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

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Citation
Helm-van Mil, A. H. M. van der. (2006, October 26). *Genetics, autoantibodies and clinical features in understanding and predicting rheumatoid arthritis*. Retrieved from https://hdl.handle.net/1887/4929

Version:	Corrected Publisher's Version
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Downloaded from:	https://hdl.handle.net/1887/4929

Note: To cite this publication please use the final published version (if applicable).

Chapter 9

Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients that carry HLA-DRB1 shared epitope alleles

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Ann Rheum Dis 2006;65(3):366-71.

ABSTRACT

Objectives. To study the gene-environment interaction between tobacco-exposure (TE) and shared-epitope (SE) alleles on autoantibodies in rheumatoid arthritis (RA) and undifferentiated arthritis (UA).

Methods. From incident cases of arthritis (n=1305), patients that did not fulfill any classification criteria at the two weeks visit, UA (n=486), as well as patients that fulfilled the ACR criteria for RA (n=407) were identified. IgM Rheumatoid Factor (RF), anti-cyclic-citrullinated peptide (CCP) antibodies and HLA-DRB1 alleles were determined.

Results. In RA an interaction was found between TE and SE for the presence of anti-CCP antibodies, as the odds ratio (OR) for anti-CCP antibodies of patients having both TE and SE was higher than the summed ORs of patients having only TE or SE (OR TE+SE- 1.07, TE-SE+ 2.49, and TE+SE+ 5.27, all relative to TE-SE-). A similar effect was found for RF, but stratification revealed that the interaction primarily associates with the anti-CCP antibody response. In patients with UA at two weeks or with persistent UA after one year no interaction between TE and SE was observed for the presence of autoantibodies.

Conclusions. TE increases the risk factor for anti-CCP antibodies only in SE-positive RA-patients. The gene-environment interaction between smoking and SE leading to autoantibodies is specific for RA and is not observed in UA.

INTRODUCTION

In the search for the etiology of Rheumatoid arthritis (RA) genetic predisposition, environmental- and dietary risk factors may present clues for pathogenesis (1-7). The most important genetic risk factor for the development of RA is the presence of HLA Class II alleles that share the conserved amino acid sequence called the Shared Epitope (SE) (8). These SE-residues constitute a part 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. The most prominent environmental risk factor for RA is smoking; smokers have increased levels of Rheumatoid Factor (RF) (9-11), are more prone to develop RA (11-14) and develop more severe disease (15-17). Interaction between environmental and genetic risk factors points to the existence of disease-specific pathogenic pathways involved in disease induction or progression.

For RA Padyukov et al recently described a gene-environment-interaction between smoking and SE that provides risk (OR 2.8; 95% CI 1.6-4.8) for RF-positive but not RF-negative RA in a large cohort of 858 RF-positive and 1048 RF-negative patients with RA (18). Recently, we identified in two large cohorts from both the USA and from Europe by different genetic-epidemiological methods (association and linkage) that HLA-DRB1 alleles are only a risk factor for RA patients that have anti-CCP antibodies and not in the absence of anti-CCP antibodies, suggesting different pathogenic pathways for anti-CCP positive and negative RA (19).

This study investigates whether the gene-environment interaction smoking-shared epitope is also present for the anti-CCP antibody response and whether this interaction was more pronounced for the development of RF compared to the development of anti-CCP antibodies. Secondly, this study aimed to assess whether the interaction smoking and SE is specific for patients with RA or is also present in undifferentiated arthritis (UA). To this end patients with arthritis that did not fulfill any classification criteria at presentation and patients with persistent UA at one-year follow-up were used. These patients have a spontaneous remission rate of about 50% (20) and might have differences in the underlying pathogenesis. If the interaction between smoking and SE that leads to autoantibody formation is specific for the pathogenesis of RA, it is hypothesized that such an interaction will not be seen in patients with an undifferentiated arthritis of which clinical follow-up has learned that these patients have not developed RA.

PATIENTS AND METHODS

Patients

For this study, the Leiden Early Arthritis Clinic (EAC), a population-based inception cohort of patients with newly diagnosed early arthritis was used (for further reading see (21)). RA was diagnosed according to the American College of Rheumatology (ACR) criteria of 1987(22). Patients who could not be properly classified according to one of the ACR-criteria at 2 weeks follow-up were categorized as having UA (20). This population of UA patients was further divided in patients that (1) had developed RA, (2) remained unclassified (persistent UA) or (3) had developed other rheumatic diseases such as spondylarthropathies, osteoarthritis, gout, reactive arthritis at one year follow-up (Figure 1).

At inclusion in the EAC-cohort, for each patient the smoking status (cigarettes, cigars) was registered as past, current or never smokers. Current and past smokers were classified as tobacco exposure positive (TE+) and never smokers as tobacco exposure negative (TE-)(11). Baseline laboratory parameters included C-reactive protein (CRP), IgM rheumatoid factor (ELISA as previously described 23), anti-CCP antibodies (ELISA, Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands and Axis-Shield, Dundee, UK) and HLA-Class II alleles. The HLA-DRB1 (sub-)typing was performed by polymerase chain reaction using specific primers and hybridisation with sequence-specific oligonucleotides. Shared epitope alleles were: DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *0410 and *1001 (24). Patients homozygous and heterozygous for shared epitope were both classified as SE positive. Missing data for the whole cohort of 1305 patients ranged for several items

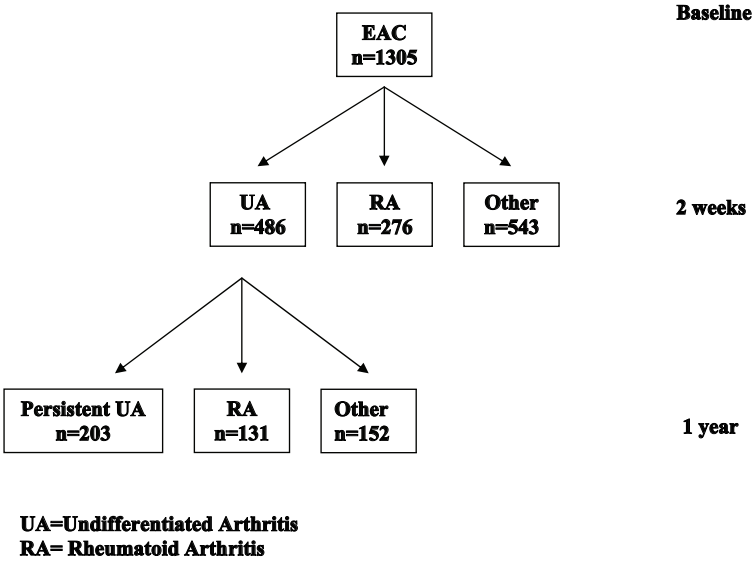

Figure 1. **Flow chart early arthritis patients**.

SE, TE anti-CCP and RF between 0%-20%. An analysis of the baseline values of patients with missing data-points revealed no differences to those patients without.

Statistical analysis

Odds ratios were calculated for the primary outcome measures RF and anti-CCP antibodies. Stratified analysis was performed for both anti-CCP-positive and anti-CCP negative as well as RF-positive and RF-negative strata. Chi-square analysis was performed on 2 by 4 tables.

RESULTS

Patient characteristics

Between 1993 and 2003, 1305 patients were included in the EAC-cohort. At two weeks follow-up, 486 patients did not fulfill any classification criteria and were thus classified as UA. At this time 276 patients fulfilled the ACR criteria for RA. Of the 486 UA patients, after one year follow-up 131 patients were diagnosed as having RA. In 203 patients the diagnosis remained UA (persistent UA) and in the other 152 patients another rheumatic disorder such as spondylarthropathy, osteoarthritis, psoriatic arthritis, etc was identified (Figure 1). The total number of patients from the cohort that was identified as having RA at one year was 407. From the patients classified as persistent UA at one year, 15% developed RA during further follow-up (K.Verpoort, unpublished data).

Baseline patient characteristics of patients that at two weeks presented with RA or UA are given in Table 1; the data on the UA patients are presented for both the UA patients

Table 1. Patient characteristics at baseline of patients that presented with RA, patients that presented with UA and developed RA after 1 year and patients that presented with UA and had other diagnoses than RA after one year follow-up.

	RA at 2 weeks (n=276)	UA→RA (n=131)	UA→non RA (n=355)	p*
Age yr (mean)	58	56	48	<0.001
Female (%)	66	64	51	n.s.
CRP (mg/l, mean)	35	29	21	0.036
IgM RF + (%)	65	52	13	<0.001
Anti-CCP + (%)	54	51	7	< 0.01
Shared Epitope + (%)	68	63	49	0.046
Tobacco exposure + (%)	47	52	50	n.s.

Shared Epitope + means presence of 1 or 2 shared epitope alleles.
Tobacco exposure + means current and past smokers as indicated in the medical history.
*P values were determined for UA→RA versus UA→non RA.
Comparison of RA at 2 weeks versus UA→RA revealed no significant differences.

that developed RA, as the UA patients that had persistent UA after one-year follow-up. UA patients that did not develop RA were younger at presentation than the patients that developed RA (mean 48 years, vs. 56 years, p<0.001). The UA patients that developed RA after one year had at baseline higher levels of CRP, RF and anti-CCP antibodies and were more often SE-positive compared to the UA-patients that had persistent UA or developed other rheumatologic diagnosis (Table 1). No differences were observed between the 131 RA patients that developed RA after 1 year and the 276 patients with RA that were diagnosed at the two weeks visit.

Interaction tobacco exposure and shared epitope in RA

In RA and UA patients the interaction between SE and TE was analyzed. Outcome parameters were RF and anti-CCP antibodies.

In all RA patients, no effect of TE on the RF status was seen in SE negative patients in contrast to a clear effect in SE positive patients. The OR for positive RF was 1.47 for TE+ SE- patients, 1.35 for TE- SE+ patients and 3.23 for TE+SE+ patients all relative to TE-SE- patients (Table 2), showing an interaction between TE and SE for the development of RF.

In SE negative RA patients, no effect of TE was seen for positive anti-CCP antibodies in contrast to a clear effect in the SE positive group. The OR for positive anti-CCP antibodies was 1.07 for TE+ SE- patients, 2.49 for TE+ SE- patients and 5.27 for TE+SE+ patients, again all relative to TE-SE- patients. As the odds ratio for anti-CCP antibodies of patients having both TE and SE was higher than the summed OR's of patients having only TE or SE, an interaction was found between TE and SE for the presence of anti-CCP antibodies. The difference between the TE- SE+ patients and the TE+ SE+ patients was significant both for IgM-RF and for anti-CCP (Table 2).

Presuming that RF-positive and anti-CCP positive patients partly overlap each other, a stratified analysis was done for both RF-positive and RF-negative and anti-CCP positive and negative patients. The results, shown in Table 2, demonstrate that when stratified for the presence/absence of anti-CCP antibodies, no significant interaction is found between TE and SE in relation to the presence of RF. When stratified for RF, in the RF negative group an interaction between TE and SE was observed for the development of anti-CCP antibodies. These data suggest that the interaction between TE and SE primarily associates with positive anti-CCP antibodies and not with positive RF.

Interaction tobacco exposure and shared epitope in UA

In the whole group of patients who presented with UA, the combination of TE and SE did not significantly increase the risk for the presence of positive RF or anti-CCP antibodies (Table 3). The OR for positive anti-CCP antibodies in TE- SE+ patients with UA was increased compared to TE-SE- (OR 3.83: 95% CI 1.33-12.53), but addition of TE to the

Table 2. Odds ratios for developing RF and anti-CCP antibodies in the presence of Tobacco Exposure (TE) and/or shared epitope alleles (SE) in all Rheumatoid Arthritis patients at 1 year.

	TE	SE	RF +	RF-	OR	95% CI	p
	–	–	23	31	1.00	-	-
	+	–	25	23	1.47	0.62-3.45	0.33
	–	+	54	54	1.35	0.66-2.75	0.37 *
	+	+	72	30	3.23	1.54-6.81	<0.001 *
							0.002 π
	TE	**SE**	**anti-CCP +**	**anti-CCP -**	**OR**		**p**
	–	–	18	34	1.00	-	-
	+	–	17	30	1.07	0.43-2.65	0.87
	–	+	58	44	2.49	1.18-5.31	0.01 #
	+	+	67	24	5.27	2.37-11.80	<0.001 #
							<0.001 π
anti-CCP	**TE**	**SE**	**RF +**	**RF-**	**OR**	**95% CI**	**p**
+	–	–	14	4	1.00	-	-
+	+	–	17	0	∞	∞	0.10
+	–	+	46	12	1.10	0.22-4.42	0.88 ¶
+	+	+	54	13	1.19	0.24-4.68	0.79 ¶
							0.23
–	–	–	7	27	1.00	-	-
–	+	–	8	22	1.40	0.38-5.32	0.57
–	–	+	6	38	0.61	0.12-2.40	0.41 ¶
–	+	+	7	17	1.59	0.39-6.34	0.45 ¶
							0.39 π
RF	**TE**	**SE**	**anti-CCP +**	**anti-CCP -**	**OR**	**95% CI**	**p**
+	–	–	14	7	1.00	-	-
+	+	–	17	8	1.06	0.26-4.34	0.92
+	–	+	46	6	3.83	0.91-16.07	0.03 ¶
+	+	+	54	7	3.86	0.96-15.11	0.02 ¶
							0.02 π
–	–	–	4	27	1.00	-	-
–	+	–	1	22	0.31	0.01-3.47	0.28
–	–	+	12	38	2.13	0.56-9.97	0.22 ¶
–	+	+	13	17	5.16	1.28-24.71	0.01 ¶
							0.04 π

* TE-SE+ versus TE+SE+: OR 2.4 (95%CI 1.3-4.4, p=0.002)
# TE-SE+ versus TE+SE+: OR 2.1 (95%CI 1.1-4.1, p=0.02)
¶ Comparison TE-SE+ versus TE+SE+ not significant
π P-value of Chi-square analysis of 2 by 4 table

Table 3. Odds ratios for developing RF and anti-CCP antibodies in the presence of Tobacco Exposure (TE) and/or shared epitope alleles (SE) in patients with Undifferentiated Arthritis (UA).

3A. All UA patients at two weeks

TE	SE	IgM RF +	IgM RF –	OR	95% CI	p
–	–	12	55	1.00	-	-
+	–	13	48	1.24	0.47-3.29	0.63
–	+	19	60	1.45	4.60-3.59	0.34 ¶
+	+	18	54	1.53	0.62-3.82	0.31 ¶
						0.75 π
TE	**SE**	**anti-CCP +**	**anti-CCP –**	**OR**	**95% CI**	**p**
–	–	6	54	1.00	-	-
+	–	9	48	1.69	0.49-6.19	0.35
–	+	20	47	3.83	1.33-12.53	0.01 ¶
+	+	21	47	4.02	1.40-13.09	0.01 ¶
						<0.01 π

3B. Subgroup of UA patients who develop RA within one year

TE	SE	IgM RF +	IgM RF –	OR	95% CI	p
–	–	6	12	1.00		
+	–	8	14	1.14	0.26-5.24	0.84
–	+	15	22	1.36	0.37-5.44	0.61 ¶
+	+	19	13	2.92	0.76-11.90	0.08 ¶
						0.87 π
TE	**SE**	**anti-CCP +**	**anti-CCP –**	**OR**	**95% CI**	**p**
–	–	8	10	1.00		
+	–	8	13	0.77	0.18-3.33	0.69
–	+	19	16	1.48	0.41-5.46	0.50 ¶
+	+	21	9	2.92	0.74-11.66	0.08 ¶
						0.12 π

3C. Subgroup of persistent UA patients at one year

TE	SE	IgM RF +	IgM RF –	OR	95% CI	p
–	–	6	37	1.00		
+	–	5	32	0.96	0.21-4.20	0.95
–	+	9	34	1.63	0.46-6.17	0.39 ¶
+	+	4	33	0.75	0.14-3.48	0.67 ¶
						0.62 π
TE	**SE**	**anti-CCP +**	**anti-CCP –**	**OR**	**95% CI**	**p**
–	–	1	36	1.00		
+	–	2	34	2.12	0.10-128	0.54
–	+	8	27	10.67	1.26-486	0.01 ¶
+	+	4	32	4.50	0.41-227	0.16 ¶
						0.03 π

¶ Comparison TE-SE+ versus TE+SE+ not significant

π P-value of Chi-square analysis of 2 by 4 table

presence of SE did not significantly increase the risk of having anti-CCP antibodies (OR 4.02 relative to OR of 3.83, table 3A).

Subsequently, we assessed whether in the subgroup UA patients that developed RA (n=131) smoking combined with presence of SE increased the risk of having anti-CCP antibodies. Therefore the non-smoking SE negative UA patients that developed RA were compared to smoking SE negative UA patients that developed RA patients (OR 0.77), as well as to non-smoking SE-positive patients with UA that developed RA (OR 1.48) and finally to smoking, SE-positive patients with UA that developed RA (OR 2.92). In this smaller group of RA patients a trend for the interaction of SE and TE to increase the risk of having anti-CCP antibodies was observed. Calculations for outcome RF showed similar results (Table 3B).

The same calculations were repeated for patients with persistent UA (n=203). Although the number of anti-CCP positive UA patients is low, no effect of tobacco exposure combined with SE on the risk of having anti-CCP antibodies or RF was observed (Table 3C). Thus, the interaction of SE and tobacco exposure was found for the presence of RF and for anti-CCP antibodies in patients with RA and in those UA patients that develop RA within one year but not in patients with persistent UA.

DISCUSSION

A strong gene-environment interaction between TE and SE for the presence of autoantibodies was observed. Intriguingly, this gene-environmental interaction was only present in patients with RA and not observed in patients with (persistent) UA. Stratified analysis for the different autoantibody responses IgM RF and anti-CCP showed that the interaction is primarily for the anti-CCP response.

A recent Swedish study demonstrated that a gene-environment interaction between SE and smoking results in an elevated risk specifically for RF-positive RA (18). These data were replicated as well as extended in the present study. Replication by a separate group in a separate cohort minimizes the risk that the current findings are false positive (25). Both the Swedish and the current data demonstrate the lack of a relation between smoking and autoantibodies in SE-negative RA, indicating that this interaction is preferential for a given pathogenetic pathway in SE-positive RA. To specify this pathogenetic pathway with regard to the specificity of the autoantibody response, the stratified analysis for anti-CCP positivity yielded no additional effect of smoking on risk to develop RF. In contrast the stratified analysis for RF indicated that smoking more than doubled the risk in the SE-positive patients to develop anti-CCP antibodies. These data suggest that the gene-environment interaction between smoking and SE leading to autoantibodies is primarily

associated with the anti-CCP response. Apart from the specificity of this gene-environment interaction, our group has recently described that SE is only a risk factor for anti-CCP positive RA and not for anti-CCP negative RA (19). The current data do not allow the analysis of smoking as a risk factor for anti-CCP positive RA versus anti-CCP negative RA because no data of smoking in a matched group of the general population are known. However given the fact that SE alone is not a risk factor for anti-CCP negative RA (19), the gene-environment interaction between smoking and SE leading to anti-CCP antibodies seems characteristic for anti-CCP positive RA. Indeed no effect of smoking was observed in the SE-negative patients (see Table 2). These data are in line with our previously reported hypothesis that different pathogenetic pathways operate in anti-CCP negative RA as compared to anti-CCP positive RA. The demonstration of these pathogenetic pathways is difficult because it is not known which proteins are citrinullated as a result of smoking, nor is it known if or how smoking breaks normal tolerance to citrinullated self-proteins.

In this study the diagnosis persistent RA was defined as the presence of arthritis that did not fulfill any of the classification criteria after one-year follow-up. This may lead to some misclassification because a small proportion of UA patients only develop RA after a longer time period. However, in previous analysis of this cohort this concerned only less than 15% of the patients with persistent UA at one year. More importantly, no interaction between smoking and shared epitope was observed at all in the persistent UA group.

A weakness of the current study is that the information on TE is limited to patient history taking and patients were not asked about the number of pack-years smoking. Therefore no conclusion on the minimal exposure can be drawn.

In summary, smoking was confirmed to be a risk factor for anti-CCP antibodies in the presence of shared epitope alleles in patients with RA. In UA no interaction between TE and SE was demonstrated for the presence of autoantibodies.

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