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

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Chapter 10

The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-CCP antibodies and are not an independent risk factor to develop rheumatoid arthritis

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ABSTRACT

Objectives. The Shared-Epitope (SE)-containing HLA-DRB1-alleles represent the most significant genetic risk factor for rheumatoid arthritis (RA). Recent studies showed that the SE-alleles only associate with RA that is characterized by the presence of anti-Cyclic Citrullinated Peptide (CCP)-antibodies, and not with anti-CCP-negative disease. Here we studied whether the SE-alleles contribute to the development of anti-CCP-positive RA, or rather associates with the presence of anti-CCP-antibodies. Therefore the influence of SE-alleles and anti-CCP-antibodies on the progression from recent onset undifferentiated arthritis (UA) to RA is investigated.

Methods. From the Leiden Early Arthritis Cohort, an inception cohort of patients with recent onset arthritis, the patients with UA at the 2-week visit were selected (n=570). SE-alleles, rheumatoid factor (RF) and anti-CCP-antibody levels were determined. Progression to RA or other diagnoses was monitored.

Results. 177 UA patients developed RA during 1-year follow-up, whereas 393 patients remained unclassified or developed other diagnoses. The SE-alleles correlated with the presence of anti-CCP-antibodies, but, after stratification for anti-CCP-antibodies, not with the presence of RF. Both in SE-positive and SE-negative UA-patients, the presence of anti-CCP-antibodies was significantly associated with the development of RA. More intriguingly however, no apparent contribution of the SE-alleles was found on the progression to RA when stratified for the presence of anti-CCP-antibodies. In anti-CCP-positive patients the presence of SE-alleles was associated with significantly higher levels of anti-CCP-antibodies, suggesting that the SE-alleles act as classical immune response genes.

Conclusions. The SE-alleles do not independently contribute to the development of RA from UA, but rather to the development of anti-CCP-antibodies.

INTRODUCTION

The most important genetic risk factor for rheumatoid arthritis (RA) are the HLA-Class II alleles. Especially the HLA-DRB1 alleles encoding for the shared epitope (SE) confer a higher risk to develop RA (1). The shared epitope hypothesis postulates that the shared epitope motif (a conserved amino acid sequence in the peptide binding pocket of the DRB1 molecule) is directly involved in the pathogenesis of RA by allowing the presentation of an arthritogenic peptide to T cells (2). Recently, it was observed by two different methods (linkage and association analysis) that the SE-alleles are only a risk factor for RA that is characterised by the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies and that the SE-alleles are not associated with anti-CCP-negative RA (3). Anti-CCP antibodies are highly specific for RA, can be detected years before the first clinical manifestation of RA (4) and are reported to be a good predictor for the development of RA (5). Because the contribution of the SE-containing HLA-alleles to the pathogenesis of RA is not well understood, the novel information on the association of SE-alleles with anti-CCP-positive disease (3) led us to evaluate the hypothesis that the SE-alleles are mainly a risk factor for anti-CCP-antibodies, rather than for (anti-CCP-positive) RA. To this end, we took advantage of a well-characterized inception cohort and studied the patients with an early arthritis that at presentation could not be classified according to the ACR-criteria (undifferentiated arthritis). Analysis of the clinical evolution, in combination with genetic and serological risk factors, of these patients prone to develop RA allows insight in the factors that are associated with progression towards RA. Accordingly this permits the analysis of the contribution of the SE-alleles to the development of RA after stratification for anti-CCP antibodies.

PATIENTS AND METHODS

Study population

For this study, the Leiden Early Arthritis Clinic (EAC) was used (see for description ref 6). In short, the EAC was started in 1993. Patients were referred to the EAC when arthritis was suspected and were included in the cohort when arthritis was found at physical examination. At baseline blood samples were taken. At present more than 1900 patients are included. Two weeks after inclusion, 313 patients had the diagnosis RA according to the 1987 ACR criteria and 570 patients had an arthritis that could not be classified according to one of the ACR criteria and were therefore called undifferentiated arthritis (UA). After one year of follow-up, the disease status of all UA patients was examined in order to determine whether they had developed RA according to the ACR-criteria. It might be possible that some patients did not fulfil the ACR criteria for RA at one year but developed RA at a

later time point. Inherent to the design of an inception cohort the duration of follow-up differs within the study population. However, at the moment of analysis the majority of patients (94%) has been followed for more than one year (mean follow-up 8 years, SD 3 years) and only 9% of the patients that did not classify as RA at one year developed RA later on in the disease course.

Laboratory investigations

Baseline laboratory parameters (determined using blood samples that were taken at inclusion) included IgM rheumatoid factor (RF, ELISA) and anti-CCP2 antibodies (ELISA, Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands). The cut-off level for anti-CCP antibody positivity was according to the manufacturers instruction set at a level of 25 arbitrary units. The HLA-DRB1 (sub)typing was performed by polymerase chain reaction using specific primers and hybridisaton with sequence specific oligonucleotides. The shared epitope alleles were HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408,*0410 and *1001. Of 438 from the 570 UA patients both data on SE-alleles and anti-CCP antibodies were available.

Statistical analysis

Odds ratios were calculated and proportions were compared by the chi-square test. Differences in means between groups were analysed using the Mann Whitney test or t-test when appropriate. The question whether SE-alleles and anti-CCP antibodies both independently contribute to progression of RA was investigated with a stratification procedure, as well as with logistic regression analysis. In this logistic regression analysis the disease outcome was entered as dependent variable and anti-CCP antibodies and SE-alleles as possibly explanatory variables; with a backward selection procedure the significant independent variables were selected. For all tests, p-values of < 0.05 were considered significant.

RESULTS AND DISCUSSION

Outcome of UA

Of 570 patients with UA at the two weeks visit, 177 developed RA during the first year of follow-up, 99 patients developed other rheumatologic diseases (reactive arthritis, psoriatic arthritis, SLE, etc) and 294 patients remained unclassified (persistent UA). For further analysis the patients with persistent UA and with other rheumatologic diagnosis were described as the non-RA group. Characteristics of the patients that developed RA and the non-RA group are depicted in Table 1. In univariate analysis, the presence of SE-alleles, RF and anti-CCP antibodies were all associated with significantly higher odds ratio's to develop RA (OR 1.8, 6.3 and 8.5 respectively, Table 1).

Table 1. Baseline characteristics of patients with undifferentiated arthritis at two weeks that did and did not develop RA during the first year of follow-up

	RA (n=177)	non-RA (n=393)	P	OR (95%CI)
Age (yr, mean ± SD)	56.3 ± 15.3	48.6 ± 16.9	<0.001	–
Gender F/M	121/56	208/185	0.001	1.9 (1.3-2.8)
SE positive*	100 (63%)	158 (49%)	0.005	1.8 (1.2-2.6)
Anti-CCP positive#	83 (51%)	38 (11%)	<0.001	8.5 (5.2-13.7)
RF positive	84 (47%)	56 (14%)	<0.001	6.3 (4.1-9.7)

* SE data was missing in 17 of the UA-RA patients and in 68 of the UA-non-RA patients
# Anti-CCP antibody data was missing in 15 of the UA-RA patients and 49 of the UA-non-RA patients

Association between SE-alleles and presence of auto-antibodies

To determine whether the SE-alleles correlate with RF, with anti-CCP antibodies or with both auto-antibodies, the associations between SE-alleles and anti-CCP and the associations between SE-alleles and RF were investigated in the 570 UA patients. In univariate analysis, the SE-alleles were associated with both RF and anti-CCP antibodies (OR 1.7, 95%CI 1.1-2.7, p=0.01 and OR 3.1, 95%CI 2.1-5.3, p<0.001 respectively). As anti-CCP positivity is correlated with RF positivity, the association between SE-alleles and anti-CCP antibodies was assessed in both the RF-positive and RF-negative patients. In RF-negative patients the presence of SE-alleles was associated with a higher odds ratio to develop anti-CCP antibodies (OR 2.9, 95%CI 1.2-6.9, p<0.01). Similarly, in RF-positive patients the presence of SE-alleles conferred a higher odds ratio to have anti-CCP antibodies (OR 5.6, 95% CI 2.1-14.6, p<0.001). These data indicate that the SE-alleles are associated with the presence of anti-CCP antibodies independent of the presence or absence of RF. Next it was assessed whether the SE-alleles are associated with RF, independent of the anti-CCP antibodies. In both the anti-CCP positive and anti-CCP negative patient groups, the SE-alleles were not associated with the presence of RF (p=0.9 and 0.2 respectively), indicating that after correction for the presence or absence of anti-CCP antibodies the SE-alleles do not confer risk to RF positivity. In conclusion, the SE-alleles are primarily correlated with the presence of anti-CCP antibodies but not with the presence of RF.

SE-alleles and anti-CCP antibodies in progression from UA to RA

Subsequently, the influence of SE-alleles on the progression from UA to RA was examined. Univariate analysis assessing the association between patient characteristics and disease outcome revealed that the presence of SE-alleles and anti-CCP antibodies at baseline were both associated with the development of RA (see Table 1). However, as the presence of SE-alleles and anti-CCP antibodies are correlated, the individual effect of SE-alleles on the development of RA was determined by stratification for anti-CCP antibodies. Both in the anti-CCP positive and in the anti-CCP negative UA-patients, the presence of SE-alleles was not associated with the development of RA (Table 2). These data are important as they

Table 2. Number of UA-patients that during one year of follow-up did not and did develop RA, stratified for baseline anti-CCP antibodies and SE-alleles

		non-RA n (%)	RA n (%)	P	OR (95%CI)
Stratification for anti-CCP antibodies					
anti-CCP –	SE –	142 (55)	37 (53)		
	SE +	118 (45)	33 (47)	0.8	1.1 (0.6-1.9)
anti-CCP+	SE –	8 (26)	21 (27)		
	SE +	23 (74)	56 (73)	0.9	0.9 (0.3-2.6)
Stratification for SE-alleles					
SE –	anti-CCP –	142 (95)	37 (64)		
	anti-CCP +	8 (5)	21 (36)	<0.001	10.1 (3.9-27.1)
SE +	anti-CCP –	118 (84)	33 (39)		
	anti-CCP +	23 (6)	56 (61)	<0.001	8.7 (4.5-17.0)

indicate that the SE-alleles do not correlate with RA development in patients with UA when stratified for the presence/absence of anti-CCP antibodies.

To assess the effect of the anti-CCP antibodies independent of SE alleles, the risk to develop RA was determined in SE-positive and SE-negative UA-patients separately (Table 2). This analysis showed that in the SE-positive as well as in the SE-negative UA patients the presence of anti-CCP antibodies was significantly associated with the development of RA (OR 8.7 and 10.1 respectively).

In a logistic regression analysis with a backward selection procedure entering the disease outcome (RA versus non-RA) as dependent variable and the SE-alleles and anti-CCP antibodies as possibly explanatory variables, the anti-CCP antibodies were the only independent factor that significantly associated with the development of RA with an odds ratio of 9.2 ($p < 0.001$). This odds ratio resulting from multivariate analysis is not importantly different from the odds ratio for anti-CCP antibodies on the development of RA as observed in univariate analysis (OR 8.5, see Table 1).

In conclusion, these data show that after stratification for SE-alleles the anti-CCP antibodies confer a high risk to develop RA, whereas after stratification for anti-CCP antibodies the SE-alleles are not associated with progression to RA. Together these data indicate that the SE-alleles primarily predispose to the presence of anti-CCP antibodies and are not an independent risk factor for the development of RA.

Association between SE-alleles and anti-CCP antibody level

In classic studies performed in mice on the genetical background associated with antibody production it has been shown that major histocompatibility alleles act as Immune Response genes (Ir-genes) that control the magnitude and specificity of antibody production in a dominant fashion (7). In these mice the magnitude of the antibody response of the first generation offspring was comparable to the magnitude of the high responding parent, denoting that in mice homozygositiy for MHC-genes did not improve the level of antibody
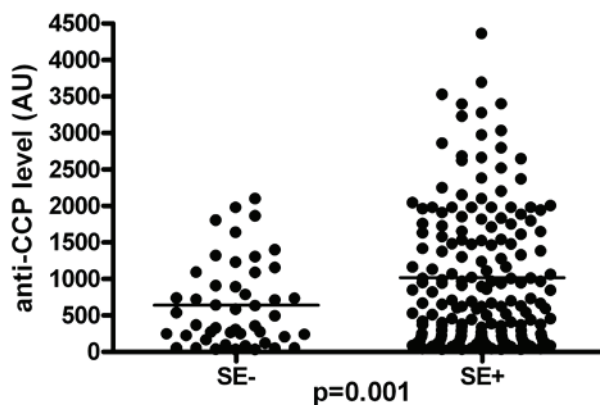
Figure 1. **Levels of anti-CCP antibodies (arbitrary units) in anti-CCP positive RA patients without and with SE-alleles.** The median anti-CCP antibody level is depicted. The mean anti-CCP antibody levels for the anti-CCP positive RA patients were: mean 1041 (SEM 134, n=46) for carrying two SE-alleles, 1029 (SEM 86, n=123) for carrying 1 SE-allele and 652 (SEM 86, n= 46) for carrying no SE-alleles. The median anti-CCP antibody levels for the subgroup anti-CCP positive UA patients that progressed to RA were: 699 (IQR 278-1282, n=13) for carrying 2 SE-alleles, 927 (IQR 251-1970, n=43) for carrying 1 SE-allele and 358 (IQR 169-1424, n=21) for carrying no SE-alleles.

production compared to heterozygosity (7). As the current data revealed that the SE-alleles are associated with anti-CCP antibodies, we wished to investigate whether the characteristics of the SE-alleles resemble such a classic Ir-gene. Thus we wished to analyse whether the level of anti-CCP antibodies present in serum was correlated to the presence of SE-alleles. To this end the correlation between the presence of SE-alleles and level of anti-CCP antibodies was assessed in all anti-CCP positive patients that at one-year follow-up had the diagnosis RA. Of a total number of 490 RA patients (313 RA diagnosed at two weeks follow-up and 177 patients that progressed from UA to RA during the first year of follow-up), 233 patients had anti-CCP antibodies of which 73% harboured SE-alleles. The anti-CCP antibody levels of anti-CCP-positive SE-positive and anti-CCP-positive SE-negative patients are depicted in Figure 1. SE-positive patients had a significantly higher level of anti-CCP antibodies (n= 169, mean 1032, SEM 72 arbitrary units) than SE-negative patients (n= 46, mean 652, SEM 86 arbitrary units, p=0.001). Patients carrying one SE-allele displayed a significantly higher level of anti-CCP antibodies (n=123, mean 1029; SEM 86 arbitrary units) compared to patients without SE-alleles (p=0.002). Patients with two SE-alleles did not have a significantly higher anti-CCP level (n=46, mean 1041, SEM 134 arbitrary units) compared to patients carrying one SE-allele (p=0.94). Thus, the current data show that in anti-CCP-positive patients the presence of SE-alleles is associated with higher levels of anti-CCP antibodies and indicate that the presence of one or two SE-alleles does not result in an apparent difference in anti-CCP antibody level. This observation is compatible with the notion that the SE-alleles are Ir-genes for the development of anti-CCP antibodies.

CONCLUSION

Recently, we reported that the SE-alleles were only associated with anti-CCP-positive RA and not with anti-CCP-negative disease, indicating that the SE-alleles are not associated with RA as such but rather with a distinct phenotype of the disease. We now extend these findings by showing that the SE-alleles are not an independent risk factor for the development of RA after correction for anti-CCP-antibody status. The SE-alleles were however associated with the presence of anti-CCP antibodies. Moreover, the presence/ absence of SE-alleles was correlated with the levels of anti-CCP antibodies, suggesting that the SE-alleles act as classic Ir-genes for the development of anti-CCP antibodies. Although no formal conclusions on causality can be drawn from this association study, these findings suggest that anti-CCP antibodies mediate the association between SE-alleles and RA. It would be of interest to replicate the findings of the present study by following the development of anti-CCP antibodies and RA in healthy asymptomatic persons with and without SE-alleles. Nevertheless, the present data constitute an important refinement of the long-known association between HLA and RA by indicating that the SE-alleles do not primarily associate with RA, but rather with anti-CCP-positivity.

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