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Smoking is not associated with autoantibody production in systemic lupus erythematosus patients, unaffected first-degree relatives, nor healthy controls

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Abstract

Objective—To examine whether smoking is associated with autoantibody production in systemic lupus erythematosus (SLE) patients, unaffected first-degree relatives (FDR) of individuals with SLE - a group at increased risk of developing SLE, or unaffected, unrelated controls.

Methods—Detailed demographic, environmental, clinical, and therapeutic information was collected by questionnaire on 1,242 SLE patients, 981 FDRs, and 946 controls in the Lupus Family Registry and Repository; a blood sample was obtained. All sera were tested for multiple lupus autoantibodies by immunofluorescence and luminex bead-based assays. Generalized estimating equations, adjusting for age, gender, and ethnicity and accounting for correlation within families, were used to assess smoking status with the dichotomous outcome variables of positivity for SLE status, positivity of ANA by immunofluorescence (1:120), positivity for 1 autoantibody by the luminex assay, and positivity for each of the 11 autoantibodies.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Results—Current smoking was associated with being positive for 1 autoantibody (excluding ANA) (adjusted OR=1.53, 95% CI 1.04–2.24) in our subjects with SLE. No association was observed in unaffected FDRs or healthy controls. Former smoking was associated with anti-Ro/SS-A60 in our unaffected FDRs. There was an increased association with anti-nRNP A seropositivity, as well as a decreased association with anti-nRNP 68 positivity, in current smokers in SLE subjects.

Conclusions—No clear association between smoking status and individual autoantibodies was detected in SLE patients, unaffected FDRs, nor healthy controls within this collection. The association of smoking with SLE may therefore manifest its risk through mechanisms outside of autoantibody production, at least for the specificities tested.

Keywords

Smoking; autoantibodies; systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by autoantibody production, acute and chronic inflammation of various tissues, and protean manifestations such as fever, rash and nephritis. The etiology and pathogenesis of SLE remains largely unidentified.

A number of case-control studies have identified an association between the presence of SLE and ever smoking^{1–3}, with the highest risk among current smokers^{3–6}. Smoking has also been associated with seropositivity for SLE-related autoantibodies, including anti-dsDNA in individuals with SLE⁷, and ANA in primary Sjogren's syndrome⁸. In addition, smoking appears to play a role in autoantibody positivity in other autoimmune diseases, namely rheumatoid arthritis (RA)^{9–19}.

These results may suggest a plausible biologic mechanism into the development of SLE, in which smoking leads to increased autoantibody production, which in turn leads to SLE in select individuals. Autoantibodies could be generated through either modification of autoantigens by tobacco smoke⁷ or through ineffective clearance of apoptotic and necrotic cells as a result of smoking, leading to loss of self-tolerance^{20–22}. In addition, cigarette smoke byproducts can enhance auto-reactive B cell activity²³. Interestingly, since this model suggests smoking leads to autoantibody production, individuals at high-risk of developing SLE but who do not have clinical features of SLE may be more likely to be autoantibody positive if they smoke.

SLE is more likely to be diagnosed in first-degree blood relatives (FDRs) of SLE patients; 5–10% of SLE patients have a second family member with SLE²⁴, the monozygotic twin concordance rate is estimated to around 24%²⁵, and the sibling risk ratio is estimated to be between 6- and 29-fold greater than the general population²⁶. In addition, prior studies in our cohort indicate that autoantibodies associated with SLE are enriched in FDRs compared to controls, including ANA by immunofluorescence and autoantibodies against dsDNA, La, Ro, Sm and nRNP^{27, 28} by luminex bead-based assays. Given that SLE-related

autoantibodies progressively accumulate before the onset of SLE ²⁹, these observations strongly suggest that important understandings of SLE pathogenesis could be gathered from studies in FDRs. Therefore, to examine whether smoking is associated with SLE through autoantibody production, we tested autoantibody seropositivity in unaffected FDRs as well as in subjects with SLE and unaffected, unrelated controls.

Materials and methods

Participant information

All protocols were approved by the Institutional Review Boards at the Oklahoma Medical Research Foundation (OMRF) and the University of Oklahoma Health Sciences Center. Study participants were enrolled in the Lupus Family Registry and Repository (LFRR) ³⁰ and provided informed consent prior to enrollment. We included 1,242 SLE patients, 981 individuals who were documented first-degree, blood relatives of SLE patients, and 946 unaffected, unrelated controls in this study. FDRs did not have clinically diagnosed SLE at the time of the study, and approximately 5% self-reported having another autoimmune disease, most commonly rheumatoid arthritis. Medical records were reviewed to confirm American College of Rheumatology (ACR) classification criteria ³¹ for all SLE subjects. Detailed clinical and demographic information was collected by interview and questionnaire. Peripheral blood samples were obtained from all study participants and processed for serum, plasma and DNA.

Assessment of smoking status

Smoking status was self-reported at the time of serum sample collection based on the questions: 1. Have you ever smoked cigarettes; and 2. Do you smoke cigarettes now? In those that reported ever smoking cigarettes, individuals were asked to report the age they started smoking, the total number of years smoked, and how many cigarettes and packs of cigarettes were smoked per day. Individuals were categorized as never smoker if they indicated they had never smoked; current smoker if they indicated they had ever smoked and smoked now; and former smokers if they reported ever smoking but did not smoke at the time of data collection. Pack-years were calculated as number of packs smoked per day multiplied by the number of years smoked.

Autoantibody assays

Antinuclear antibodies (ANA) (IIF with positive 1:120) were detected using a HEp-2 indirect immunofluorescent assay (INOVA Diagnostics, San Diego, California, USA) according to manufacturer instructions. All sera were tested for antibodies against extractable nuclear antibodies (ENA) by the Bio-Rad Bioplex ANA 2200 for the following autoantibody specificities: chromatin, dsDNA, ribosomal P, Sm, Sm/ribonucleoprotein (nRNP), nRNP A, nRNP 68, 60 kD Ro (SS-A 60), 52 kD Ro (SS-A 52), La (SS-B), Scl-70, Centromere B and Jo-1. As Scl-70, Centromere B and Jo-1 are autoantibodies more commonly found in systemic sclerosis, limited sclerosis and myositis respectively, they were excluded from analyses, as previously reported ³².

Statistical analyses

All analyses were performed in SAS 9.2 (Cary, NC). T-tests for continuous variables and chi-square tests for categorical variables were used to determine demographic differences between subjects with SLE and FDRs. Generalized estimating equations (GEE), accounting for correlation within families, were used to assess associations between a categorical measure of smoking status and the categorical outcomes of SLE status, being positive for at least one autoantibody detected by the BioRad assay, and for being positive for each of the 10 autoantibodies individually. Odds ratios and 95 % confidence intervals were determined. Models were adjusted for age, gender and race/ethnicity. Possible confounders such as diagnosis of rheumatoid arthritis and socio-economic status were examined and found not to be associated with either smoking or autoantibody status, and were therefore not included in these analyses.

Results

Unaffected FDRs were on average older, more likely to be male, and more likely to be of European American ancestry than individuals with SLE (Table 1). Unaffected FDRs were more likely to be ever smokers than subjects with SLE and healthy controls ($p=0.03$). While all groups started smoking around 17 years of age, subjects with SLE and healthy controls smoked for fewer years and had fewer pack-years of smoking than unaffected FDRs ($p<0.0001$). Among unaffected FDRs, 24.4% tested positive for 1 or more lupus-associated autoantibodies by bead based assays, while 14.5% of healthy controls tested positive. The proportion of individuals positive for each of the SLE-related autoantibodies by smoking status is shown in Figure 1. The largest proportions of ever smokers in the unaffected FDRs and healthy controls were positive for ANA, dsDNA, Chromatin, nRNP A, Ro/SS-A 60 and Ro/SS-A 52 autoantibody responses.

After adjusting for age, gender, and race/ethnicity, and accounting for correlation within families, smoking status is significantly associated with having SLE compared to unaffected FDRs (Figure 2); this association is largely driven by confounding with age, as there was no unadjusted association between smoking status and having SLE, and no indication of effect modification by age when examining age-stratified results. Compared to unaffected FDRs, subjects with SLE were 1.43 times more likely to be former smokers than never smokers (95% CI 1.12–1.81). No significant associations were found between pack-years of smoking and being positive for at least one autoantibody.

We then examined whether positivity for an autoantibody was associated with smoking status within the SLE and FDR cohorts. No significant associations between smoking and being positive for at least 1 autoantibody were found in FDRs, after adjusting for age, gender, race/ethnicity and accounting for correlation within families (Table 2). In subjects with SLE, those who were positive for 1 or more autoantibodies by bead-based assay detection were 1.53 times more likely to be a current smoker than a never smoker (95% CI 1.04–2.24).

After adjusting for age, gender, race/ethnicity, and accounting for correlation within families, no consistent association was identified between smoking and individual

autoantibodies, including ANA (Table 3). Due to small cell numbers, models examining former smokers compared to never smokers could not be run in FDRs nor former smokers or current smokers compared to never smokers in healthy controls for anti-nRNP 68 antibody. In subjects with SLE, those who ever smoked were less likely to be positive for ribosomal P (OR=0.64, 95% CI 0.43–0.94). No significant associations with ever smoking were found in unaffected FDRs nor healthy controls. In subjects with SLE, current smokers were more likely to have anti-nRNP A autoantibodies than never smokers (OR=1.46, 95% CI 1.01–2.11), but less likely to be positive for anti-nRNP 68 autoantibody (OR=0.37, 95% CI 0.20–0.68). In unaffected FDRs, former smokers were 1.98 times more likely than never smokers to be positive for anti-60kD Ro autoantibody (95% CI 1.09–3.61). Similar associations between individual autoantibodies were found when examining pack-years instead of categorical smoking variables.

Discussion

In our cohort of subjects with SLE, unaffected FDRs, and healthy controls, we did not see clear associations between smoking and autoantibody production. Smoking is a risk factor for both rheumatoid factor (RF) positive^{9, 13, 14, 17} and anti-citrullinated protein antibodies (ACPA) positive RA^{10, 12, 16, 18, 19}, with reported risk strongest in individuals at increased genetic risk^{10, 11, 15}. Assuming a similar pattern in other autoimmune diseases, one would expect to see more of an association with smoking and autoantibodies in both subjects with SLE and unaffected FDRs. We found a few significant associations, indicating that low power was not the reason for lack of associations. In addition, there was not a trend in our non-significant associations to suggest an underlying mechanism. One hypothesis is that through ineffective clearance of apoptotic and necrotic cells as a result of smoking, loss of self-tolerance^{20–22} leads to autoantibody production. The analyses with ever smoking and pack-years did not indicate that a break in tolerance was occurring in regards to smoking exposure in our cohort. We did not see an association between ever smoking and being positive for 1 autoantibody in our subjects with SLE, nor with any individual autoantibodies in our unaffected FDRs and healthy controls. In addition, we saw a similar lack of association between increasing pack-years and autoantibody positivity.

Increased seropositivity for anti-Ro/SS-A60 was associated with former smoking in our unaffected FDRs, but no significant association with any other autoantibody specificity was found. An increased rate of anti-nRNP A seropositivity, as well as a decreased association with anti-nRNP 68 positivity, was found in current smoking in SLE subjects. The decreased association with anti-nRNP 68 autoantibodies is interesting as individuals positive for anti-nRNP 68 are clinically more likely to have Raynaud's syndrome, which is worsened by smoking, and may therefore indicate that these individuals are actively quitting smoking due to clinical symptoms. The direction of these associations may indicate that autoantibody production due to smoking prior to disease onset is unlikely in our collection. However, we could not look at associations in former smokers compared to never smokers in FDRs for anti-nRNP-68 responses, so a relationship may have been missed between smoking status and positivity for this autoantibody.

An association between being a current smoker and being positive for one or more SLE-related autoantibodies was found only in SLE patients. Smoking has been reported to suppress IgG autoantibody development but leads to higher levels of autoantibodies after smoking was discontinued³³, which was found in both murine models of SLE as well as human SLE patients. Although the study was small and no further follow-up could be found, these results may indicate that former, not current, smokers are most likely to have increased autoantibody production. Since the association with 1 autoantibody was not seen in the unaffected FDRs, and SLE subjects were less likely than FDRs to smoke, smoking may not provide an initial insult leading to SLE. Rather, smoking may lead to a possible exacerbation of autoantibody production in individuals who smoke after SLE diagnosis, which may reflect the reported association between smoking and increased disease activity^{34–37}. However, no significant associations were observed in being positive for more than 2 or more than 3 SLE-related autoantibodies in the subjects with SLE, possibly indicating that the number of autoantibodies one is positive for is not directly related to smoking. Nevertheless, it may be of interest to determine if specific groupings of autoantibodies pertinent to SLE are associated with smoking status.

Previously, an increased risk was reported between current smoking and dsDNA autoantibody positivity compared to both former and never smokers in SLE subjects⁷. We did not see an association with anti-dsDNA in SLE subjects in this cohort. While both studies used self-report to assess smoking status, our population reported higher levels of both former and current smoking, but less dsDNA seropositive (30% in our study compared to 56% in the previous study). This may be due to the attenuation of anti-dsDNA titers by pharmacological treatment of SLE, the long-standing nature of disease in this collection, different methods of autoantibody detection, other unknown factors, or a combination of these. Nevertheless, we also did not observe this association in our unaffected FDRs and unrelated controls, who are unlikely to be affected by treatment. Moreover, the borderline association we did see within our FDRs indicated that current smoking was potentially protective for anti-dsDNA positivity compared to never smokers. Our population is more racially/ethnically diverse compared to the previous study, which examined only white individuals. Differences in genetics may play a role in this association, although we also did not see an association with dsDNA when limiting our subjects with SLE to those with European ancestry. However, it is possible that gene-environment interactions play a role in the formation of autoantibodies, and is masking an association in our population. It should be noted that the previous study did not find any significant associations when examining other SLE-related autoantibodies, including Sm, Ro, La and nRNP. While they hypothesized that this was due to lack of power, lack of association in both studies may indicate that the effect of smoking on autoantibody production is limited.

Our study found no significant relationship between ANA positivity by immunofluorescence nor by luminex bead-based assay and smoking status, contrary to a previous study which found an association in Sjogren's syndrome⁸. While there were different levels of ANA positivity between our subjects with SLE, our unaffected FDRs and our healthy controls, there were no significant differences by smoking status in the proportion of individuals positive for ANA in any of these groups. Taken with the dsDNA results in our population,

the lack of association may indicate that the formation of autoantibodies from cigarette smoke modification of autoantigens is not the major pathway in SLE pathogenesis.

Although we did not find clear associations between autoantibody positivity and smoking status, we found an association between smoking status and SLE, after adjusting for age, gender, and ethnicity, compared to both unaffected FDRs and healthy controls. As in previous studies conducted with unaffected population controls³⁻⁶, current smokers were at increased risk of having SLE than our healthy controls. In addition, contrary to these other studies, we found an association in former smokers as well. Our results suggest that the association seen in our subjects with SLE may manifest itself outside of autoantibody production.

While we rely on self-report to ascertain an individuals' smoking status, cotinine levels validate this measure. Cotinine levels in a small subset of these patients and FDRs suggest that less than 5% of individuals may misidentify themselves as never smokers on the questionnaire, biasing the association towards the null. We also did not capture information regarding second-hand smoke exposure.

To our knowledge, this is the first study to examine the association of smoking with autoantibodies in a group of unaffected FDRs at increased risk of SLE and unrelated, unaffected controls, as well as to provide information on the association with a large number of specific autoantibodies in SLE subjects. We saw associations between smoking and anti-Ro/SS-A60 autoantibody in our unaffected FDRs and with anti-nRNP A and anti-nRNP 68 autoantibodies in our subjects with SLE, which is notable as anti-Ro/SS-A60 and anti-nRNP A were among the first autoantibodies detected in the earliest pre-clinical serum samples of subjects with SLE³⁸. If we are observing FDRs very early in their pre-clinical disease, further follow-up of autoantibody positivity and smoking status may be reasonable. Additionally, several gene-smoking interactions have been reported to be associated with autoantibodies in RA^{39, 40}, and examination of similar interactions as well as through examining racial/ethnic differences should be carried out in our population. In addition, further exploration of other pathophysiologic pathways responsible for the smoking and SLE association is warranted. These include examining the influence of smoking on immunosuppressive properties and lymphocyte function^{41, 42}, possibly increasing susceptibility to infectious triggers. The pro-inflammatory effects of smoking should also be examined, including the free radicals within cigarette smoke and those generated by cigarette smoke,⁴³ as well as the influence on elevated T-cells⁴⁴. In conclusion, the association of smoking with SLE may manifest its risk through mechanisms outside of autoantibody production, at least for the specificities tested.

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Figure 1.

Proportion of **A)** subjects with SLE, **B)** unaffected first-degree relatives and **C)** unrelated, unaffected controls who are positive for each of the SLE-related autoantibodies, by smoking status. Significant differences ($p<0.05$) by smoking status are marked by an asterisk (*). ANA was measured by indirect immunofluorescence and the rest of the autoantibodies were measured by bead-based assays

Figure 2. Association between smoking status and systemic lupus erythematosus. **A).** Subjects with SLE were more likely to be former smokers than unaffected FDRs. **B).** Subjects with SLE were more likely to be current or former smokers than healthy controls. **C).** Unaffected FDRs were more likely to be current smokers than healthy controls. Generalized estimating equations, accounting for correlation within families, were used, adjusting for age, gender, and race/ethnicity. Significant associations are noted in bold.

Table 1

Demographics of Subjects with Systemic Lupus Erythematosus, Unaffected First-Degree Relatives, and Unrelated Unaffected Controls.

Variable (n, %)	SLE Subjects (n=1242)	Unaffected FDRs (n=981)	Healthy Controls (n=946)	p-value
Age (years): mean (median) $\pm$ sd	41.7 (42.2) $\pm$ 13.2	56.3 (56.9) $\pm$ 16.3	41.7 (42.0) $\pm$ 13.2	<0.0001
Gender:				
Female	1107 (89.3%)	616 (62.8%)	655 (69.2%)	<0.0001
Male	135 (10.7%)	365 (37.2%)	291 (30.8%)	
Race:				
European American	631 (50.8%)	596 (60.7%)	509 (53.8%)	<0.0001
African American	325 (26.2%)	196 (20.0%)	255 (27.0%)	
Hispanic	213 (17.2%)	142 (14.5%)	136 (14.3%)	
Other ^a	73 (5.9%)	47 (4.8%)	46 (4.9%)	
Ever Smoker:				
No	637 (51.3%)	458 (46.7%)	514 (54.3%)	0.03
Yes	605 (48.7%)	523 (53.3%)	432 (45.7%)	
Smoker:				
Never	637 (51.3%)	458 (46.7%)	514 (54.3%)	0.09
Former	381 (30.7%)	324 (33.0%)	261 (27.6%)	
Current	224 (18.0%)	199 (20.3%)	171 (18.1%)	
Age started smoking ^b : mean (median) $\pm$ sd	17.3 (16.0) $\pm$ 4.3	17.8 (17.0) $\pm$ 5.2	17.6 (17.0) $\pm$ 5.4	0.11
Years smoked ^c : mean (median) $\pm$ sd	16.9 (15.0) $\pm$ 12.2	24.2 (21.0) $\pm$ 16.0	16.1 (15.0) $\pm$ 12.1	<0.0001
Pack-years of smoking ^c : mean (median) $\pm$ sd	17.0 (12.0) $\pm$ 17.0	25.7 (20.0) $\pm$ 22.0	15.4 (10.0) 16.9	<0.0001
Age at diagnosis: mean (median) $\pm$ sd	30.4 (34.0) $\pm$ 11.8	N/A	N/A	N/A
SLE antibody count ^d : mean (median) $\pm$ sd	2.6 (2.0) $\pm$ 2.4	0.4 (0.0) $\pm$ 0.8	0.2 (0.0) $\pm$ 0.6	<0.0001
SLE antibody count ^d :				
0	287 (23.1%)	742 (75.6%)	809 (85.5%)	<0.0001
1	955 (76.9%)	239 (24.4%)	137 (14.5%)	

^a Other includes American Indian, Alaskan Native, Asian or Pacific Islander.

^b Data on age at initiation of smoking was missing on 46 of the SLE individuals who smoked, 63 of the FDRs who smoked, and 47 unrelated unaffected controls.

^c Data on the number of years smoked and pack-years of smoking was missing on 49 individuals with SLE, 144 FDRs who smoked, and 50 unrelated unaffected controls.

^d SLE antibody count includes only the 10 SLE-associated autoantibodies as detected by Bio-Rad Bioplex 2200 (luminex bead based assay).

Table 2

Association between Smoking Status and Autoantibody Status (Positivity for α 1 Ab) in in Subjects with SLE, Unaffected FDRs and Unrelated Unaffected Controls.

Smoking Status	Outcome: Positive for α 1 Ab		
	In SLE subjects Odds ratio (95% CI)	In Unaffected FDRs Odds ratio (95% CI)	In Healthy Controls Odds Ratio (95% CI)
Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
Current smoker	1.53 (1.04–2.24)	0.69 (0.42–1.11)	0.90 (0.53–1.53)
Former smoker	0.95 (0.70–1.30)	1.09 (0.76–1.56)	1.17 (0.76–1.81)
Ever smoker	1.15 (0.87–1.51)	0.91 (0.66–1.26)	1.04 (0.71–1.52)

Generalized estimating equations, accounting for correlation within families, were used, adjusting for age, gender, and race/ethnicity.

Significant associations are noted in bold.

Table 3

Association of Smoking Status and Individual Autoantibodies in Subjects with SLE, Unaffected FDRs, and Unrelated Unaffected Controls.

		Outcome: Positivity for Ab		
Autoantibody	Smoking Status	In SLE subjects Odds ratio (95% CI)	In Unaffected FDRs Odds ratio (95% CI)	In Healthy Controls Odds ratio (95% CI)
ANA	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	1.00 (0.56–1.80)	1.18 (0.84–1.67)	1.02 (0.62–1.67)
	Former smoker	1.13 (0.66–1.94)	1.13 (0.86–1.50)	1.24 (0.82–1.88)
	Ever smoker	1.06 (0.67–1.67)	1.16 (0.90–1.50)	1.10 (0.77–1.59)
dsDNA	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	0.87 (0.60–1.26)	0.35 (0.12–1.04)	0.34 (0.04–2.80)
	Former smoker	0.95 (0.71–1.28)	0.71 (0.37–1.34)	1.90 (0.71–5.07)
	Ever smoker	0.92 (0.72–1.20)	0.57 (0.32–1.00)	1.34 (0.52–3.46)
Chromatin	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	0.96 (0.68–1.34)	0.97 (0.50–1.87)	0.87 (0.38–2.00)
	Former smoker	1.13 (0.86–1.50)	0.98 (0.61–1.59)	1.25 (0.64–2.45)
	Ever smoker	1.08 (0.84–1.38)	0.94 (0.58–1.51)	1.04 (0.58–1.88)
Ribosomal P	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	0.58 (0.33–1.01)	0.46 (0.05–4.03)	0.84 (0.17–4.29)
	Former smoker	0.66 (0.42–1.05)	0.63 (0.12–3.35)	1.89 (0.59–6.02)
	Ever smoker	0.64 (0.43–0.94)	0.52 (0.12–2.20)	1.49 (0.51–4.40)
Sm	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	1.13 (0.75–1.71)	1.86 (0.57–6.10)	0.42 (0.05–3.77)
	Former smoker	1.04 (0.73–1.46)	0.19 (0.02–1.89)	1.34 (0.36–5.02)
	Ever smoker	1.09 (0.81–1.48)	0.83 (0.24–2.87)	0.98 (0.28–3.43)
Sm RNP	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	1.18 (0.82–1.71)	0.58 (0.19–1.82)	1.60 (0.52–4.89)
	Former smoker	1.03 (0.76–1.40)	0.97(0.36–2.56)	0.73 (0.22–2.48)
	Ever smoker	1.11 (0.85–1.45)	0.82 (0.36–1.90)	1.11 (0.43–2.88)
RNP A	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	1.46 (1.01–2.11)	1.03 (0.41–2.55)	0.79 (0.25–2.43)
	Former smoker	1.22 (0.89–1.68)	1.05 (0.52–2.09)	0.76 (0.28–2.05)
	Ever smoker	1.31 (1.00–1.71)	1.06 (0.56–2.01)	0.79 (0.34–1.83)
RNP 68	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	0.37 (0.20–0.68)	1.00 (0.10–10.37)	*
	Former smoker	0.82 (0.53–1.26)	*	*
	Ever smoker	0.66 (0.45–0.96)	0.44 (0.04–4.83)	*
Ro/SS-A60	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	1.21 (0.87–1.70)	1.15 (0.55–2.42)	1.65 (0.49–5.59)
	Former smoker	0.94 (0.70–1.26)	1.98 (1.09–3.61)	0.25 (0.03–2.00)
	Ever smoker	1.03 (0.80–1.34)	1.55 (0.90–2.65)	0.80 (0.26–2.48)
Ro/SS-A52	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)

Outcome: Positivity for Ab				
Autoantibody	Smoking Status	In SLE subjects Odds ratio (95% CI)	In Unaffected FDRs Odds ratio (95% CI)	In Healthy Controls Odds ratio (95% CI)
La/SS-B	Current smoker	0.90 (0.61–1.35)	1.11 (0.48–2.57)	1.40 (0.26–7.51)
	Former smoker	0.98 (0.71–1.35)	1.22 (0.62–2.40)	1.75 (0.45–6.87)
	Ever smoker	0.94 (0.71–1.26)	1.14 (0.63–2.08)	1.65 (0.48–5.62)
	Never smoker	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Current smoker	1.10 (0.70–1.73)	1.01 (0.37–2.76)	0.51 (0.11–2.37)
	Former smoker	0.85 (0.58–1.24)	2.11 (0.95–4.72)	1.46 (0.57–3.72)
	Ever smoker	0.94 (0.67–1.32)	1.56 (0.75–3.27)	1.10 (0.46–2.65)

* Cannot calculate as there are not enough individuals positive for the autoantibody.

Generalized estimating equations, accounting for correlation within families, were used, adjusting for age, gender, and race/ethnicity.

Significant associations are noted in bold.