Interaction between the STAT4 rs11889341(T) risk allele and smoking confers increased risk of myocardial infarction and nephritis in patients with systemic lupus erythematosus

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ABSTRACT

Objective To investigate how genetics influence the risk of smoking-related systemic lupus erythematosus (SLE) manifestations.

Methods Patients with SLE (n=776, replication cohort =836) were genotyped using the 200K Immunochip single nucleotide polymorphisms (SNP) Array (Illumina) and a custom array. Sixty SNPs with SLE association (p<5.0×10−8) were analysed. Signal transducer and activator of transcription 4 (STAT4) activation was assessed in in vitro stimulated peripheral blood mononuclear cells from healthy controls (n=45).

Results In the discovery cohort, smoking was associated with myocardial infarction (MI) (OR 1.96 (95% CI 1.09 to 3.55)), with a greater effect in patients carrying any rs11889341 STAT4 risk allele (OR 2.72 (95% CI 1.24 to 6.00)) or two risk alleles (OR 8.27 (95% CI 1.48 to 46.27)). Smokers carrying the risk allele also displayed an increased risk of nephritis (OR 1.47 (95% CI 1.06 to 2.03)). In the replication cohort, the high risk of MI in smokers carrying the risk allele and the association between the STAT4 risk allele and nephritis in smokers were confirmed (OR 6.19 (95% CI 1.29 to 29.79) and 1.84 (95% CI 1.05 to 3.29), respectively). The interaction between smoking and the STAT4 risk allele resulted in further increase in the risk of MI (OR 2.14 (95% CI 1.01 to 4.62)) and nephritis (OR 1.53 (95% CI 1.08 to 2.17)), with 54% (MI) and 34% (nephritis) of the risk attributable to the interaction. Levels of interleukin-12-induced phosphorylation of STAT4 in CD8+ T cells were higher in smokers than in non-smokers (mean geometric fluorescence intensity 1063 vs 565, p=0.0063). Lastly, the IL12A rs564799 risk allele displayed association with MI in both cohorts (OR 1.53 (95% CI 1.01 to 2.31) and 2.15 (95% CI 1.08 to 4.26), respectively).

Conclusions Smoking in the presence of the STAT4 risk gene variant appears to increase the risk of MI and nephritis in SLE. Our results also highlight the role of the IL12–STAT4 pathway in SLE-cardiovascular morbidity.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a disease characterised by loss of tolerance to self-antigens, formation of immune complexes and an activated type I interferon (IFN) system.1 A widely accepted view of the aetiology of SLE is that environmental factors trigger the disease in genetically susceptible individuals. The genetic background is complex, with more than 100 single nucleotide polymorphisms (SNPs) associated with risk...
for SLE. Exposure to certain environmental factors, including ultraviolet radiation and viral infections, is associated with SLE development and flare-ups of the disease. Several studies have evaluated smoking as a risk factor for SLE, with the largest meta-analysis to date showing a modest risk increase. While the results are not confirmed in prospective studies, both Cozier and Barbhaiya et al observed a trend of increased risk in smokers. The most extensive prospective study involving 286 cases with SLE demonstrated an association between smoking and development of SLE with increased anti-dsDNA, but no risk of overall SLE.

Although death from active SLE has decreased since the 1950s, the mortality rate still exceeds that of the general population, with cardiovascular morbidity remaining considerably high and a strong risk factor for premature mortality. Both traditional and SLE-related risk factors, such as hypertension, nephritis and high disease activity have been identified as risk factors, but cannot fully account for the excess cardiovascular disease (CVD) risk seen in patients with SLE.

To fully explain the aetiology of SLE or its comorbidities such as CVD, gene–gene or gene–environment interactions may be essential to the aetiology of SLE or its comorbidities such as CVD, gene–environment interactions may be essential to the aetiology of SLE or its comorbidities such as CVD.

**METHODS**

**Patients of the discovery and replication cohort**

The discovery cohort included 774 patients with SLE from Sweden. The replication cohort included 836 patients from Norway and Denmark. All subjects fulfilled ≥4 American College of Rheumatology (ACR)—82 and ACR-97 classification criteria for SLE and were of European descent. Clinical characteristics of the cohorts are described in Table 1 and online supplemental.

**Table 1** Prevalence of clinical manifestations in smokers (n=371) and non-smokers (n=387) in the discovery cohort

| Smokers, n (%) | Non-smokers, n (%) | OR (95% CI) | P value |
|---------------|-------------------|-------------|---------|
| Age at last follow-up, mean (SD) | 55 (15) | 50 (17) | 1.00 (0.74 to 1.34) | 0.97 |
| Disease duration, mean (SD) | 17 (11) | 16 (12) | 0.96 (0.68 to 1.36) | 0.81 |
| Male sex | 49 (13) | 48 (13) | 0.94 (0.61 to 1.45) | 0.79 |
| Deceased at follow-up | 55 (15) | 39 (11) | 1.19 (0.75 to 1.88) | 0.45 |

ACR 1982 classification criteria:

1. Malar rash
   - Smokers: 205 (55)
   - Non-smokers: 211 (57)
   - OR: 1.00 (0.74 to 1.34)
   - P value: 0.97

2. Discoid rash
   - Smokers: 83 (22)
   - Non-smokers: 80 (21)
   - OR: 0.96 (0.68 to 1.36)
   - P value: 0.81

3. Photosensitivity
   - Smokers: 262 (71)
   - Non-smokers: 246 (65)
   - OR: 1.27 (0.93 to 1.74)
   - P value: 0.13

4. Oral ulcer
   - Smokers: 103 (28)
   - Non-smokers: 96 (25)
   - OR: 1.20 (0.86 to 1.67)
   - P value: 0.27

5. Arthritis
   - Smokers: 306 (82)
   - Non-smokers: 301 (81)
   - OR: 1.11 (0.76 to 1.61)
   - P value: 0.59

6. Serositis
   - Smokers: 179 (48)
   - Non-smokers: 165 (45)
   - OR: 1.10 (0.82 to 1.47)
   - P value: 0.52

7. Renal disorder
   - Smokers: 129 (35)
   - Non-smokers: 132 (36)
   - OR: 1.10 (0.80 to 1.50)
   - P value: 0.56

8. Neurological disorder
   - Smokers: 33 (9)
   - Non-smokers: 40 (10)
   - OR: 0.86 (0.52 to 1.40)
   - P value: 0.54

9. Haematological disorder
   - Smokers: 213 (57)
   - Non-smokers: 258 (70)
   - OR: 0.62 (0.46 to 0.84)
   - P value: 0.0021

10. Immunological disorder
    - Smokers: 245 (66)
    - Non-smokers: 256 (69)
    - OR: 0.97 (0.71 to 1.33)
    - P value: 0.86

11. Anti-dsDNA
    - Smokers: 224 (61)
    - Non-smokers: 231 (63)
    - OR: 1.02 (0.76 to 1.38)
    - P value: 0.88

| Renal variables |
|-----------------|
| WHO class I–II |
| Smokers: 12 (5) |
| Non-smokers: 20 (8) |
| OR: 0.71 (0.33 to 1.50) |
| P value: 0.37 |
| WHO class III–IV |
| Smokers: 62 (21) |
| Non-smokers: 71 (22) |
| OR: 1.13 (0.75 to 1.69) |
| P value: 0.57 |
| WHO class V |
| Smokers: 15 (6) |
| Non-smokers: 16 (6) |
| OR: 1.05 (0.50 to 2.20) |
| P value: 0.90 |
| Other* |
| Smokers: 11 (4) |
| Non-smokers: 6 (2) |
| OR: 1.24 (0.50 to 3.06) |
| P value: 0.65 |
| ESRD |
| Smokers: 364 (98) |
| Non-smokers: 367 (99) |
| OR: 0.69 (0.19 to 2.49) |
| P value: 0.57 |

| Cardiovascular events |
|-----------------------|
| MI |
| Smokers: 39 (11) |
| Non-smokers: 19 (5) |
| OR: 1.96 (1.09 to 3.55) |
| P value: 0.025 |
| ICVD |
| Smokers: 45 (12) |
| Non-smokers: 30 (8) |
| OR: 1.37 (0.84 to 2.24) |
| P value: 0.21 |
| VTE |
| Smokers: 61 (16) |
| Non-smokers: 52 (14) |
| OR: 1.14 (0.76 to 1.71) |
| P value: 0.52 |
| Clinical APS |
| Smokers: 68 (20) |
| Non-smokers: 61 (18) |
| OR: 1.08 (0.73 to 1.60) |
| P value: 0.68 |
| Anti-β2GP-I IgG |
| Smokers: 58 (19) |
| Non-smokers: 57 (18) |
| OR: 0.98 (0.69 to 1.39) |
| P value: 0.91 |
| Anti-β2GP-I IgM |
| Smokers: 8 (11) |
| Non-smokers: 11 (12) |
| OR: 0.96 (0.36 to 2.55) |
| P value: 0.93 |
| LA |
| Smokers: 62 (23) |
| Non-smokers: 57 (21) |
| OR: 1.19 (0.79 to 1.80) |
| P value: 0.40 |
| aCL-IgG |
| Smokers: 86 (26) |
| Non-smokers: 90 (28) |
| OR: 1.05 (0.70 to 1.59) |
| P value: 0.80 |
| aCL-β2GP-I |
| Smokers: 34 (14) |
| Non-smokers: 33 (13) |
| OR: 0.96 (0.36 to 2.55) |
| P value: 0.93 |

Logistic regression models were used to assess differences between smokers and non-smokers. All analyses were adjusted for age at last follow-up and disease duration. P<0.05 (unadjusted for multiple comparisons) in bold.

*Patients with biopsies displaying signs of nephritis but not meeting the criteria for any of the above classes were classified as other.

ACR, American College of Rheumatology; ANA, antinuclear antibodies; Anti-β2GP-I, anti-β2-glycoprotein I; APS, antiphospholipid syndrome; dsDNA, double-stranded DNA; ESRD, end-stage renal disease; ICVD, ischaemic cerebrovascular disease; LA, lupus anticoagulant; MI, myocardial infarction; VTE, venous thromboembolism.
Genotyping and selection of SNPs

Genotyping of the discovery cohort was performed using the Illumina 200K Immunochip SNP array, for details, see online supplemental file. SNPs previously associated with SLE at genome-wide significance in the European population were selected. For SNPs not included on the Immunochip, the SNPs were filtered for independent signals, removing the variant with the lowest SLE-OR for SNPs in LD (r² ≥ 0.96) was selected. All SNPs were investigated for associations with MI (online supplemental table 2). Individuals in the replication cohort were genotyped for three single nucleotide variants using a custom assay on the MassARRAY system (see online supplemental file).

Interleukin-12-induced phosphorylation of STAT4

Interleukin 12 (IL-12)-induced phosphorylation of signal transducer and activator of transcription 4 (pSTAT4) was previously determined in 72 healthy blood donors from Uppsala Bioresource using flow cytometry. Smoking data were available from 45 of these donors, of which 20 were past or current smokers and 25 were non-smokers.

Statistical analysis

To investigate associations between smoking and clinical manifestations, logistic regression models were used. As smoking was associated with longer disease duration and higher age at follow-up, these variables were included as covariates. In addition, patients carrying two alleles of both the STAT4 and the IL12A risk variants displayed independent, positive association with MI (table 2). In contrast, patients carrying one risk allele of both the STAT4 and the IL12A risk alleles displayed a substantial higher prevalence of MI compared with those with any other allele combination (27% vs 7%) (OR 5.88 (95% CI 2.44 to 14.17), p=0.00068) (figure 1A).

Next, we stratified patients by smoking status to determine whether each of the three SNPs displayed stronger association with MI in smokers. No significant associations were found for the NCF2 or IL12A risk alleles (OR 1.58 (95% CI 0.89 to 2.78), p=0.12 and OR 1.36 (95% CI 0.89 to 2.08), p=0.15, respectively). However, the STAT4 risk allele demonstrated a stronger association in smokers (OR 2.45 (95% CI 1.46 to 4.19), p=0.00086) (figure 2A). Next, we assessed the association between smoking and MI in patients carrying the STAT4 risk allele and smoking observed in the discovery dataset through addition of a STAT4-smoking interaction term in the logistic models and by calculating the attributable proportion due to interaction, respectively. Differences in levels of pSTAT4 were assessed by Student’s t-test and by a linear regression model allowing adjustment for age and the STAT4 risk allele. PLINK was used for all analyses except the meta-analyses which were performed in

RESULTS

Smoking is modestly associated with MI

Initially, we assessed the association between smoking and clinical manifestations (table 1). We found no evidence of any associations between smoking and the ACR criteria, except the haematological criterion, which was less prevalent in smokers (table 1). Elevated levels of red and white blood cells in smokers is a well-known phenomenon. Smoking was not associated with a history of DVT or ICVD, however, a significant association between smoking and MI was observed (OR 1.96 (95% CI 1.09 to 3.55), p=0.025) (table 1).

Increased risk of MI in SLE-smokers with the STAT4 risk allele

Next, we asked whether there are subgroups of patients in which smoking plays a more prominent role in MI development. We initially examined 60 SNPs with established association with SLE (p<5.0×10⁻⁸) for association with MI (online supplemental table 2). We found that the Neutrophil Cytosolic Factor 2 (NCF2), Interleukin-12A (IL12A) and STAT4 risk alleles demonstrated a substantial higher prevalence of MI compared with those with any other allele combination (27% vs 7%) (OR 5.88 (95% CI 2.44 to 14.17), p=0.00068) (figure 1A).

Next, we stratified patients by smoking status to determine whether each of the three SNPs displayed stronger association with MI in smokers. No significant associations were found for the NCF2 or IL12A risk alleles (OR 1.58 (95% CI 0.89 to 2.78), p=0.12 and OR 1.36 (95% CI 0.89 to 2.08), p=0.15, respectively). However, the STAT4 risk allele demonstrated a stronger association in smokers (OR 2.45 (95% CI 1.46 to 4.19), p=0.00086) (figure 2A).

Next, we assessed the association between smoking and MI in patients carrying the STAT4 risk allele and smoking observed in the discovery dataset through addition of a STAT4-smoking interaction term in the logistic models and by calculating the attributable proportion due to interaction, respectively.
in patients without the risk allele (OR 1.20 (95% CI 0.49 to 2.96) p=0.53). As patients with nephritis have previously been shown to have a higher prevalence of both MI and the STAT4 risk allele,\textsuperscript{25-27} we hypothesised that the results would be similar if using nephritis, rather than MI, as the outcome variable. Without stratifying for smoking, the association between the STAT4 risk allele and nephritis reached suggestive significance (OR 1.23 (95% CI 0.98 to 1.54), p=0.072). The effect was more pronounced in the smokers only (OR 1.47 (95% CI 1.06 to 2.03), p=0.020). In addition, we found moderate evidence that patients with nephritis carrying the STAT4 risk allele were at a greater risk of developing ESRD (OR 1.85 (95% CI 0.96 to 3.59), p=0.068), and this risk was enhanced in smokers (OR 2.52 (95% CI 1.04 to 6.10), p=0.040) (figure 2B). Of note, despite the non-smoking group including more patients with nephritis (n=140 vs n=129), no evidence of an association between the STAT4 risk allele and nephritis or ESRD could be demonstrated in this group (OR 1.07 (95% CI 0.77 to 1.46), p=0.70 and OR 1.10 (95% CI 0.38 to 3.16), p=0.86, respectively). To validate our significant findings, we performed the same analyses in an independent cohort of patients with SLE (online supplemental table 1). Analysis of the genetic variants demonstrated that the IL12A risk allele was the only gene variant significantly associated with MI when not accounting for smoking (table 2). Patients with two risk alleles of both STAT4 and IL12A (n=28, 3.2% of the patients) were found to have a significantly higher prevalence of MI than patients without this combination of risk alleles (OR 7.21 (95% CI 1.36 to 38.27), p=0.020) (figure 1B). Similar to in the discovery cohort, we found a significant association between the STAT4 risk allele and MI in smokers (OR 2.11 (95% CI 1.04 to 4.26), p=0.038). In addition, smoking was associated with MI in patients carrying the STAT4 risk allele (OR 6.19 (95% CI 1.29 to 29.79), p=0.023). No evidence of these associations could be observed in the non-smoking group (OR 0.58 (95% CI 0.09 to 3.90), p=0.58 and OR 1.32 (95% CI 0.23 to 7.34), p=0.75, respectively). In patients carrying two risk alleles (n=51), the logistic regression could not be performed due to a ‘perfect separation’ between groups, with 9% of smokers having had a MI compared with 0% of never-smokers. As in the discovery cohort, we found an association between the STAT4 risk allele and nephritis in smokers (OR 1.84 (95% CI 1.05 to 3.29), p=0.035), whereas the effect size was non-significant in non-smokers (OR 0.78 (95% CI 0.41 to 1.45), p=0.44).

Meta-analysis of the two cohorts showed the risk of MI to be more than double with each additional STAT4 risk allele in smokers (OR 2.28, p=0.00010). In contrast, no association could be detected in the never-smoker group (OR 0.80, p=0.52). Similarly, in meta-analysis of patients with 2 STAT4 risk alleles (n=282, 22%), smoking was found to be a strong risk factor for MI (OR 8.27, p=0.016). For nephritis, each STAT4 risk allele increased the risk by ~50% in smokers (OR 1.52, p=0.00051), whereas no increase in risk was found in the non-smoker group (OR 0.82, p=0.33).

**Interaction between STAT4 risk allele and smoking results in a higher risk of MI and nephritis**

We subsequently performed interaction analyses on all patients and found a significant multiplicative interaction between the STAT4 risk allele and smoking on the development of MI (OR 2.14 (95% CI 1.01 to 4.62), p=0.049) as well as nephritis (OR 1.53 (95% CI 1.08 to 2.17), p=0.020) (online supplemental table 3). Next, we examined additive interaction and observed an attributable proportion due to interaction of 0.54 (95% CI 0.24 to 0.83, p=0.00019) and 0.34 (95% CI 0.080 to 0.61, p=0.0051) for MI and nephritis, respectively.

To determine whether the effect of the STAT4 risk allele on nephritis in smokers could explain the association with MI, we performed stratification of the combined dataset and investigated the association between the STAT4 risk allele and MI in smokers and non-smokers without nephritis. In the smokers, the association between the STAT4 risk allele and MI remained significant (n=428, OR 2.43 (95% CI 1.40 to 4.27), p=0.0017) (figure 3). Similarly, the association between the STAT4 risk allele and nephritis in smokers remained significant after excluding patients with MI from the analysis (n=623, OR 1.54 (95% CI 1.20 to 1.98), p=0.00076).

Lastly, as both SLE risk alleles in STAT4 and smoking have shown association with the development of aPL in previous studies,\textsuperscript{28,29} we assessed the association between the STAT4 risk allele and aPL in smokers, however, it was not significant (OR 1.56 (95% CI 0.71 to 3.72), p=0.26). Next, we performed a multiple regression model in the smoking group including the STAT4 risk allele, any aPL, nephritis, age at follow-up, and disease duration as covariates. We found the association between the STAT4 risk allele and MI to remain significant (OR 3.26 (95% CI 1.15 to 9.20), p=0.026), whereas neither aPL nor
patients with SLE. To investigate whether smoking also demonstrated association with increased cardiovascular damage in patients with SLE, we stratified association with increased cardiovascular damage in patients with SLE. Furthermore, higher levels of STAT4 expression have demonstrated association with increased cardiovascular damage in patients with SLE. To investigate whether smoking also increases the levels of pSTAT4, we analysed IL-12 stimulated CD8+ T cells from healthy blood donors who were smokers (n=20) or never-smokers (n=25). We found the levels of pSTAT4 to be higher in smokers (p=0.0063), with a mean value of 1063 compared with 565 in non-smokers (figure 4). When adjusting for age and the STAT4 risk allele, which is in LD (r^2=1.00) with a STAT4 risk variant previously shown to influence levels of pSTAT4 in these individuals, the association between smoking and levels of pSTAT4 remained significant (β=396, p=0.023).

Levels of activated STAT4 are increased in smokers

The STAT4 risk allele is associated with higher levels of pSTAT4 in CD8+ T cells from SLE patients on stimulation with IL-12. Furthermore, higher levels of STAT4 expression have demonstrated association with increased cardiovascular damage in patients with SLE. To investigate whether smoking also increases the levels of pSTAT4, we analysed IL-12 stimulated CD8+ T cells from healthy blood donors who were smokers (n=20) or never-smokers (n=25). We found the levels of pSTAT4 to be higher in smokers (p=0.0063), with a mean value of 1063 compared with 565 in non-smokers (figure 4). When adjusting for age and the STAT4 risk allele, which is in LD (r^2=1.00) with a STAT4 risk variant previously shown to influence levels of pSTAT4 in these individuals, the association between smoking and levels of pSTAT4 remained significant (β=396, p=0.023).

DISCUSSION

In the present study, we demonstrate that smoking substantially increases the risk of MI in a subset of patients with SLE carrying a variant of the STAT4 SLE-risk gene. In both the discovery and replication cohorts, the effect size increased with an increasing number of STAT4 risk alleles, with smoking giving rise to a more than 8-fold risk of MI in homozygous individuals. We believe that our results add important knowledge in the understanding of how SLE-risk alleles can modulate the effect of traditional risk factors.

The prevalence of MI is higher in SLE patients with nephritis than patients without renal manifestations and SLE-risk alleles in STAT4 have previously been linked to both nephritis and severe renal insufficiency. We, therefore, speculated that the smoking-STAT4 risk allele interaction did not directly affect MI, but rather, was a consequence of an interaction between the STAT4 risk allele and smoking on the development of nephritis. Indeed, we found that this gene-environment combination also results in a higher risk of nephritis, as well as ESRD. Interestingly, however, the STAT4 risk allele/smoking effect on MI did not decrease when adjusting the model for nephritis or when completely removing the patients with nephritis from the analysis. Similarly, the effect of the STAT4 risk allele/smoking on nephritis remained significant after excluding all patients with MI from the analysis, indicating that the associations were independent.

Based on these results, we hypothesised that the increased risk in individuals who smoke and carry the risk allele is connected with the levels of activated STAT4 in these individuals. Hagberg et al have shown that the rs7574863 STAT4 risk allele—which is in perfect LD (r^2=1.00) with the SNP used in this study—is associated with increased levels of pSTAT4 in activated CD8+ T cells of SLE patients. Therefore, we assessed whether smoking elevates pSTAT4 in this cell type and found that the levels were almost twofold higher in smokers. This observation is in line with previous findings by Di Stefano et al, who demonstrated higher levels of pSTAT4 in bronchial T cells from healthy smokers compared with non-smokers. When STAT4 is activated and phosphorylated, it homodimerises and translocates to the nucleus where it induces expression of hundreds of genes, resulting in production of IFN-γ, T-helper type 1 and 17 differentiation and activation of monocytes. Increased STAT4 mRNA expression is associated with increased cardiovascular damage in patients with SLE and several studies on animal models indicate a link between STAT4 and the development of atherosclerosis. The mechanism of how smoking leads to increased levels of activated STAT4 is unclear, however, we speculate that epigenetics may constitute the bridge between smoking, genetics and SLE. It is well known that smoking affects both overall DNA methylation and specific gene promoters. Epigenetic regulation is further believed to play an important role both in cardiovascular biology and in SLE development. Whether smoking is associated with epigenetic changes in SLE-specific genes, and if such changes are associated with specific manifestations of SLE, deserves further studies.

The analyses of individual SLE risk alleles identified the SLE-risk SNP IL12A to be associated with an increased risk of MI, and that patients in the discovery and replication cohort carrying two alleles of both the IL12A and STAT4 risk SNPs had a more than fivefold and eightfold risk of MI, respectively. The IL12A SNP is located within the fourth intron of the IL12A gene, which encodes the p35 subunit of the IL-12 protein. On binding to its receptor, IL-12 induces phosphorylation of STAT4.

Figure 3 The prevalence of myocardial infarction (MI) in patients without nephritis. To investigate whether the association between the rs11889341 (STAT4) allele and MI was dependent on the association between the STAT4—nephritis association, all patients with nephritis were excluded and the prevalence of MI was subsequently plotted for smoking (A) and non-smoking (B) patients with 0, 1 or 2 of the STAT4 risk allele. Patients with 0, 1 or 2 risk alleles in each group were compared using logistic regression, adjusting for age at follow-up and disease duration. STAT4, signal transducer and activator of transcription 4.

Figure 4 Levels of pSTAT4 in CD8+ T cells after stimulation with IL-12. The levels of IL-12–induced pSTAT4 were compared between healthy blood donors who were smokers (current or past) (n=20) and never-smokers (n=25) by Student’s t-test. IL-12, interleukin-12; pSTAT4, phosphorylated Signal Transducer and Activator of Transcription 4.
believe that the association of the IL12A risk allele, in addition to the STAT4 risk allele, with MI points to the importance of this pathway in the development of the comorbidity. Previous work has demonstrated that JAK-inhibitors efficiently block the increase in pSTAT4 levels, and ameliorate murine lupus as well as its associated vascular dysfunction.19 39 Due to the potential therapeutic strategy of JAK-inhibitors for patients with SLE displaying an altered activity in this particular pathway, we believe that further studies of the effect of this pathway on development of CVE are warranted.

This study’s strength is the large, well-characterised discovery cohort, that results were validated in a second large cohort, and the analysis of healthy control cells, which confirmed that pSTAT4 levels are higher in smokers. In addition, the quality control of genetic data was rigorous, and the patients’ long mean follow-up time of 17 years allowed for many outcome variables such as MI to be recorded. There are, however, some limitations. First, our study is based on retrospective data and we lacked data on the year of smoking cessations. As patients who were past smokers at the last follow-up may have been active smokers at the time of their CVE, we could not analyse previous and current smoking separately. Second, we did not have data on number of pack-years, which may have generated more precise results. Third, the study includes only Scandinavian patients with SLE, and whether the associations are generalisable to patients of other ethnicities needs further investigation.

CONCLUSION
We demonstrate that smokers carrying the STAT4 risk allele are at an increased risk of MI and nephritis and that the IL12A-STAT4 pathway may be important for the development of MI. Our results stress the importance of smoking cessation in SLE and particularly among those carrying this risk allele.

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Contributors SR, DL and LR designed the study. SR, NH, JKS, AA, PP, AJ, IG, AT4 levels, and ameliorate murine lupus as well

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