was collected from 56 out of these 79 RA patients. Unfortunately, in the other 23 RA patients genetic material could not be collected because of death (n=3), moving home

(n=19) or non-response (n=1). The study was approved by the local Medical Ethical Committee.
The carrier ship of SE in the Dutch population (41%) was estimated in a previous study (7) and compared with the preclinical RA patients. The presence of HLA-DRB1*04 (HLA-DR4) and SE (HLA-DRB1*04, HLA-DRB1*01 and DRB1*10) were inferred from HLA-DQA1 and DQB1 typing performed by PCR-Single Strand Conformation Polymorphism method (8, 9).
Associations between the SE-alleles and the auto antibody tests (IgM-RF and anti-CCP) were calculated by Chi square and odds ratios (OR) were determined in SPSS 14.0.

Results

A median of 10 (range, 1 – 51) serum samples for auto-antibodies measurement, per patient was available the first of which was taken at a median of 8.1 years (range, 0.1 – 14.5 years) before the onset of the symptoms. The studied group had a lower mean age and contained more men in comparison to the group of persons who dropped out, but the differences did not reach statistical significance (p=0.13 and p=0.16, respectively). Of the 56 pre-clinical RA patients, 26 were positive for anti-CCP (46%), 13 for IgM-RF (23%), 32 for HLA-DR4 (57%) and 47 for SE (84%). The numbers of patients with the test combinations are shown in table 8.1. Anti-CCP was significantly associated with the presence of HLA-DR4 (p=0.03, OR: 3.5; 95% CI 1.1-11.0). The association between anti-CCP and SE, however, did not reach significance (p=0.11, OR: 3.7; 95% CI 0.7-19.4). IgM-RF was not statistically significantly associated with HLA-DR4 (p=0.31, OR: 2.0; 95% CI 0.5-7.3) and the association with SE did just not reach significance (p=0.07, OR could not be calculated due to the absence of patients with the combination IgM-RF+ and SE-).

Table 8.1: Association between autoantibodies and HLA-DR4 and shared epitope (SE) in preclinical RA patients

	anti-CCP+	anti-CCP-	p-value	IgM-RF+	IgM-RF-	p-value
HLA-DR4+	19	13	0.03	9	23	0.31
HLA-DR4-	7	17		4	20	
SE+	24	23	0.11	13	34	0.07
SE-	2	7		0	9	

Discussion

In pre-clinical RA a positive association between anti-CCP and carrier ship of one or two HLA-DRB1*04 alleles was observed. These results are in accordance with the observation by Berglin et al. (10) who described 59 subjects with pre-clinical RA (of whom 45 were women) in whom a very high relative risk (OR 66.8, 95% CI 8.3-539.4) for future development of clinical RA was found based on the presence of anti-CCP antibodies and carrier ship of DRB1*0404 and DRB1*0401 SE alleles.

A study of carrier ship of SE alleles in anti-CCP positive arthralgia patients compared with anti-CCP positive early arthritis and established RA patients showed a significantly higher frequency of SE alleles in the arthritis patients compared with the arthralgia patients (9). These results confirm the findings of the present study which shows that a combination of SE alleles and HLA-DR4 and anti-CCP or RF predict the onset of arthritis at an early stage. In established (early) RA more studies stress the importance of the serological antibodies in combination with SE alleles. In early RA, the carrier ship of one or two SE-alleles showed a strong correlation with the production of anti-CCP antibodies (OR 3.3, 95% CI 1.8-6.0 and OR 13.3 95% CI 4.6-40.4 respectively) (11). This group also described a strong association between the number of SE-alleles and the production of anti-CCP antibodies. A small study from Germany described a stronger association of the production of anti-CCP with HLA-DR4 than with HLA-DR1 positive RA patients (12). The association of a major DR4 positive SE allele, HLA-DRB1*0401, was described as very strong because 90% of the RA patients carrying this allele were anti-CCP-positive. Not all RA patients carrying the HLA-DRB1*0401 allele, however, were anti-CCP-positive.

It can be concluded in established RA that serological tests are related to the genetic markers of RA at the MHC, but more tests can be added in the future like PTPN22 , TRAF-1 C5, STAT 4 that seem to be related to the anti-CCP positivity in RA (13). The fact that the strong relationship between the presence of SE and the serological markers anti-CCP and IgM-RF was expected but could not be confirmed in this study was probably due to the fact that the number of available DNA samples that could be obtained several years after the blood donation unfortunately was limited. Several efforts were made to isolate DNA out of the sera because some patients could not be retrieved or had passed out. Unfortunately insufficient DNA could be extracted to perform HLA-typing. However, this study in preclinical RA shows a positive association between HLA-DR4 and anti-CCP which is in accordance with the studies in arthralgia patients

and in established RA. Moreover, the prevalence of carrier ship of SE-alleles (84%) was also significantly higher in this pre-clinical RA group compared with the healthy population (42%) (7). These facts support the hypothesis that in order to detect RA at a very early stage of the disease it can be useful to add tests for genetic markers like SE to the serological tests like the anti-CCP antibodies and IgM-RF, especially if medical interventions at such an early phase of the disease are planned to inhibited the onset of RA.

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