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Interaction Between Smoking, the Shared Epitope, and Anti–Cyclic Citrullinated Peptide

A Mixed Picture in Three Large North American Rheumatoid Arthritis Cohorts

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Objective. **Recently, Swedish members of the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) provided evidence that smoking may trigger RA-specific immune reactions to citrullinated protein in carriers of HLA–DR shared epitope alleles. In an effort to confirm this interaction between smoking and shared epitope alleles, we performed a case-only analysis of 3 North American RA cohorts.**

Methods. **A total of 2,476 white patients with RA were studied, 1,105 from the North American Rheumatoid Arthritis Consortium (NARAC) family collection, 753 from the National Inception Cohort of Rheumatoid Arthritis Patients (Inception Cohort), and 618 from the Study of New Onset Rheumatoid Arthritis (SONORA). All patients were HLA–DRB1 typed, and tested for anti–cyclic citrullinated peptide (anti-CCP) and rheumatoid factor. Information about smoking history was obtained by questionnaire.**

Results. **A significant association was found between smoking and the presence of anti-CCP in the NARAC and the Inception Cohort, but not in the SONORA. The shared epitope alleles consistently correlated with anti-CCP in all 3 populations. Using multiple logistic regression analyses, shared epitope alleles were still the most significant risk factor for anti-CCP positivity. Weak evidence of gene–environment interaction between smoking and shared epitope alleles for anti-CCP formation was found only in the NARAC.**

Conclusion. **Unlike the EIRA data, we could not confirm a major gene–environment interaction for anti-CCP formation between shared epitope alleles and smoking in 3 North American RA cohorts. Our data indicate a need for further studies to address the full range of environmental factors other than smoking that may be associated with citrullination and RA.**

Rheumatoid arthritis (RA) is a complex, multifactorial autoimmune disease whose etiology involves both genetic and environmental contributions. Several

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studies that examined the epidemiology of RA have shown an association between cigarette smoking and the development of RA (1–8). In particular, smoking has previously been known to be associated with rheumatoid factor (RF)–positive RA and to interact with the HLA–DRB1 shared epitope alleles (9,10). However, more recently, case–control data from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) in Sweden have shown a striking interaction between smoking and shared epitope alleles in conferring risk for the development of anti–cyclic citrullinated peptide (anti-CCP)–positive, rather than RF-positive, RA (11). This study also demonstrated an association between smoking and the development of citrullinated antigens in bronchoalveolar lavage fluid cells from smokers. Since these antibodies are increasingly linked to the pathogenesis in a subset of RA, this study provided a possible causal link between smoking and the development of anti–CCP–positive RA.

In an effort to confirm the interaction between smoking and shared epitope alleles in relation to anti-CCP–positive RA, we performed a case-only analysis of 3 large RA patient populations: the North American Rheumatoid Arthritis Consortium (NARAC) (12,13), the National Inception Cohort of Rheumatoid Arthritis Patients (Inception Cohort) (14), and the Study of New Onset Rheumatoid Arthritis (SONORA) (15,16). Although we observed a modest association between smoking and the production of autoantibodies, we found minimal evidence of gene–environment interaction for anti-CCP formation between shared epitope alleles and smoking. Thus, we propose that environmental factors in addition to smoking are likely to play a predominant role in the development of anti-CCP–positive RA in North American populations.

PATIENTS AND METHODS

Patient population. We studied a total of 2,476 white patients with RA, including 1,105 from the NARAC family collection, 753 from the Inception Cohort, and 618 from the SONORA cohort. Information about smoking history was obtained by questionnaire at the time of enrollment from members of the NARAC and SONORA cohorts and at every visit from members of the Inception Cohort. This included the smoking status (never smoked, ever smoked, currently smoke) and, for the Inception Cohort, the amount smoked (pack-years), if applicable.

All patients met the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for RA (17) at the time of enrollment. The participants in the NARAC were members of 512 multicase families that had been established to create a resource for RA gene-mapping studies (12,13). The details of the large national Inception Cohort have been described previously (14). Briefly, white patients with a clinical diagnosis of RA of <6 months' duration were enrolled by 161 rheumatologists and were being followed up to obtain 6-month Health Assessment Questionnaire data, yearly clinical data, and 5-year radiographic data. The SONORA cohort consisted of patients with recent-onset RA who were enrolled within 12 months of diagnosis in a 5-year outcome study, which included radiographic analysis (16). Clinical data, such as age, sex, and body mass index (BMI), were retrieved from each data set.

Laboratory procedures. At the time of study entry, RF was measured using the BN II nephelometer (Dade Behring, Deerfield, IL) (RF positive >12) in the NARAC and SONORA patients, and by latex fixation (RF positive >20) in the Inception Cohort. Levels of anti-CCP were measured using a second-generation commercial anti-CCP enzyme immunoassay (Inova Diagnostics, San Diego, CA), which was performed as recommended by the vendor, using a serum dilution of 1:100. The upper limit of the reference range is 20 units. In the NARAC samples, anti-CCP levels were not titered out beyond 210 units. Thus, all anti-CCP titers >210 were truncated and assigned as 210 for analysis. In the Inception Cohort, 500 was used as a maximum. Anti-CCP levels were tested at the University of Washington, Department of Laboratory Medicine, Immunology Division, for the NARAC and the SONORA samples. Samples from the Inception Cohort were tested at the Feinstein Institute of Medical Research (Manhasset, NY) using the same methods. Specimens were assayed in a blinded manner.

HLA–DRB1 genotyping. Broad-level HLA–DRB1 typing and high-resolution DRB1*04 typing were accomplished by initial polymerase chain reaction (PCR) amplification of groups of alleles (all DRB1 alleles for broad-level typing, and group-specific amplification for DRB1*04 alleles) using biotinylated PCR primers, followed by hybridization to immobilized sequence-specific oligonucleotide probes in a linear array format as previously described (18).

Statistical analysis. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using chi-square statistics to compare the frequency of the anti-CCP positivity and RF positivity between groups stratified by variables such as the presence of shared epitopes and smoking status. The Mann-Whitney U test was applied to the distribution of anti-CCP titers and RF titers in each group. Multiple logistic regression analyses for the presence of antibodies and generalized linear model (GLM) analyses for the antibody titers were used to establish the independent risk of variables such as the shared epitope, smoking, age, sex, and BMI. All analyses were conducted using SAS software, version 9.1 (SAS Institute, Cary, NC). Attributable proportions were also calculated as a means to measure interaction between the shared epitope and smoking for anti-CCP formation in each cohort (11,19,20). *P* values less than 0.05 were considered significant.

RESULTS

Patient characteristics. Baseline characteristics of patients in the study populations are shown in Table 1. The mean age at enrollment and the percentage of

Table 1. Baseline characteristics of patients in the 3 different cohorts*

	NARAC (n = 1,105)	Inception Cohort (n = 753)	SONORA (n = 618)
Age at enrollment, mean ± SD years	55.5 ± 11.8	54.3 ± 15.3	54.9 ± 12.3
Disease duration, mean ± SD years	16.03 ± 11.8	≤0.5	≤1
Female, %	76.8	75.3	71.0
Anti-CCP positive, %	77.1	56.7	54.9
RF positive, %	77.1	67.0	62.9
SE positive, %	85.9	66.3	67.7
Ever smoked, %	58.1	40.5	65.8

* NARAC = North American Rheumatoid Arthritis Consortium; SONORA = Study of New Onset Rheumatoid Arthritis; anti-CCP = anti–cyclic citrullinated peptide; RF = rheumatoid factor; SE = shared epitope.

females among the 3 cohorts were similar. In contrast, the frequencies of positive anti-CCP, RF, and shared epitopes were higher in the NARAC population compared with those from the Inception Cohort and the SONORA group. These differences are almost certainly a result of the different inclusion criteria for the studies, particularly in the NARAC family collection, which included RA patients from multicase families with a generally earlier age at onset (mean ± SD 39.3 ± 13.4 years) and longstanding disease (16.0 ± 11.8 years). More than 95% of RA patients in the NARAC family collection also had documented erosions on hand radiographs (21). In contrast, the Inception Cohort and SONORA are composed of new-onset RA patients, with a disease duration of <1 year. The distribution of patients who had ever smoked or were smokers at the time of determination of anti-CCP or RF positivity was also different among the cohorts. Therefore, based on the different baseline characteristics of the 3 groups, further analysis of the relationship between anti-CCP and variables such as smoking was performed separately in each population.

Effects of smoking on anti-CCP and RF formation. When the effect of smoking (ever smoked) on anti-CCP and RF formation was analyzed, a significant association was found between smoking and the presence of both autoantibodies in those in the NARAC cohort and in the Inception Cohort, but no significant association was found with the presence of either autoantibody in those in the SONORA cohort (Table 2). The

Table 2. Risk of anti-CCP and RF positivity and titers of anti-CCP and RF according to smoking status (ever smoked versus never smoked) among the 3 cohorts*

	Anti-CCP		RF	
Cohort, smoking status	No. (%) positive	Median (IQR) titer†	No. (%) positive	Median (IQR) titer†
NARAC				
Never smoked	328 (73.2)	108.5 (60.4–132.5)‡	321 (71.7)	100.0 (44–220)
Ever smoked	491 (79.1)	115.6 (75.2–135.2)‡	503 (81.0)	146.0 (61–402)
P	0.03	0.018	0.0003	<0.0001
OR (95% CI)	1.38 (1.04, 1.84)	–	1.69 (1.27, 2.25)	–
Inception Cohort				
Never smoked	239 (53.4)	275.6 (109.5–367.8)	277 (63.5)	157.0 (81.5–326.0)
Ever smoked	188 (61.6)	287.1 (125.3–368.6)	216 (72.2)	204.5 (104.0–417.5)
P	0.024	0.68	0.01	0.02
OR (95% CI)	1.41 (1.04, 1.89)	–	1.49 (1.08, 2.06)	–
SONORA				
Never smoked	113 (54.3)	214.0 (71–488)	126 (60.6)	121 (61–325)
Ever smoked	220 (55.0)	259.5 (79.0–517.5)	257 (64.3)	143.0 (74–352)
P	0.87	0.02	0.37	0.0001
OR (95% CI)	1.02 (0.73, 1.44)	–	1.17 (0.83, 1.65)	–

* All odds ratios (ORs) and *P* values were obtained from the comparison with the "never smoked" group. Anti-CCP = anti–cyclic citrullinated peptide; RF = rheumatoid factor; IQR = interquartile range; NARAC = North American Rheumatoid Arthritis Consortium; 95% CI = 95% confidence interval; SONORA = Study of New Onset Rheumatoid Arthritis.
† Includes only patients who were positive for anti-CCP or RF.
‡ In the NARAC cohort, all anti-CCP titers >210 units were truncated and assigned as 210 units for analysis.

Table 3. Risk of anti-CCP and RF positivity and titers of anti-CCP and RF according to SE status among the 3 cohorts*

Cohort, SE status	Anti-CCP		RF	
	No. (%) positive	Median (IQR) titer†	No. (%) positive	Median (IQR) titer†‡
NARAC				
No SE	83 (50.9)	102.0 (43.0–127.7)	98 (60.1)	118 (63.0–247.0)
Single SE	412 (77.2)	107.6 (60.3–130.7)	408 (76.4)	122 (50.5–302.5)
Double SE	357 (87.5)	118.8 (84.7–137.1)§	346 (84.8)	120 (54.0–317.0)
OR (95% CI)¶				
Single SE	3.25 (2.3, 4.7)		2.2 (1.5, 3.1)	
Double SE	6.75 (4.4, 10.3)		3.7 (2.5, 5.6)	
Inception Cohort				
No SE	89 (35.0)	191.8 (72.2–359.4)	125 (49.8)	142.0 (60.8–342.0)
Single SE	233 (63.5)	288.8 (122.4–362.9)	260 (73.0)	187.0 (92.4–349.5)
Double SE	105 (79.5)	293.2 (153.4–371.8)¶	108 (84.4)	195.0 (91.2–403.5)
OR (95% CI)¶				
Single SE	3.22 (2.3, 4.5)			
Double SE	7.21 (4.4, 11.8)			
SONORA				
No SE	60 (30.2)	270.5 (64.0–433.0)	91 (45.7)	140.0 (49.0–295.0)
Single SE	180 (59.6)	216.5 (78.5–490.5)	202 (66.9)	138.5 (70.0–348.0)
Double SE	98 (85.2)	251.0 (90.0–596.0)	94 (81.7)	139.0 (86.0–398.0)
OR (95% CI)¶				
Single SE	3.4 (2.3, 5.0)		2.4 (1.7, 3.5)	
Double SE	13.4 (7.4, 24.3)		5.3 (3.1, 9.2)	

* All ORs and *P* values were obtained from the comparison with the group that was negative for the shared epitope (SE). See Table 2 for other definitions.
† Includes only patients who were positive for anti-CCP or RF.
‡ RF titers were not affected by SE status in the 3 cohorts.
§ Double positivity for the SE in the NARAC cohort was significantly associated with higher anti-CCP titers ($P = 0.0002$).
¶ All corresponding *P* values < 0.0001.

median RF was significantly higher in patients in all 3 cohorts who smoked. However, the median anti-CCP titer was increased only in the NARAC and SONORA cohorts.

Effects of the shared epitope on the presence of anti-CCP and RF. As shown in Table 3, the occurrence of anti-CCP and RF was strongly influenced by the shared epitope status, either single or double shared epitope, in all 3 populations. We also found a stronger effect in patients with a double dose of the shared epitope than in patients with a single shared epitope allele, suggesting a gene dosage effect on antibody formation. Among patients in the NARAC cohort, double positivity for the shared epitope was significantly associated with higher anti-CCP titers. In the Inception and SONORA cohorts, shared epitope alleles had no significant effect on anti-CCP titers. In addition, RF titers were not affected by shared epitope status.

Gene–environment interaction between smoking and HLA–DR shared epitope alleles for anti-CCP formation. In order to investigate whether there is an interaction between smoking and shared epitope alleles for anti-CCP production in RA, we compared the different combinations of smoking and shared epitope allele status for the presence of anti-CCP in all 3 RA patient populations. As shown in Table 4, there was no significant effect of smoking on anti-CCP formation within the various shared epitope groups (none, single, and double), thus arguing against a strong interaction of smoking with shared epitope alleles. In contrast, the presence of either single or double shared epitope alleles still had a significant effect on anti-CCP formation regardless of smoking status. We also extracted data from the recent study by Klareskog et al (11) in order to perform a similar case-only analysis using the Swedish data set. Consistent with that study, the data are highly suggestive of a strong interaction between shared epitope and smoking for anti-CCP production.

We also investigated the gene–environment interaction with an additive model using these data. The calculation of attributable proportion was performed on each data set (see footnote in Table 4). Only the NARAC cohort showed weak evidence of interaction using this model.

Table 4. Risk of anti-CCP positivity in patients with RA, by combined smoking and SE status*

Cohort, smoking status	No SE		Single SE		Double SE	
	No. anti-CCP–positive/ no. of patients	OR (95% CI)	No. anti-CCP–positive/ no. of patients	OR (95% CI)	No. anti-CCP–positive/ no. of patients	OR (95% CI)
NARAC†						
Never	33/66	Referent	155/213	2.67 (1.51, 4.72)	134/156	6.09 (3.15, 11.79)
Ever	39/78	1.0 (0.52, 1.93)	219/275	3.91 (2.22, 6.88)	212/241	7.31 (3.94, 13.58)
Inception Cohort†						
Never	55/163	Referent	126/209	2.98 (1.95, 4.57)	58/76	6.33 (3.4, 11.8)
Ever	34/91	1.17 (0.69, 2.0)	107/158	4.12 (2.59, 6.56)	47/56	10.25 (4.68, 22.5)
≥20 pack-years	31/66	1.77 (0.97, 3.11)	77/117	3.78 (2.29, 6.24)	39/46	10.94 (4.59, 26.06)
SONORA†						
Never	18/64	Referent	60/103	3.57 (1.82, 6.98)	35/40	17.89 (6.05, 52.89)
Ever	42/133	1.18 (0.61, 2.27)	115/192	3.82 (2.06, 7.07)	62/74	13.2 (5.80, 30.10)
EIRA‡						
Never	20/85	Referent	72/136	3.66 (1.99, 6.69)	36/54	6.5 (3.05, 13.8)
Ever	58/142	2.24 (1.23, 4.10)	192/268	8.21 (4.66, 14.5)	126/144	22.75 (11.26, 45.98)

* RA = rheumatoid arthritis; SE = shared epitope; EIRA = Epidemiological Investigation of RA (see Table 2 for other definitions).
† Attributable proportions (95% CI) are 0.298 (0.036, 0.561), 0.256 (−0.048, 0.561), and −0.030 (−0.485, 0.424) in the NARAC, the Inception Cohort, and the SONORA, respectively.
‡ Calculated from data reported by Klareskog et al (11).

Mixed picture for smoking as a risk factor for anti-CCP and RF in multiple logistic regression analysis. Since we have previously shown that multiple clinical variables such as age and sex also influence the risk for presence of antibodies in RA populations (21), we analyzed our data using multiple logistic regression. As shown in Table 5, the presence of the shared epitope was associated with risk for anti-CCP positivity in all 3 populations, but smoking had no effect on this risk in these large cohorts. Although sex and age also showed a modest effect on the presence of anti-CCP, these findings were not consistent in all data sets. However, the Inception Cohort did reveal a significant independent effect of smoking on the presence of anti-CCP when a subgroup analysis was performed using only heavy smokers (at least 20 pack-years) compared with nonsmokers (see footnote in Table 5). Due to the limits of the data collected in the other groups, we were not able to ascertain this effect in the SONORA and NARAC cohorts. Similarly, shared epitope alleles are also a significant risk factor for RF formation in multiple logistic regression analyses, but smoking is a risk factor only in the NARAC population (Table 6).

The effect of all these variables on anti-CCP titer was also analyzed by GLM analysis. Although sex and shared epitope in the NARAC population were significant risk factors, we did not find any variables that were consistently related to anti-CCP titer (data not shown). Similarly inconsistent findings were observed for RF titer across the 3 cohorts, with some effect of smoking in the NARAC cohort and an age effect in the 2 inception cohorts (data not shown).

DISCUSSION

Overall, genetics studies in RA have so far yielded only a few genes, such as HLA–DRB1 (22), protein tyrosine phosphatase N22 (*PTPN22*) (23), and

Table 5. Multiple logistic regression analysis of variables affecting anti-CCP formation*

	NARAC		Inception Cohort		SONORA	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.01 (0.99, 1.03)	NS	0.98 (0.97, 0.99)	0.002	1.04 (0.90, 1.21)	NS
Female sex	0.59 (0.39, 0.88)	0.02	0.77 (0.53, 1.10)	NS	0.73 (0.48, 1.11)	NS
Shared epitope	3.95 (2.74, 5.70)	<0.0001	3.73 (2.70, 5.15)	<0.0001	4.67 (3.14, 6.94)	<0.0001
Ever smoked	1.16 (0.85, 1.57)	NS	1.32 (0.96, 1.80)	NS†	0.92 (0.62, 1.36)	NS

* NS = not significant (see Table 2 for other definitions).
† When smoking was restricted to >20 pack-years in the Inception Cohort, the evidence of an independent effect of smoking emerged (OR 1.45 [95% CI 1.05, 2.10], $P = 0.024$).

Table 6. Multiple logistic regression analysis of variables affecting RF formation*

	NARAC		Inception Cohort		SONORA	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.05 (0.93, 1.19)	NS	0.88 (0.79, 0.98)	0.02	1.04 (0.89, 1.20)	NS
Female sex	0.68 (0.47, 1.01)	NS	0.94 (0.64, 1.38)	NS	0.71 (0.46, 1.09)	NS
Shared epitope	2.49 (1.72, 3.61)	<0.0001	3.08 (2.22, 4.29)	<0.0001	3.18 (2.16, 4.68)	<0.0001
Ever smoked	1.60 (1.19, 2.17)	0.002	1.39 (0.99, 1.95)	NS	1.09 (0.74, 1.63)	NS

* NS = not significant (see Table 2 for other definitions).

peptidyl arginine deiminase type 4 (*PADI4*) (24,25), that have been conclusively linked to risk for RA. Environmental risk factors for RA susceptibility have been even less well defined. Smoking (1–9), BMI (5,7), prior blood transfusion (5,26), hormonal factors (27,28), and decaffeinated coffee consumption (29–31) have been reported with variable consistency as risk factors. Nevertheless, a role of smoking is supported by multiple case–control studies and several cohort studies that show a modestly increased risk of RA in both males and females (1–9,32,33). In addition, it has been reported that both increased duration and intensity of smoking are related to a higher risk of RA, with prolonged increased risk after smoking cessation (4,34).

Although it has been suggested that the development of RA results from the combined roles of genetic susceptibility factors and environmental exposures, few studies have shown convincing gene–environment interactions in RA. For example, a gene–environment interaction between smoking and glutathione S-transferase (GST) polymorphisms as a predictor of RA outcome (not RA susceptibility) was demonstrated in a case-only analysis where cigarette smoking was associated with the most severe disease in patients who carried the GSTM1-null polymorphism (35). More recently, Klareskog and collaborating Swedish EIRA investigators have provided evidence that smoking might trigger RA-specific immune reactions to citrullinated proteins, and that this is more likely to occur in carriers of HLA–DR shared epitope alleles (11). Using a case–control study design, a strong interaction between smoking and shared epitope status was observed for anti-CCP–positive RA. Interestingly, this interaction was not observed in the subset of patients negative for anti-CCP, and this was confirmed by our case-only analysis of the published data (Table 4).

Unlike the EIRA study (11), we could not confirm a major gene–environment interaction between HLA–DR shared epitope alleles and smoking in 3 separate North American RA cohorts. As reported previously (36), shared epitope alleles are a risk factor for anti-CCP–positive disease, with the most prominent effect seen in individuals who are homozygous for shared epitope alleles (Table 3). While the NARAC group and the Inception Cohort showed an association of smoking status (ever smoked) with the presence of anti-CCP antibodies (Table 2), these associations were not significant after regression analysis with the other potential confounding factors, such as shared epitope, age, and sex (Table 5). However, when we reanalyzed the Inception Cohort data using only heavy smokers (>20 pack-years) as compared with nonsmokers, we did observe a significant independent effect of smoking on anti-CCP status.

In order to obtain further perspective on our findings, we tabulated the case-only data from published studies on the relationship between smoking and the

Table 7. Comparison of published studies (Swedish, Dutch, and Danish) and our data (NARAC and Inception Cohort) in terms of the relationship between smoking and anti-CCP production (case-only analysis)*

	Ever smoked		Never smoked			
	CCP positive	CCP negative	CCP positive	CCP negative	*P*	OR (95% CI)
NARAC	491	130	328	120	0.03	1.38 (1.04, 1.84)
Inception Cohort	188	117	239	209	0.024	1.41 (1.04, 1.89)
Swedish†	376	178	128	147	3.19×10^{-9}	2.43 (1.80, 3.26)
Dutch†	84	54	76	78	0.048	1.60 (1.00, 2.64)
Danish†	225	85	84	51	0.029	1.61 (1.05, 2.47)

* Values are the number of patients. See Table 2 for definitions.
† Calculated using data reported by Klareskog et al (11), Linn-Rasker et al (37), and Pedersen et al (8).

development of anti-CCP antibodies in RA (Table 7). Using a case-only analysis, the evidence for this association is strongest in the Swedish population (11), but data from other European studies (8,37) are quite consistent with the ORs we observed in NARAC and the Inception Cohort. Thus, our basic findings on this association do not appear to be inconsistent with studies in other populations.

Antibodies against citrullinated proteins, such as antiperinuclear factor (38) and antikeratin antibody (39), are less sensitive but more specific for RA than is RF and may be present for many years before the onset of clinically apparent disease (40). More recently, Kuhn et al (41) have provided evidence that anti-CCP are directly involved in the pathogenesis of autoimmune arthritis in experimental models. In addition, the significant association of *PADI4* with RA (24,25) and the enhanced expression of PADs in RA synovium (42–44) support the view that citrullination of proteins is important in the development of RA. Therefore, the work of Klareskog et al (11) provides support for an elegant hypothesis that ties smoking together with several of the known genetic risk factors, including HLA, *PADI4*, and *PTPN22*, with the latter gene being associated with B cell hyperactivity in knockout mouse models (45). It is therefore somewhat disappointing that our data do not more strongly support a role for smoking in the production of anti-CCP in the context of the shared epitope.

We have considered several explanations for this difference in results. Most significantly, our information on cigarette smoking varies in quality and timing with respect to disease onset across the 3 data sets. Because of the long disease duration at enrollment in the NARAC cohort and the inherent uncertainty of patient recall, it is difficult to assess whether smoking exposure occurred prior to disease onset. However, the other 2 cohorts contain patients with disease of recent onset, and are thus more similar to the Swedish EIRA cohort. Since the subgroup analysis of heavy smokers in the Inception Cohort revealed an independent risk of smoking for the development of anti-CCP, it is possible that the Swedish cohort contained a larger proportion of heavy smokers, thus highlighting the effect of smoking on anti-CCP production. This suggests a potential dose effect of smoking on anti-CCP development. However, a stratification of anti-CCP levels for different definitions of "positive" did not influence the results.

Since BMI has been associated with risk for RA (5,7) and obesity is an epidemic problem in North America, we analyzed our data for an effect of BMI on risk for anti-CCP–positive disease. However, we did not observe an effect of BMI on anti-CCP–positive RA (data not shown). We also considered whether other environmental exposures might obscure the smoking effect in the North American populations. One of these factors is air pollution. Although it is difficult to directly compare the various air pollution exposures between North America and Sweden, it is interesting that Sweden has one of the lowest rates of greenhouse gas emissions among European Union countries (from Sweden's Environmental Objectives: For the Sake of Our Children, 2005; online at www.internat.naturvardsverket.se). Thus, pollution exposure might be one of several confounding factors that obscure the association between anti-CCP–positive RA and smoking in the North American population. Recent data from Chang and colleagues support this view (46).

Clearly, these data indicate that additional research is needed to examine the full range of environmental factors that are responsible for induction of citrullination in the lungs. Subclinical lung disease appears to be common in the RA population (47–49), but it is not clear whether this is a cause or an effect of the systemic inflammatory process in RA. The studies by Klareskog et al (11) are seminal, in the sense that they support the former causative relationship. The current findings further suggest that there may be various environmental factors, and they may be rather ubiquitous in some geographic locations, thus obscuring the specific effects of smoking observed in Sweden. Similarly, the risk conferred by *PADI4* may be modulated by the presence of environmental inducers of citrullination, potentially explaining the much weaker association of *PADI4* with RA in North America and Europe (50), compared with Asian populations. In this model, once citrullination of self antigens has been induced, risk factors such as HLA–DR shared epitope and *PTPN22* will predispose to the development of anti-CCP, regardless of the specifics of the environmental insult. Overall, these data emphasize the importance of taking all the known genetic, demographic, phenotypic, and environmental factors into account when attempting to identify new genetic or environmental risk factors for a complex disease such as RA.

AUTHOR CONTRIBUTIONS

Dr. Hye-Soon Lee had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Hye-Soon Lee, Irigoyen, Weisman, Gregersen.

Acquisition of data. Kern, Annette Lee, Batliwalla, Khalili, Wolfe, Massarotti, Weisman, Bombardier, Criswell, Gregersen.

Analysis and interpretation of data. Hye-Soon Lee, Irigoyen, Lum, Karlson, Criswell, Vlietinck, Gregersen.
Manuscript preparation. Hye-Soon Lee, Irigoyen, Gregersen.
Statistical analysis. Hye-Soon Lee, Irigoyen, Lum, Criswell, Vlietinck, Gregersen.

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