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Gene-environment interaction between DRB1 shared epitope and smoking regarding risk of ACPA-positive rheumatoid arthritis - all alleles are important

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Abstract

Objective—An interaction effect for developing Rheumatoid Arthritis (RA) was previously observed between HLA-DRB1 shared epitope (SE) alleles and smoking. We aimed to further investigate this interaction between distinct SE alleles and smoking regarding risk of developing RA with and without antibodies toward citrullinated protein antigens (ACPA).

Methods—Data regarding smoking habits and HLA-DRB1 genotypes from 1319 patients and 943 controls from the EIRA study were utilized where 972 patients and 488 controls were SE positive. Subsequently, 759 cases and 328 controls were subtyped for specific alleles within the *04 group. The odds ratios (OR) with 95% confidence interval (95% CI) were calculated by means of logistic regression. Interaction was evaluated by calculating attributable proportion due to interaction (AP) with 95% CI.

Results—A strong interaction between smoking and SE alleles in development of ACPA-positive RA was observed regarding both all DRB1*04 SE alleles taken as a group (RR: 8.7 95% CI: 5.7-13.1) or the *0401 and*0404 alleles (RR: 8.9 95% CI: 5.8-13.5) and the *01 and *10 alleles as specific, separate groups (RR: 4.9 95% CI: 3.0-7.8) with similar strength of interaction for the different groups AP: 0.4 (0.2-0.6), 0.5 (0.3-0.7) and 0.6 (0.4-0.8), respectively.

Conclusion—A statistically significant interaction is evident between distinct DRB1 SE alleles and smoking in development of ACPA-positive RA. Interaction is present in the *04 group as well as in the *01/*10 group, demonstrating that regardless of fine specificity all SE alleles strongly interact with smoking in providing an increased risk for ACPA-positive RA.

Recent progress in genetic studies of Rheumatoid Arthritis (RA) has revealed several new loci as risk factors for disease development (1-7). However, all newly found variations outside the HLA locus provide only limited, although statistically significant, increased risk for RA. The strongest association with ACPA (antibodies to citrullinated protein) -positive RA was repeatedly reported for the HLA-DRB1 gene and it is evident that this genetic locus plays a central role in susceptibility to disease in different Caucasian populations. RA is a complex disease with many different factors involved and it is rational to discern which of the combinations of these factors results in the most aggressive form of the disease. Our own and other previous reports demonstrated an unexpected high increase in risk associated with

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exposure to smoking in the presence of shared epitope alleles of the HLA-DRB1 gene, with regard to susceptibility to ACPA-positive and/or rheumatoid factor (RF) positive RA, which we considered as strong evidence for an interaction (8-16).

According to the current state of knowledge, the association between the HLA-DRB1 variations and susceptibility to ACPA-positive RA is related to more than one allele (*0101, *0401, *0404, *0405, *0408, *1001, *1402). These alleles share a common amino acid sequence ($Q/R^{70}K/RRAA^{74}$) in the third hypervariable region of the DRB1 molecule and have therefore been denoted the 'shared epitope' (SE) (17-20). The SE residues constitute a part of the antigen-binding site forming the fourth anchoring pocket 4 (P4) in the HLA groove. The epitope motif hypothetically serves as a binding site for arthritogenic peptides allowing presentation to $CD4^+$ T cells and generation of T cell autoimmune responses and may possibly induce certain B cells to differentiate into plasma cells, duly leading to the production of ACPA (15).

ACPA occur in approximately 60% of RA patients, 2% of healthy populations and is rather rare in patients with other inflammatory diseases (15,21). The occurrence of ACPA is observed several years before onset of disease (22) and is closely linked to the presence of SE alleles. More specifically, the association between SE and RA, which is the strongest genetic risk factor for disease, is exclusively observed within the ACPA-positive patient subset (8,9,15).

Several environmental factors have been described with ambiguous results, predisposing or protecting against development of RA (16,23-27). However, the main environmental risk factor for RA detected to date is smoking (8,13). A strong gene-environment interaction between tobacco exposure and SE for the ACPA-positive subset has been repeatedly demonstrated in several studies within Europe (8,10-13), whereas neither smoking nor SE confers an increased risk of ACPA-negative RA. However, when replication of the demonstrated gene-environment interaction was assessed in three North American cohorts by Lee *et al* (28), evidence of a gene-environment interaction between smoking and SE alleles for ACPA formation could only be observed in one of these. This discrepancy could possibly be explained by different recruitment procedures of controls and patients, diverse methodologies for evaluation of smoking and due to the existence of different sorts of environmental exposure. In a recent study, van der Helm-van Mil *et al* (8) performed gene-environment analyses stratifying for the *01, *04 and *10 groups in an investigation of 421 patients using ACPA-negative patients as controls. Interestingly, through using a multiplicative model they observed an interaction between tobacco exposure and DRB1*01 and *10 in ACPA-positive RA, but no interaction was evident for the *04 alleles.

Herein we employ a large population-based case-control study to scrutinize the gene-environment interaction between smoking and SE alleles in RA. The goal of our investigation was to ascertain whether all HLA-DRB1 SE alleles (HLA-DRB1*01, HLA-DRB1*04, HLA-DRB1*10) present a similar interaction effect or if the interaction is restricted to a particular DRB1 SE group. In addition, we assessed the relevance of studying the different subtypes of *04. More specifically we focused on *0401, *0404, *0405 and *0408, i.e. the 'true' SE alleles in the *04 group. Hence in this population-based study we report findings from analyzing *01, *10 and *04 separately as well as subtypes of *04, in the context of smoking and ACPA status in RA.

Patients & Methods

Study population

This work derives from a population-based case-control study entitled the Epidemiological Investigation of Rheumatoid Arthritis (EIRA). Individuals for whom information regarding smoking habits, ACPA status and SE data were available were included in the study (1319 cases and 943 controls) and all were of Caucasian ethnicity. The current report is based on incident cases of RA from different parts of Sweden recruited between May 1996 and December 2005. A person who fulfilled the American College of Rheumatology (ACR) 1987 criteria and who had never had a previous RA diagnosis was defined as a case. For each potential case a control subject was randomly selected from the Swedish national population registry, taking into consideration the subject's age, sex and residential area. The EIRA study design, including identification of patients and controls, data collection and definition of smoking habits has been described elsewhere (29). The ethics committee of Karolinska Institutet approved the study. Distribution of age, gender and ACPA status is depicted in Table 1.

Definition of smoking status

Cases and controls were classified into ‘ever smokers’ or ‘never smokers’ according to their reported smoking habits. Briefly, subjects who reported that they regularly smoked cigarettes during or before the year they were included in the EIRA study were defined as *ever smokers* and those who reported that they had never smoked tobacco before or during the inclusion year were defined as *never smokers*.

Genetic analysis

2-digit and 4-digit HLA-DRB1 typing was conducted using sequence-specific primer polymerase chain reaction (SSP-PCR) (DR low-resolution kit (2-digit); DRB1*04 subtyping kit (4-digit), Olerup SSP, Saltsjöbaden, Sweden) and the PCR products were loaded into 2% agarose gels. An interpretation table was used to determine the specific genotype according to the manufacturers' instructions.

Statistical analyses

We calculated odds ratios for RA associated with combinations of different SE alleles and smoking together with 95% confidence intervals (CI) using unconditional logistic regression models. As a first step we analyzed the DRB1*04 SE alleles, i.e. the combination of any of the subtypes *0401, *0404, *0405 and*0408 as a risk factor. In the following analyses we excluded the more rare *0405 and *0408 subtypes and only considered the *0401 allele and/or the *0404 allele as risk factors. Finally, we studied the interaction between HLA-DRB1*01 and/or *10 and smoking, since there were too few carriers of HLA-DRB1*10 to make analyses with sufficient power. When the effect of different HLA-DRB1 SE alleles was estimated, individuals without HLA-DRB1 SE alleles of any type were used as a reference group.

Odds Ratios were interpreted as relative risks (RRs) due to the recruitment procedure. Interaction, defined by departure from additivity of effects as described by Rothman and others (by many investigators termed “biologic interaction”) (30-32), was evaluated between different HLA-DRB1 SE alleles and smoking by calculating the attributable proportion due to interaction (AP) together with a 95% CI (33). All analyses were made using the software package SAS 9.1.3.

Results

Interaction between smoking and HLA-DRB1*04 alleles in ACPA-positive RA

Due to considerable difference in the frequency of different SE alleles, even some analyses for interaction in a relatively large study will lack power. For this reason we first tested for interaction in individuals having any of the SE alleles from the DRB1*04 group. As evident from Table 2 we observed a strong interaction between smoking and the SE allele group *0401, *0404, *0405 or *0408 in individuals with at least one of these alleles with regard to risk of developing ACPA-positive RA. The interaction between HLA-DRB1*04 and smoking in ACPA-positive disease remained when the analysis was limited to only the *0401 and *0404 alleles (Table 3). The relative risk of disease associated with combinations of *0401/*0404 alleles and smoking was very high (Ever smoker, single SE, RR: 6.6 95% CI: 4.3-10.2; Ever smoker, double SE, RR: 39.6 95% CI: 18.6-84.4) as illustrated in Fig 1. The attributable proportion due to interaction between smoking and a single *0401 or *0404 allele was 0.4 (95% CI 0.2-0.6), and between smoking and double alleles was 0.8 (0.6-0.98), demonstrating a significant gene-environment interaction with involvement of the DRB1*04 group of alleles.

Interaction between smoking and HLA-DRB1*01 and *10 alleles in ACPA-positive RA

To test for interaction between smoking and non-DRB1*04 SE alleles we performed additional analyses using non-smoking, non-SE positive individuals as a reference group. In addition, due to the design of the HLA typing assay, we were able to perform discrimination of the DRB1*0103 allele within the *01 group, which was considered to be a non-SE allele. As is apparent from our data, the SE alleles HLA-DRB1*01 and *10 exhibited interaction with smoking in ACPA-positive RA (Table 4).

Interaction between smoking and HLA-DRB1 SE alleles in ACPA-negative RA

Our data confirmed previous findings of no increased risk for smokers regarding development of ACPA-negative RA (Table 2, 4). No single group of the SE alleles (DRB1*01, *04 or *10) displayed either independent risk or interaction with smoking regarding risk of developing ACPA-negative RA.

Discussion

Our data illustrates that regardless of the fine specificity of the SE alleles of DRB1, the interaction between these genetic risk factors and smoking is evident. When comparing the RR between ever smokers and carriers of any SE (i.e. single or double SE) for the DRB1*01/10 group (RR: 4.9 95% CI: 3.0-7.8) and the DRB1*04 group (RR: 8.7 95% CI: 5.7-13.1), respectively, the latter group has a higher RR, although this difference is not statistically significant.

This study was designed to address whether an interaction between smoking and SE alleles, as previously observed, is present for all HLA-DRB1 SE alleles (HLA-DRB1*01, HLA-DRB1*04, HLA-DRB1*10) or if it is restricted to any particular DRB1 group (34-36). In a first step we separately analyzed two groups of SE alleles DRB1*01; *10 and DRB1*04 (instead of only one combined group, which previously also included few individuals with unidentified DRB1*04 non-SE alleles (*0402 and *0403)). We subsequently specifically focused on *0401 and *0404 alleles which are the most common alleles within the DRB1*04 group.

The observed independent effects of smoking and SE alleles in our study are in concordance with previously published reports in that the results are consistent with the finding that

smoking and SE is primarily associated with ACPA-positive RA (10-13). However, conclusions from another study in which ACPA-positive RA cases were compared with ACPA-negative (without healthy controls), were somewhere different and interaction of DRB1*04 alleles with smoking was not determined (8). This discrepancy may be due to differences in study design and the ways of assessing interaction. Our study is based on a case-control cohort of relatively large size and we believe that it might represent a better estimate of independent, as well as combined influences from genetic and environmental risk factors.

As an attempt to measure the interaction between smoking and SE alleles we used the attributable proportion due to interaction (AP) and demonstrated significant gene-environment interactions for both single and double SE alleles in ACPA-positive disease. Interestingly, we observed an increased relative risk for any of the SE allele groups, DRB1*01;*10 and *04. However, the relative risk was highest for carriers of double DRB1*0401 and/or *0404 alleles (RR: 39.6 95% CI: 18.6-84.4 AP: 0.8 95% CI: 0.6 - 0.98). It was previously reported that the DRB1*01 allelic group provides less risk for developing ACPA-positive RA in comparison with the DRB1*04 allelic group, as also observed in our study (Tables 2-4 and Fig 1). Although the different SE alleles are associated with different magnitudes of increased risk of ACPA-positive RA, their interaction with smoking seems to be similar according to the magnitude of the AP values (Tables 2-4).

The molecular mechanisms underlying the observed risk and interaction concerning smoking and SE alleles are still unknown, but some speculations have been published. One hypothesis is that long-term exposure to cigarette smoke may induce mechanisms that accelerate deimination of arginine to citrulline in autoantigens present in the lungs. Since citrullination increases the binding of modified peptides to antigens containing the SE motif and thereby enhancing the immunogenicity of the proteins, a break of tolerance towards citrullinated proteins might be induced in those individuals carrying the SE alleles (15,37). Another possibility concerns the presence of substances in smoke which might act as adjuvants, triggering the innate immune system to contribute to development of arthritis, similar to what has been reported in animal models of adjuvant-induced arthritis (38,39). However, the possibility still remains that the HLA-DRB1 gene involvement in the gene-environment interaction is not indigenous but rather depends on another genetic factor in linkage disequilibrium (LD) in this locus, such as variations in HLA-DQ (40,41). Finally, we cannot exclude the possibility of a pure genetic interaction between the HLA-DRB1 gene and the putative gene involved in the behavioral trait, which includes smoking.

By taking advantage of using a large population-based case control study of RA, we also re-investigated the possibility of an independent effect of SE alleles or of an interaction between SE alleles and smoking in the development of ACPA-negative RA. We conclude that the SE alleles do not seem to confer an increased risk of ACPA-negative RA, neither on their own or in combination with smoking.

In conclusion, we demonstrated that regardless of fine specificity, all SE DRB1 alleles strongly interact with smoking in the development of ACPA-positive RA.

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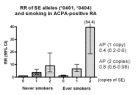

Fig 1.
Relative risk and 95% CI in smokers and non-smokers having zero, one or two copies of the SE alleles HLA-DRB1*0401 and *0404.

Table 1

Baseline characteristics

	Controls (943)	Cases (1319)
Age (mean ± SD)	52.1±11.8	51.1±12.4
Women (%)	72.4	71.5
ACPA-positive (%)	1.9	60.7

Table 2

Interaction between smoking and DRB1*04 alleles (*0401, *0404, *0405, *0408)

	ACPA positive				ACPA negative		
				No SE			
	ca/co	**RR**	**95% CI**		**ca/co**	**RR**	**95% CI**
Never smokers	38/154	1.0*	…….		90/154	1.0*	…….
Ever smokers	82/299	1.1	0.7–1.7		137/299	0.8	0.6–1.1
				Single *04‡			
	ca/co	RR	95% CI		ca/co	RR	95% CI
Never smokers	79/83	3.9	2.4–6.3		49/83	1.0	0.6–1.5
Ever smokers	214/145	6.4	4.2–9.7		82/145	1.0	0.7–1.4
	AP:	0.37	(0.1–0.6)				
				Double *04†			
	ca/co	RR	95% CI		ca/co	RR	95% CI
Never smokers	34/15	8.7	4.2–17.7		15-aug	1.0	0.4–2.4
Ever smokers	102/14	31.1	15.8–61.0		14-okt	1.4	0.6–3.2
	AP:	0.72	(0.5–0.95)				
				Any *04§			
	ca/co	RR	95% CI		ca/co	RR	95% CI
Never smokers	115/98	4.8	3.0–7.5		58/98	1.0	0.7–1.5
Ever smokers	322/162	8.7	5.7–13.1		94/162	1.0	0.7–1.5
	AP:	0.4	(0.2–0.6)				

Ca/Co represents the number of cases and controls carrying the SE gene.

ACPA = anti-citrulline protein status. RR = relative risk. 95% CI = 95% confidence interval. AP = attributable proportion due to interaction. SE = shared epitope.

‡Single *04 indicates the presence of a single *0401, *0404, *0405 or *0408 SE allele.

†Double *04 indicates presence of two *0401, *0404, *0405 and/or *0408 SE allele.

§Any *04 indicates the presence of either one or two *0401, *0404, *0405 and/or *0408 SE allele.

RR adjusted for age, sex and residential area.

* As reference group we used never smokers without HLA-DRB1*01, *04 and *10 SE alleles.

Table 3

Interaction between smoking and DRB1*04 alleles (*0401, *0404)

		ACPA positive	
		No SE	
	ca/co	RR	95% CI
Never smokers	38/154	1.0*	…….
Ever smokers	82/299	1.1	0.7–1.7
		Single *0401/*0404‡	
	ca/co	RR	95% CI
Never smokers	77/83	3.8	2.4–6.2
Ever smokers	218/144	6.6	4.3–10.2
	AP:	0.4 (0.2–0.6)	
		Double *0401/*0404†	
	ca/co	RR	95% CI
Never smokers	30/13	9.0	4.2–19.2
Ever smokers	okt-92	39.6	18.6–84.4
	AP:	0.8 (0.6–0.98)	
		Any *0401/*0404§	
	ca/co	RR	95% CI
Never smokers	107/96	4.6	2.9–7.2
Ever smokers	309/154	8.9	5.8–13.5
	AP:	0.5 (0.3–0.7)	

Ca/Co represents the number of cases and controls carrying the SE.

ACPA = anti-citrulline protein status. RR = relative risk. 95% CI = 95% confidence interval. AP = attributable proportion due to interaction. SE = shared epitope.

‡Single *0401/’0404 indicates the presence of a single HLA-DRB1*0401 or *0404 SE allele.

†Double *0401/*0404 indicates presence of two HLA-DRB1*0401 and/or *0404 SE alleles.

§Any *0401/*0404 indicates the presence of either one or two HLA-DRB1*0401 and/or *0404 SE alleles.

RR adjusted for age, sex and residential area.

*As a reference group we used never smokers without HLA-DRB1*01, *04 and *10 SE alleles.

Table 4

Interaction between smoking and DRB1*01/DRB1*10

	ACPA positive					
		No SE			**Any *01/*10[§]**	
	ca/co	**RR**	**95% CI**	**ca/co**	**RR**	**95% CI**
Never smokers	38/154	1.0[*]	…….	27/67	1.8	1.0 – 3.2
Ever smokers	82/299	1.2	0.8 – 1.9	99/91	4.9	3.0 – 7.8
				AP:	0.6	(0.4 – 0.8)
	ACPA negative					
		No SE			Any *01/*10[§]	
	ca/co	RR	95% CI	ca/co	RR	95% CI
Never smokers	90/154	1.0[*]	…….	37/67	0.9	0.5 – 1.5
Ever smokers	137/299	0.8	0.6 – 1.2	69/91	1.4	0.9 – 2.2

Ca/Co represents the number of cases and controls carrying the SE gene.

ACPA = anti-citrulline protein status. RR = relative risk. 95% CI = 95% confidence interval. AP = attributable proportion. SE= shared epitope.

[§]Any *01/*10 indicates the presence of either one or two HLA-DRB1*01 and/or *1001 SE alleles.

RR adjusted for age, sex and residential area.

[*]As a reference group we used individuals without HLA-DRB1*01, *04 and *10 SE alleles.