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

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Chapter 2

An independent role for protective HLA Class II alleles in rheumatoid arthritis severity and susceptibility

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ABSTRACT

Objectives. The most important genetic risk factor for rheumatoid arthritis (RA) is located within the Human Leucocyte Antigen (HLA)-region. HLA-DRB1 alleles encoding for the shared epitope (SE) predispose to RA and to more severe disease. Other HLA-DRB1 alleles harbouring a different epitope, encoded by the amino acids DERAA, have been associated with protection. Due to small cohort sizes, the protective effect on disease severity is still controversial and has never been discerned from non-predisposition (not carrying SE-alleles). This study investigates the effect of the DERAA-encoding alleles on RA severity and susceptibility in a large prospective cohort and differentiates protective effects from non-predisposition by comparing subgroups of patients with an equal amount of predisposition alleles.

Methods. In 440 early RA patients and 423 healthy controls the HLA class II alleles were determined. To study the effect of HLA on disease severity, radiological joint destruction (modified Sharp-van der Heijde method) was determined during 4-years follow-up.

Results. The presence of DERAA-encoding HLA-DRB1-allelles conferred a lower risk to develop RA both in the presence and in the absence of SE-alleles (OR 0.6). In the presence of one SE-allele, the group of patients that carried DERAA had significant less severe radiological destruction at all time points compared to DERAA-negative patient-group with one SE-allele. Additionally, the protective effect of DERAA was detected in the groups of patients that were prone to more severe disease due to the presence of anti-CCP-antibodies or smoking.

Conclusions. DERAA-encoding HLA-DRB1-alleles independently protect against RA and are associated with less severe disease.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex genetic disorder with an estimated heritability of 60% (1). Human Leucocyte Antigens (HLA) Class II molecules are the most powerful recognized genetic factors and contribute to at least 30% of the total genetic effect (2). Extensive evidence exists showing the association between certain frequently occurring HLA-DRB1 alleles (*0101, *0102, *0401, *0404, *0405, *0408, *0410 *1001, *1402) and susceptibility to and severity of RA (3-5). The indicated alleles share a conserved amino acid sequence (QKRAA, QRRAA or RRRAA; also called the shared epitope) at position 70-74 in the third hypervariable region of the DRβ1 chain. These residues are part of an α-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 a peptide to arthritogenic T cells.

Although the predisposing effects of shared epitope encoding HLA-DRB1 alleles are generally accepted, controversy exists on the existence of protective effects by certain HLA-DRB1 alleles. These alleles contain, instead of the shared epitope, another common anchor-region consisting of the amino acids DERAA. HLA-DRB1 alleles that express this DERAA sequence (DRB1*0103, *0402, *1102, *1103, *1301, *1302, *1304) may protect against RA (6-8). There is some evidence that patients carrying the DERAA sequence have also less erosive disease. However, there are few studies addressing the effect of DERAA on disease severity, and interpretation is hampered either by a retrospective design with variable disease duration (9,10) or by small numbers of patients carrying the DERAA-sequence. Wagner et al. performed a prospective study, but only 7 DERAA-positive patients were followed for 4 years (11). Moreover, it is not clear whether the effect of DERAA encoding HLA-DRB1 alleles is truly protective or is due to the effect of the concomitant absence of predisposing shared epitope encoding HLA-DRB1 alleles.

A number of the initial reports on the protective effects of the DERAA haplotype are based on the Leiden Early Arthritis Clinic (6,12). This cohort started in 1993 and has since then expanded considerably. Presently more than 1800 patients are included. By using this expanded cohort we aim to assess the association of DERAA encoding HLA-DRB1 alleles with 1) RA severity, taking advantage of the fact that at present a substantial number of patients is followed prospectively and 2) susceptibility to RA. This large cohort allows the determination of the possible protective effects of DERAA encoding HLA-DRB1 alleles in the presence of an equal number of shared epitope encoding HLA-DRB1 alleles, thereby permitting differentiation between protection and non-predisposition. Furthermore, the available clinical data allowed to determine whether RA patients exhibiting an extreme of the phenotypic spectrum by achieving clinical remission harbour a different distribution

of HLA-alleles compared to patients with persistent disease. The present data show that HLA-DRB1 alleles encoding the DERAA-sequence are associated with less severe disease at all time points during 4 years follow-up and confer a lower risk to develop RA.

PATIENTS AND METHODS

Study Population

In 1993 an Early Arthritis Clinic was started at the Department of Rheumatology of the Leiden University Medical Center, the only referral center for Rheumatology in a health care region of about 400,000 inhabitants in the western part of the Netherlands (13). General practitioners were encouraged to refer patients directly when arthritis was suspected. Patients referred to the early arthritis clinic could be seen within two weeks and were included in the program when the physician's examination of the patient revealed arthritis and the symptoms had lasted less then two years. For every patient, routine diagnostic laboratory screening was performed. A 44 joint count of swollen joints was performed on entering the study and yearly thereafter. The smoking history was assessed. In this study smokers were patients that smoked (cigarettes, cigars) at inclusion or patients that had smoked previously. The numbers of smoked pack years was not addressed. Non-smokers have never smoked. At present more than 1800 patients are included in the Early Arthritis Clinic, of which approximately 1650 patients have at least one year of follow-up. 440 of these patients fulfilled the diagnosis of RA according to the American College of Rheumatology 1 year after inclusion in the study (376 definite RA and 64 probable RA, according to the ACR criteria of 1987 and 1958 respectively) and had DNA available for genotyping. As it was observed that in the current inception cohort, over 2/3 of probable RA patients developed definite RA in the next year, these probable RA patients were included in the study. A small proportion of the patients involved in the present study (about one third) were also included in previous studies examining the association between HLA-DRB1 alleles and RA using the Leiden Early Arthritis Clinic (6). Informed patient consent was obtained and the study was approved by the local medical ethics committee. A random panel of 423 healthy unrelated Dutch individuals served as control.

HLA genotyping

The HLA class II alleles were determined in all RA patients and controls. The HLA-DRB1 (sub)typing was performed by polymerase chain reaction using specific primers and hybridisation with sequence-specific oligonucleotides. The predisposing shared epitope alleles were DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *0410 *1001, and *1402. The DERAA encoding alleles were HLA-DRB1*0103, *0402, *1102, *1103, *1301, *1302, and *1304. For clarity, this study uses consequently the term DERAA-encoding alleles.

Table 1. HLA-DRB1 genotypes of RA patients and healthy controls

Group	DRB1 genotype	RA patients (N=440)		Controls (N=423)	
		n	%	n	%
A	SE/SE	70	15.9	26	4.9
B	SE/x	187	42.5	124	30.5
C	SE/DERAA	27	6.1	29	6.9
D	x/x	112	25.5	149	35.2
E	x/DERAA	36	8.2	87	20.6
F	DERAA/DERAA	8	1.8	8	1.9

Group B vs. C: OR 0.6, 95%CI 0.3-1.1, p=0.1.
Group D vs. E+F: OR 0.6, 95%CI 0.4-0.97, p=0.03.
Group A+B vs. D: OR 2.3, 95%CI 1.6-3.2, p<0.001.
Patients and controls were categorized according to the HLA-DRB1 genotype.
SE alleles are DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *1001, *1402.
DERAA alleles are DRB1*0103, *0402, *1102, *1103, *1301, *1302, *1304.
x means all other DRB1 alleles.

However this study does not differentiate between the direct effect of these alleles and the effect of other alleles in linkage with the DERAA-encoding HLA-DRB1 alleles; the observed effects might therefore also be the result of a haplotype containing the DERAA-encoding alleles. For the analysis a division in six groups according to the HLA-DRB1 alleles was made: homozygous for shared epitope (SE/SE, group A), one shared epitope allele (SE/x, group B), one shared epitope and one DERAA allele (SE/DERAA, group C), no shared epitope or DERAA alleles (x/x, group D), one DERAA allele (x/DERAA, group E) and two copies of a DERAA encoding allele (DERAA/DERAA, group F), see Table 1.

Radiographic progression

Radiographs of hands and feet were made at baseline, at one year and yearly thereafter. Radiographs were scored using the modified Sharp-Van der Heijde method (14). The rheumatologist that scored the radiographs was blinded to the clinical data and unaware of the study question. At inclusion radiographs were scored of 324 patients, 305 patients had radiographs at 1 year follow-up, 259 patients at 2 year follow-up, 216 patients at 3 year follow-up and 197 patients at 4 years follow-up. The fact that at the moment of analysis not all patients had achieved 4 years follow-up is inherent to the design of an inception cohort.

Extremes of the phenotypes: clinical remission

Comparing the extreme phenotypes of a disease can elucidate the presence or absence of an association between an allele and disease severity (15,16). For this study patients that developed clinical remission, the best clinical course possible, were selected. Patients in remission had no signs of arthritis in the absence of disease-modifying drugs and were therefore discharged from the outpatient clinic. Patients were only discharged after they

had been at least one year in remission without the use of disease-modifying drugs. Eighty patients achieved clinical remission; all had fulfilled the ACR criteria for RA (62 patients for definite RA and 18 patients for probable RA, according to the ACR criteria of 1987 and 1958 respectively).

Statistical analysis

To differentiate the protective effects from the effects due to non-predisposition, analysis was performed using subgroups of patients with an equal amount of predisposing shared epitope alleles. To determine the effect of the DERAA-encoding alleles in the presence of one shared epitope allele the subgroups SE/DERAA and SE/x were compared (group B vs. C, see table 1); to assess the effect of the DERAA-encoding alleles in the absence of shared epitope alleles the subgroups X/DERAA and DERAA/DERAA were compared with x/x (group E+F vs. D, see table 1). An alternative method to identify the causative HLA-factor truly responsible for the association is described by Svejgaard and Ryder (17). This method uses a two-by-four table that is subsequently analysed using various two-by-two tables involving stratification of each of the two factors against each other. The association of DERAA with RA susceptibility was analysed and presented according to both methods. For the analysis of the severity data, subgroups with an equal amount of predisposing shared epitope alleles were compared. Odds ratio's (OR) with 95% confidence intervals (95% CI) were calculated using the method of Woolf Haldane; p values were calculated using the chi square test. Differences in means between groups were analysed with the Mann Whitney test or t-test when appropriate. In all tests, p values less than 0.05 were considered significant.

RESULTS

Susceptibility

To study the effect of the presence of DERAA on the susceptibility to RA, patients and controls were divided in 6 groups according to their HLA-DRB1 status (Table 1). In total 71 RA patients (16%) and 124 controls (29%) carried DERAA encoding HLA-DRB1 alleles. (OR 0.5, 95%CI 0.3-0.7, p<0.0001) First, the effect of DERAA in the absence of shared epitope allele was assessed by comparing group D with E+F. DERAA positive persons had a significantly lower risk to develop RA (OR 0.6, 95%CI 0.4-0.97, p=0.03). Comparing group B with group C revealed that in the presence of one shared epitope allele the DE-RAA-encoding alleles reduce the risk to develop RA, although the observed effect was not statistically significant (OR 0.6, 95%CI 0.3-1.1, p=0.1).

Additionally the same data were analysed according to the Svejgaard approach (see Table 2). The presence of DERAA conferred a significant lower risk to develop RA both

Table 2. HLA-DRB1 genotypes of RA patients and healthy controls, analysed according to the approach of Svejgaard and Ryder (17)

Shared epitope encoding DRB1	DERAA encoding HLA-DRB1	Number of patients (N=440)	Number of controls (N=423)
+	+	27	29
+	–	257	150
–	+	44	95
–	–	112	149

Comparison	Entries of 2x 2 table				
	a	b	c	d	
SE + vs. –	284	156	179	244	OR 2.5 95%CI 1.9-3.3, p<0.0001
DERAA + vs. –	71	369	124	299	OR 0.5 95%CI 0.3-0.7 p<0.0001
SE in DERAA +	27	44	29	95	OR 2.0 95%CI 1.0-4.0, p=0.03
SE in DERAA –	257	112	150	149	OR 2.3 95%CI 1.6-3.2, p<0.0001
DERAA in SE +	27	257	29	150	OR 0.5 95%CI 0.3-0.99 p=0.03
DERAA in SE –	44	112	95	149	OR 0.6 95%CI 0.4-0.97, p=0.03

in shared epitope negative and shared epitope positive patients (OR 0.6, 95%CI 0.4-0.97, p=0.03 and OR 0.5, 95%CI 0.3-0.99, p=0.03 respectively).

As anti-CCP antibodies are highly associated with RA, we wished to analyse whether the presence of DERAA was correlated with the anti-CCP status of patients. Therefore the effect of DERAA on the risk to develop RA was assessed in anti-CCP positive and anti-CCP negative RA patients separately. The presence of DERAA conferred a lower risk to develop both anti-CCP positive RA (OR 0.3, 95%CI 0.1-0.4) and anti-CCP negative RA (OR 0.7, 95%CI 0.5-1.0).

The effect on disease susceptibility of shared epitope alleles in the absence of DERAA was assessed by comparing group A+B versus D (Table 1) and similarly according the Svejgaard approach (Table 2). Shared epitope positive persons had an odds of 2.3 to develop RA compared to shared epitope negative patients (95%CI 1.6-3.2, p< 0.001).

As the HLA-DRB1 alleles are in linkage disequilibrium with certain HLA-DQ alleles (DQ3 and DQ5, see ref 22), the above-described analysis was also performed using HLA-DRB1-DQ genotypes. Similar results on predisposition to RA were found using HLA-DRB1-DQ genotypes instead of using HLA-DRB1 alleles solely (Table 3).

Table 3. HLA-DRB1 and -DQ genotypes of RA patients and healthy controls

Group	DQ genotype	DRB1 genotype	RA patients (N=440)		Controls (N=423)	
			n	%	n	%
1 a	3/3	SE/SE	31	7.0	9	0.9
b		SE/x	10	2.2	2	1.7
c		x/x	2	0.5	2	0.5
2 a	3/5	SE/SE	29	6.6	11	2.6
b		SE/x	5	1.1	2	0.5
3 a	5/5	SE/SE	9	2.0	6	1.4
b		SE/x	1	0.2	0	0
4 a	3/x	SE/SE	1	0.2	0	0
b		SE/x	100	22.7	58	13.7
c		x/x	16	3.6	16	3.8
5 a	5/x	SE/x	71	16.1	62	14.7
b		x/x	1	0.2	0	0
6 a	3/x	SE/DERAA	14	3.2	12	2.8
b		x/DERAA	3	0.7	7	1.7
7 a	5/x	SE/DERAA	13	3.0	17	4.0
b		x/DERAA	1	0.2	0	0
8	x/x	x/x	93	21.4	131	31.0
9	x/x	x/DERAA	32	7.3	80	18.9
10	x/x	DERAA/DERAA	8	1.8	8	1.9
Predisposition alleles + (gr1-5)			276		168#	
Predisposition alleles – (gr8)			93		131#	
Protection alleles – (gr 4b+5a)			171		120*	
Protection alleles + (gr 6a+7a)			27		29*	
Protection alleles – (gr 8)			93		131°	
Protection alleles + (gr9+10)			40		88 °	

Patients were categorized according to the presence or absence of DQ3 or DQ5 heterodimers and subdivided for DRB1 alleles. SE alleles are DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *1001, *1402. DQ3 means DQB1*0301, *0302, *0303, *0304, *0401, or *0402 in combination with DQA1*03. DQ5 means DQB1*0501 in combination with DQA1*0101 or *01040. DERAA alleles are DRB1*0103, *0402, *1102, *1103, *1301, *1302, *1304. x means all other DQ or DRB1 alleles.
OR 2.3 95%CI 1.7-3.3, p<0.001.
* OR 0.7, 95%CI 0.4-1.2, p=0.14
° OR 0.6, 95%CI 0.4-1.0, p=0.05

All the above-mentioned results did not change when the 64 patients with probable RA were excluded and the 376 patients with definite RA were analysed (comparison of group B with C OR 0.6, 95%CI 0.3-1.1, p=0.06; comparison of group D with E+F OR 0.6, 95%CI 0.4-1.0, p=0.04; comparison of groups A+B with D OR 2.1, 95%CI 1.1-4.0, p=0.01).

In conclusion, these data show that carriership of DERAA-encoding HLA-DRB1 alleles protects to develop RA in individuals with a shared epitope allele as well as in individuals without shared epitope alleles.

Severity

To asses the influence of the presence of DERAA encoding HLA-DRB1 alleles on radiological joint destruction, Sharp-van der Heijde scores during 4 years of follow-up were compared in subgroups of patients with an equal of amount of shared epitope encoding HLA-DRB1 alleles, thereby excluding a possible confounding effect due to a difference in predisposing alleles. Although the rate of joint destruction in the whole group of shared epitope negative patients was very low, the effect of carrying one or two DERAA alleles in the absence of shared epitope alleles was determined by comparing the radiological scores of group E+F versus D. The mean (± SEM) Sharp-van der Heijde scores at inclusion and 1, 3 and 4 years of follow up were respectively 2.9 ± 0.6, 8.1 ± 1.8, 12.4 ± 2.4, and 15.1 ± 3.6 in the patients not carrying a protection allele (group D) and 5.0 ± 2.2, 7.8 ± 3.4, 8.6 ± 3.7, and 15.2 ± 7.3 in the patients with one or two protection alleles (group E+F) (p = 0.4, 0.9, 0.3 and 0.9 respectively). Thus, the presence of DERAA-encoding alleles in patients with absence of shared epitope does not result in significantly lower radiological scores. As anti-CCP antibodies are associated with more severe disease (18), we assessed the influence of DERAA on disease severity in anti-CCP positive and negative patients separately. This analysis revealed that in shared epitope negative anti-CCP positive RA patients, the presence of DERAA associates with significantly less severe disease at all points in time except inclusion (see Figure 1). In shared epitope negative, anti-CCP negative patients the rate of joint destruction was too low to observe differences between DERAA positive and negative patients.
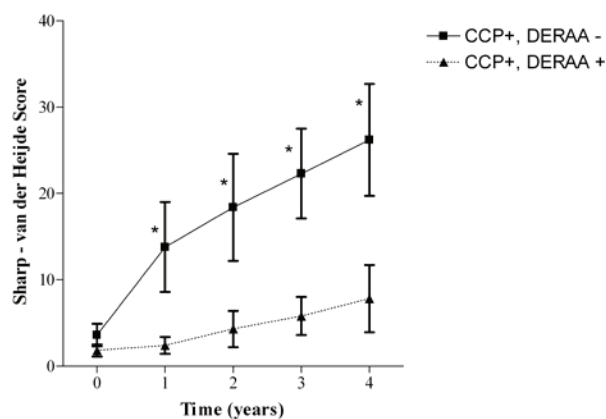

Figure 1. Sharp-van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of shared epitope negative anti-CCP positive RA patients in the presence and absence of DERAA-encoding alleles. * P <0.05.

Because patients not carrying shared epitope alleles have a less destructive disease compared to shared epitope positive patients (comparison of groups D versus A+B, Figure 2), we subsequently assessed the effect of DERAA on disease severity in shared epitope positive patients, as in this group of patients with more severe disease the window to read out an eventual protective effect is larger. Moreover, by analysing the subgroups of patients with an equal amount of shared epitope alleles a possible confounding effect due to differences in predisposing alleles was excluded. Comparing the Sharp-van der Heijde scores of group B with group C (see Table 1 for the division in groups) showed significant lower Sharp-van der Heijde scores at all time points during 4 years follow-up in the DERAA positive group (Figure 3, p<0.001 at inclusion, 1 and 2 years follow-up, p<0.01 at 3 years and p<0.05 at 4 years follow-up). Thus, DERAA-encoding alleles protect against severe disease in the presence of one shared epitope allele.

Considering the association between anti-CCP antibodies and RA severity (18), we wished to assess whether the observed protective influence of DERAA is dependent on the presence or absence of anti-CCP antibodies. Therefore, the effect of DERAA in the presence of one shared epitope allele was analysed in anti-CCP positive and negative patients separately. The protective effect of DERAA remained in both anti-CCP positive and negative RA patients (Figure 4). Not only anti-CCP antibodies are known to associate with RA severity, also the environmental factor smoking is reported to correlate with more severe disease (19). To further confirm the protective effects of the DERAA-encoding alleles we analysed the effects of DERAA in patients that were prone to more severe disease due to smoking. Therefore, the effect of DERAA in the presence of one shared epitope allele was assessed for smokers and non-smokers separately. Non-smoking patients that were DERAA-positive showed a trend for lower radiological scores (p=0.06 and 0.07 at 1 and 4
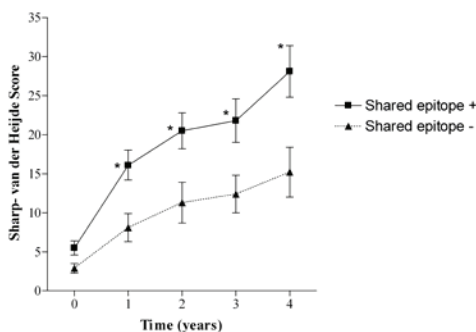
**Figure 2. Sharp- van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of RA patients with or without shared epitope alleles in the absence of DERAA –encoding alleles. *** P<0.05.
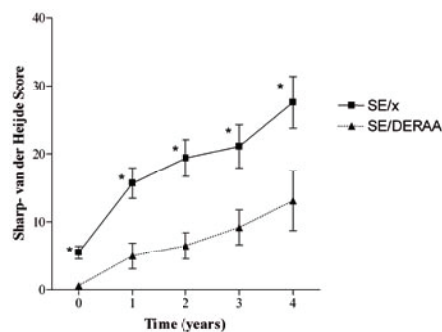
**Figure 3. Sharp- van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of RA patients with and without DERAA-encoding HLA-DRB1 alleles in the presence of one shared epitope allele. *** P<0.05.
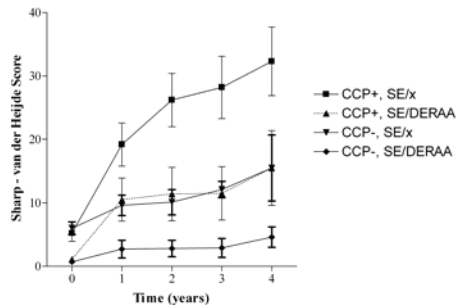
Figure 4. Sharp-van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of RA patients with and without DERAA-encoding alleles in the presence of one shared epitope encoding allele, for anti-CCP positive and anti-CCP negative patients separately.
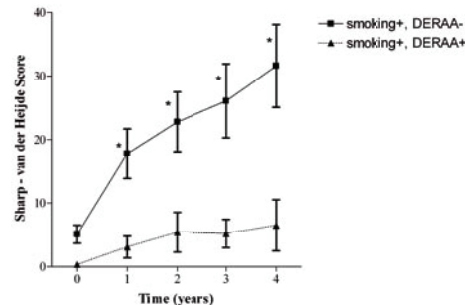
**Figure 5. Sharp-van der Heijde scores (mean and SEM) at inclusion and 4 years follow-up of shared epitope positive smoking RA patients in the presence or absence of DERAA -encoding alleles. *** P<0.05.

year follow-up respectively). In smokers the presence of DERAA correlated with significant lower Sharp-Van der Heijde scores at all time points except inclusion (p<0.05, Figure 5). As smoking might correlate with anti-CCP antibodies (non published data S. Linn -Rasker) a Mantel-Haenszel analysis revealed that a trend to a protective effect of DERAA in both anti-CCP positive and negative smoking RA patients was present (data not shown).

In conclusion, 1) DERAA-encoding HLA-DRB1 alleles are associated with less severe joint destruction in patients that also carry a HLA-DRB1 encoding shared epitope allele, 2) this protective effect remains after correction for anti-CCP antibodies and 3) DERAA-encoding alleles also exhibit a protective effect in severe disease that is associated with smoking.

Extremes of the phenotypes: clinical remission

To assess a possible association between HLA and clinical remission, we identified 80 patients that obtained clinical remission without the use of disease modifying drugs. Clinical remission was achieved after a mean follow-up of 3.9 years (SD 2.5 years). The patients in the remission group were in 62% of cases female, had a mean age of 57.7 ± 15.4 years (mean ± SD) and were in 12% anti-CCP antibody positive. The 360 patients that did have persistent RA were in 66% of cases female, had a mean age of 55.4 ± 16.4 years and were in 57% anti-CCP antibody positive. There was no different distribution of DERAA-encoding HLA-DRB1 alleles in patients that obtained remission compared to patients with persistent RA. Overall, 18% of patients that obtained remission carried DERAA alleles, versus 16% of the RA patients with persistent disease. Likewise, when the distribution of DERAA in the presence or absence of shared epitope alleles was evaluated, no differences were found in the remission or persistent RA group. In addition, the distribution of shared epitope encoding HLA-DRB1 alleles in the absence of DERAA alleles was studied in the

remission and persistent RA group. Fifty-five percent of patients that achieved clinical remission carried shared epitope alleles, compared to 73% of the patients with persistent RA. This indicates that shared epitope alleles occurred significantly less frequent in the patients that achieved clinical remission (OR 0.5, 95%CI 0.3-0.8, p=0.003). In conclusion, RA patients that achieve clinical remission have significantly less frequent shared epitope alleles but do not carry more DERAA-encoding HLA-DRB1 alleles.

DISCUSSION

This study investigates the associations between HLA Class II alleles and RA and describes the protective effects of DERAA-encoding HLA-DRB1 alleles on RA severity and susceptibility. The question whether the effect of DERAA is truly protective or only the result of the absence of predisposing shared epitope-encoding HLA-DRB1 alleles has been surrounded with some controversy. In the current study the comparison of subgroups allowed to differentiate the effects of protection and non-predisposition. This study shows that the DERAA-encoding HLA-DRB1 alleles independently reduce the risk to develop RA.

More importantly however, our study shows in a large prospective cohort that DERAA encoding alleles are associated with less severe radiological destruction in patients that were predisposed to severe RA by the presence of shared-epitope alleles at all time point during 4 years of follow-up. The protective effect of DERAA remained after stratification for anti-CCP antibodies. Stratification for smoking, another risk factor for severe disease, showed that DERAA particularly protects in patients that are also predisposed to more severe disease by smoking. All together, these data indicate that the protective influence of DERAA can be detected in patients that are prone to severe disease, by either the presence of shared epitope alleles, anti-CCP antibodies or smoking. In patients with a low rate of joint destruction such as shared epitope negative and anti-CCP negative RA patients, the current data set is not sufficiently powered to answer the question whether a protective effect of DERAA is present in these patients. Intriguingly, the differences in Sharp-van der Heijde scores between DERAA-positive and negative patients (in presence of a shared epitope allele) are as large as the differences in Sharp-van der Heijde scores between shared epitope positive and negative patients (see Figure 2 and 3). Thus, the protective effect of DERAA-encoding alleles on radiological joint destruction seems to be of a similar magnitude as the predisposing effect of shared epitope alleles.

The chance to achieve clinical remission is lower for patients carrying a predisposition allele, but is not higher in patients carrying protection alleles. Although we cannot explain these observations at this moment, these findings suggest that the disease-pro-

moting mechanisms that are associated with shared epitope alleles are distinct from the mechanisms involved in tempering disease-progression. In this respect, it is tempting to speculate that the protective pathways associated with the expression of DERAA-encoding HLA-alleles are able to dampen the effector pathways underlying bone and cartilage breakdown, but that they do not affect the principal pathway that drives chronicity.

Although the number of patients with 4 years follow-up in the current study is higher compared to previous studies on the protective effect of DERAA on RA severity, the present study lacked sufficient power to address the question of a dose effect of DERAA. This is due to the finding that homozygosity for DERAA in RA-patients is rare (2% of the RA patients in this cohort). Of these 8 patients, 5 patients had at the moment of analysis a follow-up of 2 years and only 2 had a follow-up of 4 years. Remarkably, the total Sharp-van der Heijde score of these patients was 1.0 ± 1.0 at inclusion, 1.6 ± 1.0 and 0 ± 0 at 2 and 4 years follow-up (mean $\pm$ SEM), indicating that RA patients with two copies of DERAA seems to have a non-destructive disease course. As the radiological scores of the patients that are homozygous for DERAA are lower than the patients heterozygous for DERAA, a gene-dose effect is possible. However, the number of homozygous patients is too low for definite conclusions.

Although so far not much data are available on the association between protective HLA Class II alleles and RA severity, well-designed studies are available on the association between protective HLA alleles and disease susceptibility (8,20,21). However, the definition of protective alleles differs in these studies. De Vries et al. considered alleles with amino acid D at position 70 as protecting. In this way more alleles than those encoding for $D^{70}ERAA$ were classified as protective (e.g HLA-DRB1 *07, *1201, *1501) (20). Reviron et al. concluded on a different hypothesis (electric charge of the HLA pocket) that alleles with a neutral or negative charge in their P4 pocket protect to develop RA. These alleles contain not only the DERAA-encoding HLA-DRB1 alleles but also other HLA- alleles, among which HLA-DRB1*08 (21). Our results confirm and extend these observations by focussing on the DERAA-encoding HLA-alleles and by analysing the effects of these alleles on disease severity. The observed effects of the presence of DERAA might be the direct result of the DERAA-encoding alleles or might be the result of HLA-haplotypes that contain the DERAA-encoding HLA-DRB1 alleles.

The known predisposing effect of the shared epitope alleles on RA susceptibility and severity is confirmed in this study. Previously, it has been hypothesised by our group that predisposition to RA is not only controlled by shared epitope alleles but is also conferred by HLA-DQ alleles (22). Support for a role of HLA-DQ came from studies on collagen induced arthritis in HLA-DQ transgenic mice (23). The so-called RA-protection hypothesis

further implied that DERAA is only protective in the presence of certain DQ3 or DQ5 heterodimers (22). The data of the current study were analysed both using HLA-DRB1 genotypes and using HLA-DR-DQ genotypes, revealing similar results. The predisposing HLA-DQ and -DRB1 alleles are strongly associated in our population; therefore differentiation of the individual effects of HLA-DR and HLA-DQ was not feasible. As the present study reveals that DERAA protects against RA not only in patients with predisposing HLA-DR alleles or HLA-DR-DQ genotypes but also confers a lower risk to develop RA in patients without these predisposing genotypes, the previously published RA-protection hypothesis should be amended.

It has been demonstrated that peptides carrying the DERAA motif are naturally processed by human APC and it has been suggested that the protective effect of DERAA is mediated by a specific protective T cell response (24). Although our results clearly show that the presence of a predisposing haplotype is not required to observe the protective effect associated with DERAA, it is conceivable that the DERAA-sequence itself is presented towards T-cells with protective activities. Interestingly alleles carrying the DERAA sequence, particularly DRB1*13 alleles, not only protect from (severe) RA but have also been associated with a milder disease outcome in other diseases, such as a reduced progression to active chronic hepatitis C and B (25,26), a lower incidence of cervical carcinoma (27) and a reduced rejection of renal transplants (28). These findings are intriguing and point to the importance to elucidate the biological pathways underlying these associations, as they might unveil new insights on immune regulation in relation to the HLA-system.

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