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

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Chapter 8

HLA-DR3 is associated with anti-CCP antibody negative rheumatoid arthritis

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ABSTRACT

Objectives. Recent data have shown that the most prominent and longest known genetic risk factors for rheumatoid arthritis (RA), HLA-DRB1 shared epitope alleles, are only associated with RA that is characterized by the presence of antibodies against cyclic citrullinated peptide (anti-CCP antibodies) and not with anti-CCP-negative RA. We undertook this study to investigate whether anti-CCP-negative RA is associated with other HLA-DRB1 alleles.

Methods. HLA typing was performed for 377 patients from the Leiden Early Arthritis Clinic who were diagnosed as having RA within the first year of followup (206 anti-CCP-positive patients and 171 anti-CCP-negative patients), 235 patients who, after 1 year, had undifferentiated arthritis (UA) (28 anti-CCP-positive patients and 207 anti-CCP-negative patients), and 423 healthy control subjects. Odds ratios (ORs) with 95% confidence intervals (95% CIs) for HLA-DRB1 allele frequencies were determined for all patient groups compared with the healthy control group.

Results. HLA-DR3 was more frequently present in the anti-CCP-negative RA group than in the control group (OR 1.84, 95% CI 1.26-2.67). This was not the case for anti-CCP-positive RA (OR 0.92, 95% CI 0.60-1.40). HLA-DR3 was also more frequently present in anti-CCP-negative UA patients (OR 1.59, 95% CI 1.10-2.28), but not in anti-CCP-positive UA patients (OR 0.68, 95% CI 0.17-1.92).

Conclusions. HLA-DR3 is associated with anti-CCP-negative arthritis and not with anti-CCP-positive arthritis. These data show that distinct genetic risk factors are associated with the presence of anti-CCP antibodies in RA and indicate that different pathogenetic mechanisms underlie anti-CCP-positive and anti-CCP-negative RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a multifactorial autoimmune disease with a complex genetic background. As in other autoimmune diseases, an association between RA and the HLA complex has long been observed in many different populations and is thought to account for approximately one-third of the genetic component of RA susceptibility (1). There is extensive evidence for the association between certain frequently occurring HLA-DRB1 alleles, the so-called "shared epitope" (SE)-encoding alleles (DRB1*0101, *0102, *0104, *0401, *0404, *0405, *0408, *0413, *0416, and *1001), and susceptibility to RA (2). These SE alleles encode for a common amino acid sequence in the third hypervariable region of the DRB1 molecule (QKRAA, QRRAA, or RRRAA).

In recent years, many studies on antibodies against cyclic citrullinated peptide (CCP) showed that these antibodies are highly specific and predictive for RA (3), that they can be detected years before onset (4), and that they are associated with joint destruction (5). Furthermore, the presence or absence of these antibodies seems to be a stable trait (6). Anti-CCP antibodies are detected in SE-positive as well as in SE-negative RA patients. Carriership of SE alleles in RA is associated with the presence of anti-CCP antibodies (7). Interestingly, when we recently compared anti-CCP-positive and anti-CCP-negative RA patients with healthy controls, we found that HLA-DRB1 alleles encoding the SE were only associated with RA in the presence of anti-CCP antibodies and were not associated with anti-CCP-negative RA (8). These data indicate that the SE-encoding alleles are not associated with RA as such, but rather with anti-CCP-positive RA. These observations indicate that distinct phenotypic manifestations of the disease are associated with distinct genetic risk factors. They also raise the question of whether anti-CCP-negative RA is associated with HLA-DRB1 alleles other than SE-encoding alleles. We therefore investigated the possible associations of particular HLA-DRB1 alleles with anti-CCP-positive RA and anti-CCP-negative RA. To verify the results and to study whether the results were specific for RA, we also performed the same analysis in a group of patients with undifferentiated arthritis (UA).

PATIENTS AND METHODS

Study population

In 1993 an Early Arthritis Clinic (EAC) was started at the Department of Rheumatology of the Leiden University Medical Center, as described previously (9). The population studied here consisted of 377 patients who, within the first year of follow-up, fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for RA (10) and 235 patients who, after 1 year of follow-up, could

not be properly classified according to one of the ACR criteria sets and were therefore categorized as having UA. For every patient within the cohort, routine diagnostic laboratory screening was performed, including measurements of IgM-rheumatoid factor (IgM-RF). Informed patient consent was obtained, and the study was approved by the local medical ethics committee. Four hundred twenty-three healthy Dutch individuals served as controls. The control subjects were normal healthy donors of both sexes who were randomly selected and were ages 55 years and younger.

HLA genotyping

HLA class II alleles were determined in all patients and controls. The HLA-DRB1 (sub)typing was performed by polymerase chain reaction using specific primers and hybridization with sequence-specific oligonucleotides.

Anti-CCP autoantibodies

Serum antibodies directed against CCP were assessed with a commercial enzyme-linked immunosorbent assay (Immunoscan RA, Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). Anti-CCP antibodies were measured in serum collected within 4 months after the first visit (94%) or, when serum was not available within this time period, in the first stored serum sample available thereafter.

Statistical analysis

Odds ratios (ORs) were calculated using the Epi Info Statcalc computer program (Centers for Disease Control and Prevention, Atlanta, GA) to compare HLA-DR allele frequencies between the patient groups and the control population. ORs were reported with 95% confidence intervals (95% CIs), which excluded the value of 1 in case of statistical significance. Exact confidence limits were used as described by Mehta et al (11). For HLA-DR3, both allele frequencies and genotype frequencies were compared between the patient groups and the control population using the same methods described above.

RESULTS

To find possible associations of HLA-DRB1 alleles with anti-CCP-positive or anti-CCP-negative RA, we analyzed HLA-DRB1 allele frequencies and the presence of anti-CCP antibodies in 377 RA patients of the Leiden EAC. Two hundred six of the RA patients had anti-CCP antibodies and 171 were anti-CCP negative. Other patient characteristics are presented in Table 1. After we determined HLA-DRB1 allele frequencies, we calculated ORs and 95% CIs for both patient groups compared with a control group of 423 healthy individuals (see Table 4).

Table 1. Baseline characteristics of the 377 rheumatoid arthritis (RA) patients and 235 undifferentiated arthritis (UA) patients within the study.

	RA	UA
Age, mean (range) years	57 (14-92)	48 (16-88)
Female, %	66	58
IgM rheumatoid factor positive, %	55	14
Anti-CCP* antibody positive, %	55	12
Presence of erosions on radiographs of hands and feet**, %	35	18
Duration of symptoms, median (range) weeks	19 (0.6–104)	23 (0.14-104)

* CCP = cyclic citrullinated peptide

** radiological data were available for 58% of the 377 RA patients and for 51% of the 235 UA patients

Table 2. Association of HLA-DR3 alleles with anti-CCP positive or negative rheumatoid arthritis (RA) and undifferentiated arthritis (UA)

DR3	**anti-CCP+ RA N=206**		**anti-CCP- RA N=171**		**anti-CCP+ UA N=28**		**anti-CCP- UA N=207**		**Co N=423**
	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)
Genotype frequency									
DR3/DR3	0 (0)	-	5 (2.9)	3.64 (0.77-18.62)	0 (0)	-	4 (1.9)	2.3 (0.4-12.52)	4 (0.9)
DR3/x	39 (18.9)	1.02 (0.65-1.59)	49 (28.7)	1.83 (1.18-2.82)	4 (14.3)	0.73 (0.18-2.21)	55 (26.6)	1.62 (1.07-2.45)	78 (18.4)
x/x	167 (81.1)	1.0	117 (68.4)	1.0	24 (85.7)	1.0	148 (71.5)	1.0	341 (80.6)
DR3/DR3 or DR3/x	39 (18.9)	0.97 (0.62-1.51)	54 (31.6)	1.92 (1.25-2.92)	4 (14.3)	0.69 (0.17-2.10)	59 (28.5)	1.66 (1.10-2.48)	82 (19.4)
Allele frequency	39 (9.5)	0.92 (0.60-1.40)	59 (17.3)	1.84 (1.26-2.67)	4 (7.1)	0.68 (0.17-1.9)	63 (15)	1.59 (1.10-2.28)	86 (10.2)

RA= rheumatoid arthritis; UA= undifferentiated arthritis; Co=controls; CCP= cyclic citrullinated peptide; OR= odds ratio; 95% CI= 95% confidence interval.
ORs were calculated comparing double-dose DR3 carriers (DR3/DR3), single-dose DR3 carriers (DR3/x) or at least single-dose DR3 carriers with carriers of no DR3 alleles (x/x) in patients versus controls and comparing allele frequencies in all groups versus controls.

As described previously, the SE alleles DRB1*0401, *0404, *0405, and *0408 were associated with predisposition for anti-CCP-positive RA, as were DR9 and DR10. Interestingly, HLA-DR3 was associated only with predisposition for anti-CCP-negative RA (OR 1.84, 95% CI 1.26-2.67) (Table 2). This association was not found for anti-CCP-positive RA, indicating that HLA-DR3 is only associated with RA in the absence of anti-CCP antibodies.

To confirm these findings in another group of patients and to address the question of whether the association is only found in anti-CCP-negative RA or whether it is also present in another form of arthritis, we also analyzed the association of HLA-DR3 with UA. In a group of 235 patients who, 1 year after their first visit, were categorized as having

Table 3. HLA-DR3 allele frequencies in rheumatoid arthritis with and without anti-CCP antibodies and IgM-RF.

Population	HLA-DR3 allele frequency		
	N (DR3 alleles/ total alleles)	%	OR (95% CI)
RA			
anti-CCP + ; RF +	32 / 342	9.4	0.91 (0.58-1.42)
anti-CCP + ; RF -	7 / 70	10.0	0.98 (0.37-2.24)
anti-CCP – ; RF +	16 / 74	21.6	2.44 (1.25-4.53)
anti-CCP – ; RF –	43 / 268	16.0	1.69 (1.11-2.54)
Controls	86 / 846	10.2	1.0

RA= rheumatoid arthritis; CCP= cyclic citrullinated peptide; RF= rheumatoid factor; OR= odds ratio; 95% CI= 95% confidence interval

ORs were calculated comparing allele frequency of the diseased group with allele frequency in the healthy control group

UA (Table 2), HLA-DR3 was more frequently present in anti-CCP-negative patients (n = 207) than in healthy controls (OR 1.59, 95% CI 1.10-2.28), suggesting that HLA-DR3 is not specifically associated with anti-CCP-negative RA, but rather with anti-CCP-negative arthritis. No association was observed between HLA-DR3 and anti-CCP-positive UA (n = 28) (OR 0.68, 95% CI 0.17-1.92) (Table 2). Analysis of HLA-DR3 allele frequencies in UA patients thus confirmed the results found in RA patients and indicated that association with HLA-DR3 also occurs in anti-CCP-negative UA. Analysis of whether HLA-DR3 is associated with development of RA in patients who presented initially with anti-CCP-negative UA did not show that HLA-DR3 increased the risk for developing RA (data not shown), indicating that HLA-DR3 is not a prognostic risk factor for the development of RA in this group of patients.

Since the presence of anti-CCP antibodies is linked to the presence of RF and since RF is associated with the SE (12), we next compared the frequencies of HLA-DR3 in IgM-RF-positive and IgM-RF-negative RA patients with that in controls. Indeed, HLA-DR3 was also associated with RF-negative RA (OR 1.66, 95% CI 1.13-2.43). However, after dividing the study population into those with and without anti-CCP antibodies, the association was lost for the anti-CCP-positive group (Table 3). Anti-CCP-negative, RF-positive RA patients and anti-CCP-negative, RF-negative RA patients both harboured HLA-DR3 significantly more frequently than did control subjects (OR 2.44, 95% CI 1.25-4.53 and OR 1.69, 95% CI 1.11-2.54, respectively). In contrast, anti-CCP-positive, RF-positive RA patients and anti-CCP-positive, RF-negative RA patients did not (OR 0.91, 95% CI 0.58-1.42 and OR 0.98, 95% CI 0.37-2.24, respectively) (Table 3). These data indicate that anti-CCP status, rather than RF status, is the predominant disease trait associated with HLA-DR3.

Table 4. HLA-DRB1 allele frequencies in 206 anti-CCP positive RA patients, 171 anti-CCP negative RA patients and 423 healthy controls.

DRB1	anti-CCP positive RA (N=412)			anti-CCP negative RA (N=342)			Controls (N=846)	
	N	%	OR (95% CI)	N	%	OR (95% CI)	N	%
DR1								
01(00)	33	8.0		31	9.1		6	0.7
0101	28	6.8		13	3.8		92	10.9
0102	0	0		3	0.9		2	0.2
0103	0	0		1	0.3		1	0.1
Total	*61*	*14.8*	*1.28 (0.89-1.83)*	*48*	*14.0*	*1.20 (0.81-1.76)*	*101*	*11.9*
DR3								
03(00)	0	0		0	0		2	0.2
0301/ [a]	9	2.2		5	1.5		0	0
0301	30	7.3		53	15.5		84	9.9
0302/ [b]	0	0		1	0.3		0	0
Total	*39*	*9.5*	*0.92 (0.60-1.40)*	*59*	*17.3*	***1.84 (1.26-2.67)***	*86*	*10.2*
DR4								
04(00)	9	2.2		2	0.6		0	0
0401	84	20.4	**2.56 (1.80-3.63)**	33	9.6	1.07 (0.67-1.66)	77	9.1
0402	0	0		1	0.3		2	0.2
0403	2	0.5	0.34 (0.04-1.53)	2	0.6	0.41 (0.04-1.85)	4	0.5
0403/ [c]	0	0		0	0		8	0.9
0404/ [d]	0	0		0	0		18	2.1
0404	25	6.1	**3.02 (1.69-5.49)**	12	3.5	1.89 (0.89-3.94)	2	0.2
0408	7	1.7		3	0.9		3	0.4
0405	5	1.2	**10.4 (1.15-492)**	0	0	-	1	0.1
0406	1	0.2		0	0		0	0
0407	1	0.2		4	1.2		8	0.9
Total	*134*	*32.5*	***2.83 (2.12-3.79)***	*57*	*16.7*	*1.18 (0.82-1.67)*	*123*	*14.5*
DR7								
07(00)	6	1.5		5	1.5		91	10.8
0701	28	6.8		27	7.9		0	0
Total	*34*	*8.3*	*0.75 (0.48-1.14)*	*32*	*9.4*	*0.86 (0.54-1.33)*	*91*	*10.8*
DR8								
08(00)	2	0.5		2	0.6		16	1.9
0801	3	0.7		1	0.3		10	1.2
0803	0	0		0	0		1	0.1
Total	*5*	*1.2*	***0.37 (0.11-0.99)***	*3*	*0.9*	***0.27 (0.05-0.88)***	*27*	*3.2*
DR9								
09(00)	2	0.5		1	0.3		10	1.2
0901	11	2.7		5	1.5		0	0
Total	*13*	*3.2*	***2.72 (1.09-7.00)***	*6*	*1.8*	*1.49 (0.44-4.57)*	*10*	*1.2*
DR10								
10(00)	8	1.9		2	0.6		4	0.5
1001	12	2.9		1	0.3		0	0
Total	*20*	*4.9*	***10.7 (3.56-43.4)***	*3*	*0.9*	*1.86 (0.27-11.1)*	*4*	*0.5*

DRB1	anti-CCP positive RA (N=412)			anti-CCP negative RA (N=342)			Controls (N=846)	
	N	%	OR (95% CI)	N	%	OR (95% CI)	N	%
DR11								
11(00)	8	1.9		6	1.8		5	0.6
1101/ [e]	2	0.5		6	1.8		79	9.3
1101	8	1.9	**0.29 (0.14-0.55)**	6	1.8	**0.41 (0.21-0.75)**	0	0
1104	2	0.5		2	0.6		0	0
1102/ [f]	0	0	0.34 (0.01-2.82)	1	0.3	1.24 (0.20-5.84)	6	0.7
1102	1	0.2		2	0.6		0	0
Total	*21*	*5.1*	***0.45 (0.26-0.75)***	*23*	*6.7*	***0.61 (0.36-0.99)***	*90*	*10.6*
DR12								
12(00)	6	1.5		4	1.2		7	0.8
1201	1	0.2		1	0.3		16	1.9
1202	0	0		1	0.3		4	0.5
Total	*7*	*1.7*	*0.52 (0.19-1.25)*	*6*	*1.8*	*0.54 (0.18-1.36)*	*27*	*3.2*
DR13								
13(00)	0	0		3	0.9		6	0.7
1301/ [g]	2	0.5		9	2.6		116	13.7
1301	4	1.0		13	3.8		0	0
1302	13	3.2		14	4.1		1	0.1
1303	2	0.5		4	1.2		3	0.4
Total	*21*	*5.1*	***0.31 (0.18-0.50)***	*43*	*12.6*	*0.82 (0.55-1.20)*	*126*	*14.9*
DR14								
14(00)	6	1.5		9	2.6		0	0
1401	0	0		1	0.3		27	3.2
1404	1	0.2		0	0		0	0
Total	*7*	*1.7*	*0.52 (0.19-1.25)*	*10*	*2.9*	*0.91 (0.39-1.97)*	*27*	*3.2*
DR15								
15(00)	33	8.0		33	9.6		128	15.1
1501	16	3.9		17	5.0		1	0.1
Total	*49*	*11.9*	*0.75 (0.52-1.08)*	*50*	*14.6*	*0.95 (0.65-1.37)*	*129*	*15.2*
DR16								
16(00)	1	0.2		2	0.6		5	0.6
Total	*1*	*0.2*	*0.41 (0.01-3.68)*	*2*	*0.6*	*0.99 (0.09-6.08)*	*5*	*0.6*
Total alleles	412	100		342	100		846	100

RA= rheumatoid arthritis; UA= undifferentiated arthritis; CCP= cyclic citrullinated peptide; OR= odds ratio; 95% CI= 95% confidence interval.

ORs were calculated comparing allele frequencies of the diseased group with allele frequencies in the healthy control group. HLA typings without subtyping are presented as x(00). Subtypings without conclusive result are presented as

[a] 0301/ for subtyping HLA-DRB1*0301, *0304 or *0305; [b] 0302/ for subtyping HLA-DRB1 *0302 or *0303 or *0307; [c] 0403/ for subtyping HLA-DRB1*0403, *0406 or *0407; [d] 0404/ for subtyping HLA-DRB1*0404, *0408 or *0419; [e] 1101/ for subtyping HLA-DRB1*1101 or *1104; [f] 1102/ for subtyping HLA-DRB1*1102 or *1103; [g] 1301/ for subtyping HLA-DRB1*1301 or *1302.

DISCUSSION

The data presented herein show that distinct genetic risk factors are associated with distinct subtypes of RA as defined by the presence of anti-CCP antibodies. SE-encoding alleles are associated with anti-CCP-positive RA and not with anti-CCP-negative disease. In contrast, anti-CCP-negative disease is associated with HLA-DR3, while this association is not found in anti-CCP-positive RA. Although HLA-DR3 or SE expression is not required for the development of anti-CCP-negative or anti-CCP-positive RA, respectively, our findings are important because they indicate that distinct pathogenic mechanisms may underlie anti-CCP-positive and anti-CCP-negative RA.

In a previous study of a relatively small number of patients (n = 44), it was found that HLA-DR3 frequencies in RA patients differ from the frequencies observed in controls (13). Likewise, in a group of 85 Arab RA patients, HLA-DR3 conferred a risk for RA susceptibility (14), and a more recent study showed a significantly increased frequency of HLA-DR3 in patients with synovitis of recent onset (15). In a Caucasian population of 167 RA patients, an association with HLA-DR3 was observed after excluding SE alleles from the analysis (16). Our findings represent an extension of those findings by establishing that HLA-DR3 is associated only with a particular subset of RA. Our analysis also confirmed that particular HLA-DRB1 alleles, such as DR8, DR11, and DR13, protect against RA (17). We now show that these alleles tend to be associated with protection against both anti-CCP-positive and anti-CCP-negative disease. Therefore, our data indicate that the HLA alleles conferring protection do so independently of the anti-CCP status, while the alleles that predispose to RA are associated with distinct RA phenotypes (anti-CCP-positive or anti-CCP-negative RA).

It is debatable, however, whether the association of HLA-DR3 with anti-CCP-negative RA (and UA) is attributable to the HLA-DR3 gene itself. Other genetic factors in high linkage disequilibrium with HLA-DR3 may also underlie the observed association. HLA-DR3 is known to be part of a conserved ancestral haplotype (A1;B8;DRB1*03, also known as the 8.1 haplotype (18) that occurs frequently in Caucasian individuals and has been reported to be associated with RA (19). Jawaheer et al described an additional genetic risk factor present within the major histocompatibility complex (MHC) that is part of this conserved haplotype (20). Their finding concerned a certain allelic combination of tumor necrosis factor (TNF) polymorphisms and another polymorphism on the HLA class III region of chromosome 6. Likewise, the class III MHC TNF-lymphotoxin region was described as appearing to influence susceptibility to RA separately from the HLA-DR region (21), and a microsatellite marker (MIB*350) that is also part of an ancestral haplotype associated with DRB1*0301 was described as a risk factor for RA independently of DRB1*0301 (22).

In summary, HLA-DR3 is associated with anti-CCP-negative RA and UA and not with anti-CCP-positive RA or UA. The data presented herein indicate that separate genetic risk

factors are associated with different phenotypes, which suggests that various pathogenetic mechanisms underlie anti-CCP-positive and anti-CCP-negative disease.

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