

## ORIGINAL ARTICLE

## Interplay between alcohol, smoking and HLA genes in RA aetiology

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**ABSTRACT**

**Objectives** The relationship between alcohol consumption and risk for rheumatoid arthritis (RA) is incompletely understood. We aimed to determine the influence of alcohol on anticitrullinated protein antibody (ACPA) positive and ACPA-negative RA and investigate potential interactions between alcohol consumption, smoking and the presence of human leucocyte antigen (HLA)-DRB1-shared epitope (SE).

**Methods** A Swedish population-based case–control study with incident cases of RA was used (3353 cases, 2836 matched controls). Subjects with different HLA-DRB1-SE status, smoking and alcohol consumption were compared regarding risk of ACPA-positive and ACPA-negative RA, by calculating OR with 95% CI employing logistic regression. Interaction on the additive scale between alcohol, HLA-DRB1-SE and smoking was estimated by calculating the attributable proportion (AP) due to interaction.

**Results** Compared with non-drinking, low and moderate alcohol consumption was dose dependently associated with a reduced risk of ACPA-positive and ACPA-negative RA. Independent of smoking habits, non-drinking and the presence of HLA-DRB1-SE interacted to increase the risk of ACPA-positive RA. Among HLA-DRB1-SE positive subjects, there was also a significant interaction between non-drinking and smoking with regard to risk for ACPA-positive RA. A three-way interaction was observed between alcohol, smoking and HLA-DRB1-SE with regard to risk for ACPA-positive RA (AP 0.7, 95% CI 0.6 to 0.8) that remained significant when the influence from the two-way interactions was removed (AP 0.4, 95% CI 0.2 to 0.6).

**Conclusions** Our findings emphasize the need to investigate complex interactions between several environmental and genetic factors in order to better understand the etiology of RA. Whereas of great interest in an aetiological perspective, the finding of a protective role of alcohol on risk for RA must, however, be interpreted with caution in a clinical and public health perspective.

**INTRODUCTION**

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting about 1% of the population. Autoantibodies such as rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA) are present in the majority of RA cases.<sup>1–3</sup> ACPA and/or RF positive and negative RA differ in several important

**Key messages****What is already known about this subject?**

► Previous studies have been inconclusive regarding the impact of alcohol consumption on the risk of rheumatoid arthritis (RA) and knowledge concerning a potential interplay between alcohol consumption, smoking and the presence of human leucocyte antigen (HLA)-DRB1-shared epitope (SE) in RA aetiology is very limited.

**What does this study add?**

► Low and moderate alcohol consumption is dose dependently associated with a reduced risk of both anticitrullinated protein antibody (ACPA) positive and ACPA-negative RA.

► Independent of smoking habits, non-drinking and the presence of HLA-DRB1-SE interact to increase the risk for ACPA-positive RA.

**How might this impact on clinical practice?**

► Our findings emphasise the need to investigate several environmental and genetic factors together in research on aetiology and pathogenesis of RA.

► The mechanisms behind the protective effects of alcohol consumption on risk for RA need to be further investigated.

aspects and have different underlying genetic and environmental risk factors.<sup>4–6</sup>

The relationship between alcohol and RA risk incompletely understood. Alcohol consumption suppresses the synthesis of inflammatory cytokines and chemokines<sup>7</sup> and has been shown to reduce inflammation and response to antigens in both animals and humans.<sup>8–10</sup> Several previous studies, but not all, have indicated an inverse relationship between alcohol consumption and risk for RA.<sup>11–14</sup> There are also inconsistent results concerning the impact of alcohol consumption on risk for the subsets of RA defined by the presence or absence of RF and/or ACPA.<sup>11–15</sup> Several systematic reviews and meta-analyses have supported the concept of an inverse relationship between alcohol consumption and RA,<sup>16–17</sup> but knowledge is still lacking concerning the effects of different

doses of alcohol consumption in RA overall as well as in different subsets of the disease and the potential interplay between alcohol and other risk factors. In order to address some of these remaining questions, we used an updated and enlarged data set from the Swedish population-based case–control study Epidemiologic Investigations of Rheumatoid Arthritis (EIRA) to determine the influence of alcohol on RA risk overall and on disease subsets based on the presence or absence of ACPA. We also set out to investigate potential interactions between alcohol consumption, smoking and the presence of human leucocyte antigen (HLA)-DRB1-shared epitope (SE).

## METHODS

### Study design and study subjects

This report is based on data from EIRA, which is a population-based case–control study comprising the population aged 18–91 years in the middle and southern parts of Sweden. Incident cases of RA were recruited via all hospital-based rheumatology units in the study area during the period between November 1996 and September 2014. Cases were diagnosed by a rheumatologist according to the American College of Rheumatology 1987 criteria. The mean duration between the first notice of any signs of joint inflammation by the patient and the diagnosis of RA by a rheumatologist was 0.9 years (SD 1.2). For cases recruited between November 1996 and October 2005, one control was randomly selected from the national population register, frequency matched by age in 5-year age strata, gender and residential area (EIRA I). For cases recruited between October 2005 and September 2014, two controls were selected using the same matching criteria (EIRA II).

### Data collection

Information regarding environmental and lifestyle factors was collected using a standardised questionnaire filled out at the time of diagnosis. The response proportion was 92% for the cases and 75% for the controls. All participants who filled out a questionnaire were asked to donate blood samples and those who did not donate blood were excluded as were those who could not provide detailed information on alcohol consumption or smoking habits. A flow chart presenting the number of cases and controls is presented in the online supplementary table 1.

### Definition of exposures

Information on alcohol consumption was obtained by asking about current and previous alcohol consumption habits, which allowed quantification of the average alcohol intake in gram per week.

In EIRA1, subjects provided information on alcohol status during the last 12 months (drinking or non-drinking). They reported their current alcohol intake and stated, on a seven-point scale, whether this consumption was higher, equal or lower than their usual alcohol consumption. In EIRA2, subjects were asked

whether they were drinkers, past drinkers or never drinkers. Drinkers reported their habitual drinking habits and stated whether their alcohol consumption was higher, equal or lower compared with 5 years ago. Past drinkers were asked at what age they stopped drinking alcohol. All subjects were categorised into the following four groups based on the distribution of current alcohol intake in gram per week among controls; non-drinkers, low consumption (below or equal to the median), moderate consumption (above the median but below or equal to the 75th percentile) and high consumption (above the 75th percentile).

Detailed information on smoking was obtained by asking about current and previous smoking habits. For each case, the year of disease onset was defined as the index year, and the corresponding controls were given the same index year. Subjects who had smoked before or during the index year were defined as ever smokers and those who had never smoked before or at the index year were defined as never smokers.

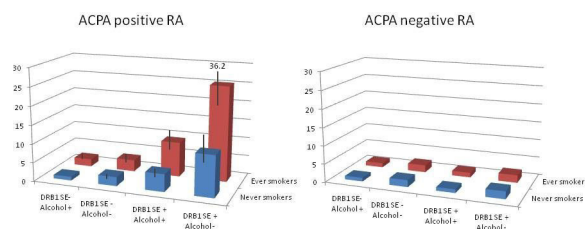
### Biological analyses

HLA-DRB1 genotypes were obtained as previously described.<sup>18</sup> The HLA-DRB1\*01, HLA-DRB1\*04 and HLA-DRB1\*10 alleles were classified as the SE alleles.

Cases were categorised into anticyclic citrullinated peptide 2 (CCP2) positive or anti-CCP2 negative based on the result of Immunoscan-RA Mark2 ELISA test (anti-CCP2 test). An antibody level exceeding 25 AU/mL was regarded as ACPA positive. RF positivity or RF negativity was determined locally, by the unit entering the case into the study.

### Statistical analysis

Using unconditional logistic regression models,<sup>19</sup> the occurrence of ACPA-positive and ACPA-negative RA in subjects with different alcohol consumption habits was compared with that in non-drinkers by calculating OR with 95% CI. Women and men were also analysed separately. Trend test for a dose–response relationship regarding alcohol consumption and risk of ACPA-positive and ACPA-negative RA was performed by using a continuous variable for the amount of alcohol consumed per week (g) in a logistic regression model. In order to illustrate the influence of the weekly amount of alcohol consumption (number of glasses of alcohol per week) on



**Figure 1** Dose–response relationship between the weekly amount of alcohol consumption and risk of developing RA. ACPA, anticyclic citrullinated protein antibodies; RA, rheumatoid arthritis.

**Table 1** OR with 95% CI of developing RA for different categories of alcohol consumers compared with never drinkers, in total and stratified by gender

Alcohol consumption	Total		ACPA-positive RA		ACPA-negative RA	
	CA/CO*	OR (95% CI)†	CA/CO*	OR (95% CI)†	CA/CO*	OR (95% CI)†
Never	930/431	1.0 (reference)	621/431	1.0 (reference)	309/431	1.0 (reference)
Low	1324/1180	0.57 (0.49 to 0.66)	883/1180	0.54 (0.46 to 0.64)	441/1180	0.62 (0.51 to 0.76)
Moderate	624/627	0.49 (0.41 to 0.58)	393/627	0.43 (0.36 to 0.52)	231/627	0.59 (0.47 to 0.74)
High	475/598	0.38 (0.31 to 0.45)	296/598	0.33 (0.27 to 0.41)	179/598	0.47 (0.37 to 0.60)
		<0.0001‡		<0.0001‡		0.005‡
Women						
Never	733/353	1.0 (reference)	492/353	1.0 (reference)	241/353	1.0 (reference)
Low	1038/963	0.57 (0.48 to 0.68)	699/963	0.54 (0.45 to 0.65)	339/963	0.63 (0.51 to 0.79)
Moderate	421/461	0.47 (0.39 to 0.57)	265/461	0.41 (0.33 to 0.51)	156/461	0.60 (0.46 to 0.78)
High	212/295	0.37 (0.29 to 0.47)	131/295	0.31 (0.24 to 0.41)	81/295	0.49 (0.36 to 0.68)
		<0.0001‡		<0.0001‡		0.003‡
Men						
Never	197/78	1.0 (reference)	129/78	1.0 (reference)	68/78	1.0 (reference)
Low	286/217	0.57 (0.41 to 0.80)	184/217	0.55 (0.38 to 0.79)	102/217	0.60 (0.39 to 0.91)
Moderate	203/166	0.53 (0.38 to 0.76)	128/166	0.51 (0.35 to 0.75)	75/116	0.58 (0.37 to 0.90)
High	263/303	0.39 (0.28 to 0.54)	165/303	0.36 (0.25 to 0.52)	98/303	0.43 (0.28 to 0.66)
		<0.0001‡		<0.0001‡		0.2‡

Low alcohol consumption means less than approximately four glasses of alcohol per week; moderate alcohol consumption means approximately four to seven glasses of alcohol per week; high alcohol consumption means approximately eight or more glasses of alcohol per week.

\*Number of exposed CA and CO.

†Adjusted for age, gender, residential area, ancestry, smoking, human leucocyte antigen-DRB1 shared epitope and study.

‡P value for trend,

ACPA, anticitrullinated protein antibodies; CA, cases; CO, controls; RA, rheumatoid arthritis.

RA risk, we used polynomial regression of order four to fit the regression lines to the estimates of ORs. One glass of alcohol was defined as 12 g of alcohol.

Potential interactions were analysed using departure of additivity of effects as criterion of interaction and were evaluated by calculating AP with 95% CI. Before calculating measures of interaction on the additive scale, preventive factors should be recoded into risk factors.<sup>20</sup> Non-drinking was thus considered a risk factor. We investigated the interaction between non-drinking and HLA-DRB1-SE with regard to risk of ACPA-positive and ACPA-negative RA and stratified the analyses by smoking status. The interaction between smoking and HLA-DRB1-SE was also calculated and stratified by alcohol consumption. Similarly, the potential interaction between non-drinking and smoking was calculated and stratified by HLA-DRB1-SE status. Finally, we investigated a potential three-way interaction between alcohol, smoking and HLA-DRB1-SE with regard to ACPA-positive and ACPA-negative RA.<sup>21</sup>

The relationship between alcohol, smoking and HLA-DRB1-SE was further investigated with regard to each RA subgroup based on both RF and ACPA status.

All analyses were adjusted for age in 5-year age strata, gender, residential area (one of 15 counties), ancestry,

study (EIRA I and EIRA II) and when appropriate for smoking, alcohol consumption or HLA-DRB1 status. Ancestry was dichotomised into Swedish or non-Swedish. A subject who was born in Sweden, whose parents had not immigrated, was classified as Swedish.

Adjustments were also made for educational level (university degree or not), exposure to passive smoking (yes or no) and body mass index at inclusion in the study (more or less than 25 kg/m<sup>2</sup>). However, these factors had minor influence on the results and were not kept in the final analyses.

In order to evaluate the presence of bias due to potential reverse causation, we performed sensitivity analyses restricted to participants who reported that they had not changed their alcohol consumption during the last 5 years prior to diagnosis. All analyses were conducted using Statistical Analysis System (SAS) V.9.4.

## RESULTS

The analyses on alcohol consumption and risk of RA included 3353 cases and 2836 matched controls. Characteristics of cases and controls, by alcohol consumption habits, are presented in the online supplementary

**Table 2** OR with 95% CI of developing RA for subjects with different combinations of HLA-SE and alcohol consumption, compared with HLA-SE negative subjects who consume alcohol, in total and stratified by smoking status

HLA-SE	RA		ACPA-positive RA		ACPA-negative RA		
	Non-drinking	CA/CO*	OR (95% CI)†	CA/CO*	OR (95% CI)†	CA/CO*	OR (95% CI)†
-	-	652/1139	1.0 (reference)	259/1139	1.0 (reference)	393/1139	1.0 (reference)
-	+	241/220	1.7 (1.4 to 2.1)	91/220	1.7 (1.3 to 2.3)	150/220	1.7 (1.3 to 2.2)
+	-	1771/1266	2.4 (2.2 to 2.8)	1313/1266	4.6 (3.9 to 5.4)	458/1266	1.0 (0.9 to 1.2)
+	+	689/211	5.3 (4.3 to 6.5)	530/211	11.0 (8.8 to 13.7)	159/211	1.8 (1.4 to 2.4)
			AP 0.4 (0.2 to 0.5)§		AP 0.5 (0.4 to 0.6)§		AP -0.03 (-0.4 to 0.3)§
<b>Never smokers</b>							
-	-	214/476	1.0 (reference)	72/476	1.0 (reference)	142/476	1.0 (reference)
-	+	120/116	2.0 (1.4 to 2.7)	44/116	2.2 (1.4 to 3.5)	76/116	1.8 (1.2 to 2.6)
+	-	528/524	2.2 (1.8 to 2.8)	367/524	4.8 (3.6 to 6.6)	161/524	1.0 (0.8 to 1.4)
+	+	251/108	4.7 (3.4 to 6.3)	171/108	10.5 (7.2 to 15.4)	80/108	2.0 (1.3 to 2.8)
			AP 0.2 (-0.02 to 0.5)§		AP 0.4 (0.2 to 0.6)§		AP -0.04 (-0.5 to 0.4)§
<b>Ever smokers</b>							
-	-	438/663	1.0 (reference)	187/663	1.0 (reference)	251/663	1.0 (reference)
-	+	121/104	1.6 (1.2 to 2.2)	47/104	1.6 (1.1 to 2.4)	74/104	1.7 (1.2 to 2.3)
+	-	1243/742	2.5 (2.2 to 2.9)	946/742	4.5 (3.7 to 5.5)	297/742	1.0 (0.9 to 1.3)
+	+	438/103	6.3 (4.8 to 8.1)	359/103	12.8 (9.6 to 17.1)	79/103	1.8 (1.3 to 2.5)
			AP 0.5 (0.3 to 0.6)§		AP 0.6 (0.5 to 0.7)§		AP -0.02 (-0.5 to 0.4)§

\*Number of exposed CA and CO.

†Adjusted for age, gender, residential area, ancestry, smoking and study.

‡Adjusted for age, gender, residential area, ancestry and study.

§AP due to interaction with 95% CI.

ACPA, anticitrullinated protein antibodies; AP, attributable proportion; CA, cases; CO, controls; HLA-SE, human leucocyte antigen-shared epitope; RA, rheumatoid arthritis.

**Table 3** OR with 95% CI of developing RA for subjects with different combinations of alcohol and smoking habits, compared with never smokers who consume alcohol, in total and stratified by HLA-SE status

Non-drinking	Smoking	RA		ACPA-positive RA		ACPA-negative RA	
		CA/CO*	OR (95% CI)†	CA/CO*	OR (95% CI)†	CA/CO*	OR (95% CI)†
–	–	742/1000	1.0 (reference)	439/1000	1.0 (reference)	303/1000	1.0 (reference)
–	+	1681/1405	1.7 (1.5 to 1.9)	1133/1405	2.0 (1.7 to 2.3)	548/1405	1.2 (1.0 to 1.4)
+	–	371/224	2.1 (1.7 to 2.6)	215/224	2.2 (1.7 to 2.8)	156/224	2.0 (1.5 to 2.5)
+	+	559/207	3.5 (2.9 to 4.3)	406/207	4.8 (3.8 to 5.9)	153/207	2.0 (1.5 to 2.6)
			0.2 (0.03 to 0.4)			0.3 (0.2 to 0.5)	–0.08 (–0.4 to 0.3)
<b>HLA-SE negative</b>							
–	–	214/476	1.0 (reference)	72/476	1.0 (reference)	142/476	1.0 (reference)
–	+	438/663	1.5 (1.2 to 1.9)	187/663	2.1 (1.5 to 2.8)	251/663	1.4 (1.0 to 1.8)
+	–	120/116	2.2 (1.6 to 3.0)	44/116	2.6 (1.6 to 4.1)	76/116	2.3 (1.6 to 3.3)
+	+	121/104	2.4 (1.8 to 3.4)	47/104	3.2 (2.1 to 5.1)	74/104	2.4 (1.6 to 3.5)
			–0.04 (–0.4 to 0.4)			–0.09 (–0.5 to 0.4)	–0.1 (–0.6 to 0.4)
<b>HLA-SE positive</b>							
–	–	528/524	1.0 (reference)	367/524	1.0 (reference)	161/524	1.0 (reference)
–	+	1243/742	1.7 (1.5 to 2.0)	946/742	2.0 (1.7 to 2.4)	297/742	1.2 (1.0 to 1.5)
+	–	251/108	2.2 (1.7 to 2.9)	171/108	2.3 (1.7 to 3.1)	80/108	1.9 (1.3 to 2.8)
+	+	438/103	4.1 (3.2 to 5.4)	359/103	5.3 (4.0 to 7.1)	79/103	1.9 (1.3 to 2.8)
			0.3 (0.1 to 0.5)			0.4 (0.2 to 0.6)	–0.1 (–0.6 to 0.4)

\*Number of exposed CA and CO.

†Adjusted for age, gender, residential area, ancestry, HLA-SE status and study.

‡Adjusted for age, gender, residential area, ancestry and study.

ACPA, anticitrullinated protein antibodies; CA, cases; CO, controls; HLA-SE, human leucocyte antigen-shared epitope; RA, rheumatoid arthritis.

table 2. Compared with non-drinkers, alcohol consumption was associated with a close to 50% reduced risk of ACPA-positive RA (OR 0.53, 95% CI 0.42 to 0.67) and a 30% reduced risk of ACPA-negative RA (OR 0.65, 95% CI 0.50 to 0.85) with an inverse dose–response relationship up to a level of approximately 12 drinks per week ( $p < 0.0001$  in both RA subsets) after which the trend towards protection was interrupted (table 1, figure 1).

With regard to ACPA-positive RA, there was a significant interaction between non-drinking and HLA-DRB1-SE, irrespective of smoking habits (table 2) and between smoking and HLA-DRB1-SE, irrespective of alcohol consumption (online supplementary table 3). There were no interactions with regard to ACPA-negative RA.

Furthermore, among HLA-DRB1-SE positive subjects, there was also a significant interaction between non-drinking and smoking with regard to ACPA-positive RA (table 3). No interaction was observed among HLA-DRB1-SE negative subjects.

A three-way interaction was observed between non-drinking, smoking and HLA-DRB1-SE with regard to ACPA-positive RA (AP 0.7, 95% CI 0.6 to 0.8) that remained significant when the influence from the two-way interactions was removed (AP 0.4, 95% CI 0.2 to 0.6) (table 4). The interaction between HLA-DRB1-SE and non-drinking, among never smokers and ever smokers, is illustrated in figure 2.

Both smoking and non-drinking independently increased the risk of ACPA-negative RA, but there was no interaction between the two risk factors (AP –0.08, 95% CI –0.4 to –0.3) (table 4). The results did not change significantly when the cases were further categorised by RF status (online supplementary table 4-6).

The inverse association between alcohol consumption and RA risk remained similar when we restricted the analyses to include subjects who had not changed their alcohol consumption during the last 5 years ( $p$  for trend 0.0002). The interaction between non-drinking and DRB1 SE with regard to ACPA-positive RA, and between non-drinking and smoking among DRB1 SE positive subjects, also remained similar.

## DISCUSSION

Our results demonstrate an inverse dose–response relationship between low and moderate alcohol consumption and risk of both ACPA-positive and ACPA-negative RA, with a possible reduction of the protective effect at higher consumption when both subsets of RA were analysed together. With regard to ACPA-positive RA, interactions were observed between HLA-DRB1-SE and alcohol and between HLA-DRB1-SE and smoking. Among HLA-DRB1-SE positive subjects, there was also a significant interaction between non-drinking and smoking with

**Table 4** OR with 95% CI of developing RA for subjects with different combinations of alcohol consumption, smoking habits and HLA-SE status, compared with HLA-SE negative never smokers who consume alcohol

	Non-drinking	Smoking	Smoking	HLA-SE	Total			ACPA-positive RA			ACPA-negative RA		
					CA/CO*	OR (95% CI)†	OR (95% CI)†	CA/CO*	OR (95% CI)†	OR (95% CI)†	CA/CO*	OR (95% CI)†	OR (95% CI)†
-	-	-	-	-	214/476	1.0 (reference)	1.0 (reference)	72/476	1.0 (reference)	1.0 (reference)	142/476	1.0 (reference)	1.0 (reference)
-	-	+	+	-	438/663	1.5 (1.2 to 1.9)	1.5 (1.2 to 1.9)	187/663	2.0 (1.5 to 2.8)	2.0 (1.5 to 2.8)	251/663	1.2 (1.0 to 1.6)	1.2 (1.0 to 1.6)
+	-	-	-	-	120/116	2.1 (1.5 to 2.9)	2.1 (1.5 to 2.9)	44/116	2.4 (1.5 to 3.8)	2.4 (1.5 to 3.8)	76/116	1.9 (1.3 to 2.7)	1.9 (1.3 to 2.7)
+	+	+	+	-	121/104	2.4 (1.7 to 3.3)	2.4 (1.7 to 3.3)	47/104	3.1 (2.0 to 4.8)	3.1 (2.0 to 4.8)	74/104	2.0 (1.4 to 2.8)	2.0 (1.4 to 2.8)
-	-	-	-	+	528/524	2.2 (1.8 to 2.7)	2.2 (1.8 to 2.7)	367/524	4.7 (3.5 to 6.3)	4.7 (3.5 to 6.3)	161/524	1.0 (0.8 to 1.5)	1.0 (0.8 to 1.5)
-	+	+	+	+	1243/742	3.9 (3.2 to 4.7)	3.9 (3.2 to 4.7)	946/742	9.3 (7.0 to 12.3)	9.3 (7.0 to 12.3)	297/742	1.3 (1.0 to 1.6)	1.3 (1.0 to 1.6)
+	-	-	-	+	251/108	5.0 (3.7 to 6.7)	5.0 (3.7 to 6.7)	171/108	11.0 (7.6 to 15.9)	11.0 (7.6 to 15.9)	80/108	2.1 (1.4 to 3.0)	2.1 (1.4 to 3.0)
+	+	+	+	+	438/103	9.2 (6.9 to 12.2)	9.2 (6.9 to 12.2)	359/103	25.3 (17.7 to 36.2)	25.3 (17.7 to 36.2)	79/103	2.1 (1.5 to 3.1)	2.1 (1.5 to 3.1)
						AP 0.6 (0.4 to 0.7)‡	AP 0.6 (0.4 to 0.7)‡		AP 0.7 (0.6 to 0.8)‡	AP 0.7 (0.6 to 0.8)‡		AP 0.008 (-0.4 to 0.4)‡	AP 0.008 (-0.4 to 0.4)‡
						AP 0.3 (0.06 to 0.5)§	AP 0.3 (0.06 to 0.5)§		AP 0.4 (0.2 to 0.6)§	AP 0.4 (0.2 to 0.6)§		AP 0.003 (-0.5 to 0.5)§	AP 0.003 (-0.5 to 0.5)§
						EOR 2.8 (0.1 to 5.5)¶	EOR 2.8 (0.1 to 5.5)¶		EOR 10.0 (2.6 to 17.5)¶	EOR 10.0 (2.6 to 17.5)¶		EOR 0.01 (-1.3 to 1.3)¶	EOR 0.01 (-1.3 to 1.3)¶

\*Number of exposed CA and CO.

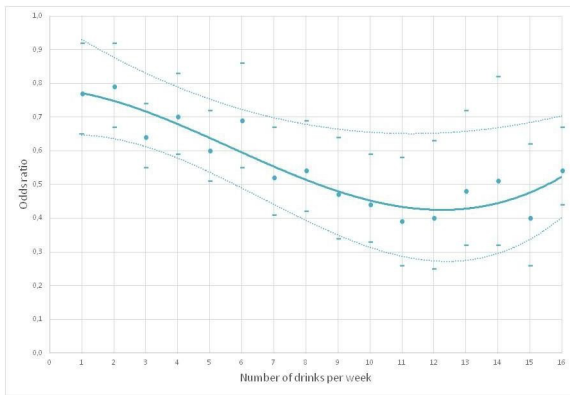
†Adjusted for age, gender, residential area, ancestry and study.

‡AP due to interaction (95% CI) of the OR due to second-order and third-order interaction between the three risk factors.

§AP due to interaction (95% CI) of the OR due to third-order interaction between the three risk factors.

¶EOR (95% CI) of triple exposed due to third-order interaction between the three risk factors.

ACPA, anticitrullinated protein antibodies; AP, attributable proportion; CA, cases; CO, controls; EOR, excess OR; HLA-SE, human leucocyte antigen-shared epitope; RA, rheumatoid arthritis.



**Figure 2** Interaction between HLA-DRB1 shared epitope and non-drinking with regard to risk of developing ACPA-positive and ACPA-negative RA, among never smokers and ever smokers. Based on data from table 4. ACPA, anticitrullinated protein antibodies; HLA, human leucocyte antigen; RA, rheumatoid arthritis.

regard to ACPA-positive RA. A significant three-way interaction was observed among the triple exposed regarding risk of developing ACPA-positive RA.

Several autoimmune diseases have been inversely associated with alcohol consumption, such as thyroid diseases,<sup>22,23</sup> systemic lupus erythematosus,<sup>24</sup> autoimmune diabetes<sup>25</sup> and multiple sclerosis.<sup>26</sup> Alcohol has dose-dependent immunomodulatory properties and has been shown to attenuate innate inflammatory responses in humans which is compatible with our finding that alcohol consumption is inversely related both to ACPA-positive and ACPA-negative RA.<sup>7,8</sup> In studies on mice, alcohol reduces the incidence and severity of collagen-induced arthritis with a simultaneous downregulation of leucocyte migration and upregulation of testosterone secretion.<sup>10</sup> Since most environmental factors for RA identified so far have mainly been related to ACPA-positive RA, it is especially interesting that alcohol is inversely related to ACPA-negative RA with about the same magnitude as for ACPA-positive RA.

A similar interaction between non-drinking and smoking has been observed with regard to risk of developing multiple sclerosis.<sup>26</sup> Alcohol thus seems to have a similar impact on RA and multiple sclerosis, which are both complex Th1 driven inflammatory diseases. However, knowledge concerning the molecular mechanisms underlying the specific interactions presented in this report are very limited.

Our study was designed as a case-control study with incident cases and information regarding environmental exposures and lifestyle factors was collected retrospectively. Recall bias was minimised by using incident cases of RA. We took great effort to obtain information on lifestyle factors and environmental exposures in an identical way for the cases and the controls. Furthermore, the questionnaire contained a wide range of questions regarding many potential environmental risk factors and no section in the questionnaire was given prime focus.

Reverse causation may be a concern since patients with RA may have been less inclined to consume alcohol due to their decreased general well-being before the diagnosis. However, in EIRA2, only 4% more cases than controls reduced their alcohol consumption in the 5-year period preceding the RA diagnosis, but without abstaining from alcohol. When the analyses were restricted to subjects who reported that they had not changed their alcohol consumption during the 5 years prior to diagnosis, our findings remained similar.

The observed interaction between DRB1-SE and non-drinking with regard to ACPA-positive RA also alleviates some potential biases in the interpretation of the influence from alcohol consumption on RA risk, since the HLA alleles are not likely to determine exposure habits. We thus consider it unlikely that our findings would be affected by recall bias since such a bias would then depend on HLA types.

Our classification of alcohol in categories of low, moderate and high consumption was based on the distribution among all controls. We examined other ways to classify alcohol exposure. A classification based on the distribution among controls by gender or applying the classification that is used by Statistics Sweden<sup>27</sup> did only marginally change the results.

When analysing the association between alcohol and RA risk, never drinkers of alcohol were used as the reference category in the main analyses. We also performed analyses of the association between alcohol and risk of RA among drinkers (ie, never drinkers were excluded). Also in this group, there was a statistically significant inverse dose-response relationship between alcohol and RA risk (p value for trend <0.0001).

A potential selection bias may arise when recruiting cases and controls. The proportion of respondents with regard to participation in EIRA was 92% for cases and 75% for controls. Since the structure of the Swedish public healthcare system provides equal access to medical services for all Swedish citizens, we know that almost all cases of RA are referred to public rheumatology units,<sup>28</sup> and it is not likely that the few unidentified cases would cause a substantial bias in our calculations. Selection bias among controls is also likely to be modest since controls were selected from the population and the prevalence of smoking and alcohol consumption among controls, seen as an indicator of life style, was in line with that of the general population at equivalent ages.<sup>29</sup> Furthermore, a previous methodological study from our group has shown that there is no significant differences between those who provided a blood sample and those who did not,<sup>28</sup> indicating that selection bias did not take place in this step.

In summary, our findings suggest that low and moderate alcohol consumption is inversely associated with both ACPA-positive and ACPA-negative RA. We find the results challenging with regard to how we should relate to and communicate these striking protective effects on RA from a habit with destructive effects for other disease conditions. The findings are also challenging

with regard to mechanism of action of the protection which are still poorly understood. Better understanding of such mechanisms may help to define ways to achieve protection against RA also by other means than alcohol consumption.

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