EXTENDED REPORT

HLA-DRB1*04/*13 alleles are associated with vascular disease and antiphospholipid antibodies in systemic lupus erythematosus

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ABSTRACT

Background and objectives Vascular disease is common in systemic lupus erythematosus (SLE) and patients with antiphospholipid antibodies (aPL) are at high risk to develop arterial and venous thrombosis. Since HLA class II genotypes have been linked to the presence of pro-thrombotic aPL, we investigated the relationship between HLA-DRB1 alleles, aPL and vascular events in SLE patients.

Methods 665 SLE patients of Caucasian origin and 1403 controls were included. Previous manifestations of ischaemic heart disease, ischaemic cerebrovascular disease (ICVD) and venous thromboembolism (together referred to as any vascular events (AVE)) were tabulated. aPL were measured with ELISA. Two-digit HLA-DRB1 typing was performed by sequence-specific primer-PCR.

Results HLA-DRB1*04 was more frequent among SLE patients with ICVD compared to unaffected patients. This association remained after adjustment for known traditional cardiovascular risk factors. HLA-DRB1*13 was associated with AVE. All measured specificities of aPL—cardiolipin IgG and IgM, β_2-glycoprotein-1 IgG, prothrombin (PT) IgG and a positive lupus anticoagulant test were associated with HLA-DRB1*04—while HLA-DRB1*13 was associated with IgG antibodies (β_2-glycoprotein-1, cardiolipin and PT). In patients with the combined risk alleles, HLA-DRB1*04/*13, there was a significant additive interaction for the outcomes AVE and ICVD.

Conclusions The HLA-DRB1*04 and HLA-DRB1*13 alleles are associated with vascular events and an aPL positive immune-phenotype in SLE. Results demonstrate that a subset of SLE patients is genetically disposed to vascular vulnerability.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterised by the production of autoantibodies, a heterogeneous clinical presentation, a remarkable female predominance (90%) and a very high relative risk for both cardiovascular disease (CVD)[1] and venous thromboembolism (VTE).[2] With better treatment for lupus itself, cardiovascular co-morbidity has become the major cause of the shorter life expectancy seen in these patients.[3 4] The mechanisms behind the enhanced CVD risk in SLE are still obscure. It is only to a minor part explained by abundance of traditional 'CVD risk factors',[5] and it seems to be unaffected by modern treatment as it has not declined since the 1960s.[3] Thus, the high incidence of CVD seems to be associated with SLE per se. Pro-thrombotic antiphospholipid antibodies (aPL) occur in 30–50% of SLE patients and prospective studies have demonstrated that their occurrence predict both VTE[2] and CVD.[6 7]

In rare cases inherited defects of the early components of the complement cascade seem to cause SLE,[8] but in most cases genetic predisposition is assumed to play a contributory role. Common loci, within the major histocompatibility complex (MHC) region on chromosome 6 are known to predispose to SLE, and to other autoimmune diseases.[9] In Caucasians SLE has in particular been associated with HLA-DRB1*03 and HLA-DRB1*15.[10] Recently, a growing number of other SLE susceptibility genes[11–13] have been identified in large international collaborations, but the strongest evidence of association with SLE remains in the MHC region also in light of these genome-wide association studies.[12 13]

Previously genetic variations in mannose-binding lectin,[14] Fcgamma receptor IIA genes[15] and CRP[16] have been associated with thrombotic vascular disease in SLE. We recently reported a surprisingly strong association between a variant of the autoimmune risk gene signal transducer and activator of transcription 4 (STAT4), and occurrence of stroke in SLE patients. We also found that the STAT4 genotype was associated with pro-thrombotic aPL.[17]

In SLE and in the primary antiphospholipid syndrome (PAPS), aPL occurrence has previously been associated with HLA-DRB1 genotypes, in particular with HLA-DRB1*04 and HLA-DRB1*13 and in a few studies with HLA-DRB1*07.[18–21] Our objective was to investigate whether these or other genetic variants in the HLA-DRB1 region are associated with manifest vascular disease in SLE. Prothrombotic aPL predict both arterial and venous events in SLE.[2 6 7 22 23] As HLA-DRB1 genotypes have been reported to contribute to autoantibody specificities including aPL,[18–21] we investigated whether a possible association between HLA-DRB1 alleles and vascular events in SLE was linked to the occurrence of a set of aPL.

METHODS

Study population

SLE patients of European Caucasian origin from three Swedish hospitals were included. All fulfilled at least four of the American College of Rheumatology (ACR) classification criteria for SLE.[24] If related only, the first case in each family was included. The first group was from the Karolinska University Hospital (n=364), the second group was from Lund University Hospital (n=161) and the third group was from Uppsala University Hospital (n=140). All included patients were interviewed and examined by a rheumatologist and medical records were scrutinised. The number of ACR 1982 revised SLE criteria,[24] treatment for hypertension, occurrence of diabetes and ever habitual smoking were tabulated. Blood samples were consecutively collected and stored at −70°C. A total of 1403 European Caucasian controls from the mid-south of Sweden were genotyped to give a comparison to the local genetic background. These were derived from a population-based study,[25] from which controls were selected throughout the middle and southern parts of Sweden. All study participants gave informed consent to participate and the regional ethics boards approved the study.

Outcomes

Vascular events were objectively verified in each case, and defined as follows:

1. *Ischaemic heart disease (IHD)*: myocardial infarction (MI), confirmed by electrocardiography and a rise in plasma creatine kinase, muscle and brain fraction (CK-MB) or troponine T and/or angina pectoris confirmed by exercise stress test.
2. *Ischaemic cerebrovascular disease (ICVD)*: stroke including cerebral infarction, confirmed by CT or MRI and/or transitory ischaemic attacks, defined as transient focal symptoms from the brain or retina with a maximum duration of 24 h.
3. *Ischaemic peripheral vascular disease (IPVD)*: intermittent claudication and/or peripheral arterial thrombosis or embolus confirmed by angiogram or Doppler flow studies.
4. *VTE*: deep vein thrombosis, confirmed by venography or ultrasonography and/or pulmonary embolism, confirmed by radionucleotide lung scanning or angiogram.

With *any arterial event (AAE)* we refer to the occurrence of one or more of 1–3, and with *any vascular event (AVE)* we refer to the occurrence of one or more of 1–4.

Antibodies and lupus anticoagulant

Antibodies against (a) cardiolipin (CL, IgG and IgM), β_2-glycoprotein-1 (β_2GP-1, IgG) were determined in 661 patients and anti-prothrombin (aPT, IgG) were analysed in 593 patients by ELISA (Orgentec, Mainz, Germany). All analyses were performed in one laboratory. The cut-off levels corresponded to the 99th percentile of healthy blood donors. Lupus anticoagulant (LAC) was determined with a modified Dilute Russel Viper Venom method (Biopool, Umeå, Sweden) using Bioclot LAC in 363 patients from Stockholm.

Genotyping

HLA-typing was performed by sequence-specific primer PCR assay (SSP-PCR) (DR low-resolution kit; Olerup SSP, Saltsjöbaden, Sweden) and the PCR products were loaded into 2% agarose gels for electrophoresis. An interpretation table was used to determine the specific genotype according to the manufacturer's instructions.[26] The HLA-DRB1 allelic groups studied were DRB1*01, DRB1*03, DRB1*04, DRB1*07, DRB1*08, DRB1*09, DRB1*10, DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*15 and DRB1*16.

The *STAT4* SNP rs10181656 had previously been genotyped using the GoldenGate assay (Illumina, San Diego, California, USA) or the SNPstream system (Beckman-Coulter, Fullerton, California, USA).[17]

Statistical analysis

Patient characteristics and allele frequencies between cases and controls were compared with χ^2 tests. Continuous variables were analysed using analysis of variance. OR and 95% CI were calculated from 2×2 contingency tables. Meta-analyses were done with RevMan 5.1.4 (Review Manager (RevMan), V.5.1; The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, 2011). Interaction between HLA-DRB1*04 and HLA-DRB1*13 alleles was estimated through attributable proportion (AP) due to interaction, which is an estimate of the proportion of excess risk when two factors are present simultaneously.[27] Patients without both alleles were used as the reference group. Univariable and multivariable-adjusted logistic regression analyses were used to estimate the impact of known CVD risk factors for having presented with previous ICVD. In the multivariable model HLA-DRB1*04, 1 or 2 versus 0 STAT4 risk alleles[17] and covariates with $p \leq 0.05$ were entered. Due to co-linearity between aPL of different specificities we chose, based on lowest p value in univariable anaysis, aCL IgG as a representative of aPL. Data processing was performed using JMP' software (SAS Institute, Carey, North Carolina, USA).

RESULTS

Patients

Patient characteristics are shown in table 1. A total of 211 patients had presented with at least one vascular event (venous and/or arterial, AVE), 139 patients had experienced AAE and 78 of these patients presented with ICVD; 67 with stroke. Sixty-nine patients had a history of IHD; 55 of them were diagnosed with MI, the remaining patients had isolated symptoms of angina pectoris (table 1). Associations between aPL and vascular events are reported in supplementary table S1.

Associations between HLA-DRB1 alleles and SLE

First we investigated the associations between HLA-DRB1 alleles and the risk of developing SLE. The HLA-DRB1*03 allele was enriched among SLE patients as compared to controls. Due to the strong influence of HLA-DRB1*03 we stratified for the HLA-DRB1*03 allele in all further calculations. This means that DRB1*03 positives were excluded from the testing of DRB1*01, DRB1*04, DRB1*07, DRB1*08, DRB1*13 and DRB1*15. In these calculations we could confirm the association between HLA-DRB1*15 and SLE (table 2, see supplementary table S2).

Associations of HLA-DRB1 alleles and vascular events among patients with SLE

We next explored the HLA-DRB1 genotypes in subgroups of SLE patients as defined by occurrence of previous vascular events. In meta-analysis for the whole patient material, HLA-DRB1*13 was associated with increased risk for AVE, while HLA-DRB1*04 conferred enhanced risk of ICVD (table 3). HLA-DRB1*13 was associated with stroke and VTE in the larger Stockholm cohort, though this association was not significant in meta-analysis (see supplementary table S3). Having the combined risk alleles HLA-DRB1*04/*13 was

Table 1 Basic characteristics of systemic lupus erythematosus (SLE) patients

	Stockholm	Lund	Uppsala	p Value	Total
Number of patients	364	161	140		665
Women	324 (89.0)	138 (86.2)	123 (87.9)	ns	586 (88.1)
Age at follow-up, years±SD	48.7±14.8	54.5±16.0	51.6±15.3	0.0002	50,7±15.5
Age at diagnosis, years±SD	33.2±13.9	39.1±15.4	31.1±14.9	<0.0001	34.2±14.8
Number of SLE criteria, median (range)	6 (4–10)	6 (4–10)	5 (4–9)	ns	6 (4–10)
Any vascular event	115 (31.6)	63 (39.1)	33 (23.6)	0.02	211 (31.7)
Any arterial event	75 (20.6)	45 (27.9)	21 (14.9)	0.02	139 (20.9)
Ischaemic cerebrovascular disease	46 (12.6)	18 (11.2)	14 (10.0)	ns	78 (11.7)
Stroke	37 (10.1)	16 (10.0)	14 (10.0)	ns	67 (10.1)
Ischaemic heart disease	40 (10.9)	21 (13.1)	8 (5.7)	ns	69 (10.4)
Myocardial infarction	32 (8.8)	17 (10.6)	6 (4.3)	ns	55 (8.3)
Ischaemic peripheral ischaemic vascular disease	15 (4.1)	14 (8.7)	3 (2.1)	0.02	32 (4.8)
Venous thromboembolism	54 (14.8)	35 (21.7)	18 (12.9)	ns	107 (16.1)
Hypertension (treated)	138 (37.9)	57 (35.4)	40 (28.6)	ns	233 (35.1)
Diabetes	9 (2.5)	11 (6.9)	9 (6.8)	0.03	29 (4.4)
Smoking ever	189 (52.1)†	82 (50.9)	50 (36.0)†	0.004	321 (48.4)†
Treatment with steroids	206 (57.6)	ND	79 (56.4)	ns	ND
Treatment with low dose aspirin	64 (17.6)	ND	17 (12.2)	ns	ND
Treatment with warfarin	60 (16.5)	ND	15 (10.8)	ns	ND
+aCL IgG	97 (26.7)	28 (17.4)	20 (14.7)‡	0.004	145 (21.9)
+aCL IgM	92 (25.3)	35 (21.9)	24 (17.6)‡	ns	151 (22.8)
+aβ_2GP-1	80 (22.0)	38 (23.6)	26 (19.1)‡	ns	144 (21.8)
+aPT	46 (14.9)§	20 (12.4)	14 (11.4)¶	ns	80 (13.5)
≥2 +aPL	93 (25.5)	29 (18.0)	20 (14.7)‡	0.01	142 (21.5)
+Lupus anticoagulant test‡	78 (21,5)	ND	ND	ND	
Number of carriers of at least one risk allele (G) of STAT4 SNP rs10181656	174 (60.2)**	66 (46.1)††	65 (56.5)‡‡	0.02	547 (55.8)
Number of HLA-DRB1*03 positive carriers	166 (45.6)	79 (49.1)	70 (50.0)	ns	315 (47.4)
Number of HLA-DRB1*04 positive carriers	111 (30.5)	49 (30.2)	37 (26.4)	ns	197 (29.6)
Number of HLA-DRB1*15 positive carriers	131 (36.0)	55 (34.2)	55 (36.2)	ns	241 (36.2)
Number of HLA-DRB1*13 positive carriers	92 (25.3)	41 (25.3)	33 (23.6)	ns	166 (25.0)
Frequency of HLA-DRB1*04/*13 genotype	15 (4.1)	5 (3.1)	4 (2.9)	ns	24 (3.6)

Only p values ≤0.05 are reported. Treatment is reported if present at inclusion.
†1 missing.
‡1 missing.
§55 missing.
¶17 missing.
**75 missing.
††18 missing.
‡‡25 missing.
+, positive for; a, anti; β_2GP-1, beta$_2$-glycoprotein-1; CL, cardiolipin; ND, not determined; PT, prothrombin; ≥2+aPL, at least two positive antiphospholipid tests out of: aCL IgG/IgM or, B$_2$GP-1 IgG; SNP, single nucleotide polymorphism; STAT4, signal transducer and activator of transcription 4.

Table 2 Number of positive HLA-DRB1 allele carriers in systemic lupus erythematosus (SLE) patients from the three groups versus controls

		Meta-analyses		
DRB1 allele	Patients, N (%)	Controls, N (%)	OR (95% CI)	p Value
*01	67 (19)	251 (24)	0.76 (0.57 to 1.00)†	0.05
*03	315 (47)	343 (24)	1.81 (1.53 to 2.15)	<0.00001
*04	129 (37)	445 (42)	0.81 (0.64 to 1.02)†	0.07
*07	54 (15)	186 (18)	0.86 (0.64 to 1.17)†	0.35
*08	42 (12)	116 (9)	1.40 (0.99 to 1.98)†	0.06
*13	91 (26)	312 (29)	0.84 (0.65 to 1.08)†	0.18
*15	175 (50)	344 (32)	2.09 (1.67 to 2.62)†	<0.00001

Given the strong association between SLE and HLA-DRB1*03 and the known linkage disequilibrium in the region, all but *03 groups, were stratified for presence of HLA-DRB1*03 alleles.
†The presented calculations are based on the *03 negative strata. N represents number of cases positive for the particular DRB1 allele, (%) represents percentage of the total number, positive for the allele.

associated with a high risk for AVE (AP: 30.52, 95% CI 0.10 to 0.94), AAE (AP: 30.65, 95% CI 0.32 to 0.98) and ICVD (AP: 0.63, 95% CI 0.27 to 0.99) and detailed analysis showed that this high risk is due to a significant gene–gene interaction between HLA-DRB1*04 and HLA-DRB1*13 alleles (table 4).

Associations of HLA-DRB1 alleles and aPL antibodies among patients with SLE

A possible association between aPL and the HLA-DRB1 alleles, which were associated with vascular events, was then investigated. In meta-analyses, HLA-DRB1*04 was associated with aPL of all measured specificities and with a positive test for LAC in the patient group from Stockholm. HLA-DRB1*13 was also associated with IgG antibodies targeting β_2GP-1, aCL and PT (table 5). Carriers of the combined alleles (*04/*13) were associated with higher ORs for LAC positivity, aCL IgG and aβ_2GP-1 IgG and the simultaneous occurrence of ≥positive aPL tests (≥2aPL, table 5, see supplementary table S4). No significant interaction was detected.

Table 3 Associations between HLA-DRB1 alleles and vascular events among patients with systemic lupus erythematosus (SLE)

	Meta-analyses		
	N (%)	OR (95% CI)	p Value
DRB1*01			
AVE	24 (11.4)	0.83 (0.51 to 1.38)	0.48
AAE	16 (11.4)	0.84 (0.47 to 1.50)	0.55
ICVD	8 (10.3)	0.74 (0.34 to 1.60)	0.45
Stroke	7 (10.4)	0.81 (0.37 to 1.79)	0.60
IHD	7 (10.1)	0.65 (0.29 to 1.48)	0.30
MI	5 (9.1)	0.63 (0.25 to 1.57)	0.32
IPVD	4 (12.5)	0.96 (0.34 to 2.68)	0.94
VTE	12 (11.2)	0.83 (0.43 to 1.60)	0.59
DRB1*03			
AVE	93 (44.1)	0.82 (0.59 to 1.15)	0.25
AAE	65 (46.1)	0.94 (0.64 to 1.36)	0.73
ICVD	34 (43.6)	0.85 (0.53 to 1.37)	0.50
Stroke	28 (41.8)	0.78 (0.47 to 1.30)	0.34
IHD	33 (47.8)	0.96 (0.58 to 1.58)	0.87
MI	27 (49.1)	1.01 (0.58 to 1.76)	0.96
IPVD	17 (53.1)	1.20 (0.59 to 2.46)	0.62
VTE	48 (44.9)	0.88 (0.58 to 1.34)	0.55
DRB1*04			
AVE	68 (32.2)	1.18 (0.83 to 1.68)	0.36
AAE	48 (34.0)	1.28 (0.86 to 1.90)	0.22
ICVD	33 (42.3)	1.88 (1.16 to 3.05)	0.01
Stroke	27 (40.3)	1.70 (1.01 to 2.86)	0.05
IHD	25 (36.2)	1.37 (0.81 to 2.32)	0.23
MI	22 (40,0)	1.62 (0.92 to 2.87)	0.09
IPVD	12 (37.5)	1.42 (0.68 to 2.96)	0.35
VTE	34 (31.8)	1.12 (0.72 to 1.74)	0.63
DRB1*13			
AVE	67 (31.8)	1.67 (1.15 to 2.41)	0.006
AAE	42 (29.8)	1.36 (0.90 to 2.07)	0.14
ICVD	25 (32.1)	1.49 (0.89 to 2.48)	0.13
Stroke	22 (32.8)	1.54 (0.90 to 2.65)	0.12
IHD	19 (27.5)	1.20 (0.68 to 2.11)	0.53
MI	15 (27.3)	1.12 (0.60 to 2.09)	0.71
IPVD	7 (21.9)	0.86 (0.37 to 1.99)	0.73
VTE	34 (20.5)	1.50 (0.95 to 2.35)	0.08
DRB1*15			
AVE	66 (31.3)	0.74 (0.52 to 1.04)	0.09
AAE	46 (32.6)	0.83 (0.56 to 1.23)	0.36
ICVD	27 (34.6)	0.93 (0.56 to 1.54)	0.76
Stroke	24 (35.8)	0.98 (0.58 to 1.66)	0.94
IHD	24 (34.8)	0.95 (0.56 to 1.60)	0.85
MI	21 (38.2)	1.12 (0.63 to 1.98)	0.70
IPVD	5 (15.6)	0.32 (0.12 to 0.84)	0.02
VTE	35 (32.7)	0.84 (0.54 to 1.31)	0.45

N represents number of cases positive for the particular DRB1 allele and positive for the vascular phenotype; (%) represents percentage of the total number positive for the phenotype (eg, how many patients out of all who have had stroke are DRB1*01 positive).
AAE, any arterial event; AVE, any vascular event; ICVD, ischaemic cerebrovascular disease; IHD, ischaemic heart disease; IPVD, ischaemic peripheral vascular disease; MI, myocardial infarction; VTE, venous thromboembolism.

Risk factors for ischaemic cerebrovascular events among SLE patients

We finally analysed occurrence of HLA-DRB1*04 alleles and the risk of ICVD in the context of other known risk factors for ICVD. Age at inclusion in this study, gender, diabetes, treatment for hypertension and aCL IgG antibodies, investigated in the majority of patients, were evaluated. We asked if the previously reported association between the SLE risk allele rs10181656(G) in *STAT4* and ICVD/aPL,[17] was independent of HLA-DRB1*04. In multivariable logistic regression we therefore entered presence of one or two STAT4 rs10181656 risk alleles (yes/no) together with the traditional risk factors, which were associated with ICVD in univariable analysis (table 6). Results demonstrate that age, treatment for hypertension, aCL IgG, *STAT4* rs10181656(G) and HLA-DRB1*04 all remained independently associated with a history of ICVD (table 6).

DISCUSSION

The major finding in the present investigation is that the HLA-DRB1*04 and the HLA-DRB1*13 alleles confer increased risk for vascular events among SLE patients. The observed association between HLA-DRB1*04 and ICVD was independent of available traditional risk factors and of the previously reported association with a *STAT4*[17] risk allele. In patients with the combined, *04/*13, genotype the risk alleles were associated with a gene–gene interaction regarding risk of vascular events. Positivity in the functional LAC test was associated with both the HLA-DRB1*04 and the HLA-DRB1*13 alleles. These alleles and aPL of different specificities were consistently associated, extending previous reports.[18 20 28]

It has been suggested that HLA-DRB1*04 confers protection with respect to development of SLE[29]; on the other hand a positive signal of association to SLE from HLA-DRB1*0401 has previously been reported.[9] The impact of HLA-DRB1*04 has hitherto not been in focus of genetic studies in SLE, but associations with other autoimmune conditions, in particular rheumatoid arthritis (RA) and type 1 diabetes, are well recognised.[9 30] Several subtypes of HLA-DRB1*04 belong to the group of HLA-DRB1 alleles, collectively referred to as the shared epitope (SE), which confers the largest known genetic contribution to RA.[9 30] One cannot exclude that genetic risk may correspond to only one or a limited number of alleles from the HLA-DRB1*04 group. However, it most likely associates with the allele most common in Caucasians, the *0401 allele. To get sufficient power to analyse different HLA-DRB1*04 alleles, one should increase the number of observations dramatically with phenotypes under investigation or study non-Caucasian populations with different patterns of HLA-DRB1*04 alleles.

Three studies have to date demonstrated that the HLA-DRB1*04 is in a dose dependent manner associated with premature mortality in RA, and in particular with mortality caused by CVDs.[31–33] Despite the fact that HLA-DRB1*04 is not a risk gene for SLE per se, it was associated with vascular events and with aPL among SLE patients in this study. HLA-DRB1*04 thus seems to be associated with vascular vulnerability both in RA[31–33] and in SLE. In the general population the HLA region has not been recognised as a risk region for ICVD, IHD or VTE,[30 34] with the exception of a recently reported weak association between IHD and HLA-DRB1*01.[35] Thus the HLA-DRB1 region seems to be of greater importance for vascular events among patients with autoimmune conditions than in the general population.

We have previously noted that the risk profiles for ICVD and IHD differ in SLE.[17] We therefore split arterial events into subgroups. A history of ICVD and IHD was approximately equally common. In a longitudinal design we recently demonstrated that 43% of deaths among SLE patients in Stockholm were caused by IHD, but only 2% by ICVD.[22] Consequently a cross-sectional design, which is limited to include only survivors

Table 4 Interaction between HLA-DRB1*04 and HLA-DRB1*13 with respect to vascular events and antiphospholipid antibodies (aPL) in all patients

Outcome	HLA-DRB1allele combinations		Number +/− for outcome	OR (95% CI)	p Value	Interaction measure (AP) (95% CI)
Any vascular event	No*04	No*13	91/237	1.0 (ref)		
	*04	No*13	53/118	1.17 (0.78 to 1.75)		
	No*04	*13	52/88	1.54 (1.01 to 2.34)	0.04	
	*04	*13	15/11	3.55 (1.57 to 8.02)	0.001	0.52 (0.10 to 0.94)†
Any arterial event	No*04	No*13	63/265	1.0 (ref)		
	*04	No*13	36/135	1.12 (0.71 to 1.78)		
	No*04	*13	30/110	1.15 (0.70 to 1.87)		
	*04	*13	12/14	3.61 (1.59 to 8.17)	0.001	0.65 (0.32 to 0.98)†
Ischaemic cerebrovascular disease	No*04	No*13	29/299	1.0 (ref)		
	*04	No*13	24/147	1.68 (0.95 to 3.00)	0.07	
	No*04	*13	16/124	1.33 (0.70 to 2.54)		
	*04	*13	9/17	5.46 (2.23 to 13.34)	4.4×10^{-5}	0.63 (0.27 to 0.99)†
Venous thromboembolism	No*04	No*13	45/283	1.0 (ref)		
	*04	No*13	28/143	1.23 (0.74 to 2.06)		
	No*04	*13	28/112	1.57 (0.94 to 2.64)	0.09	
	*04	*13	6/20	1.88 (0.72 to 4.95)		0.04 (−0.94 to 1.0)
aβ_2GP1 IgG§	No*04	No*13	42/303	1.0 (ref)		
	*04	No*13	54/114	3.21 (2.03 to 5.08)		
	No*04	*13	35/105	2.26 (1.37 to 3.73)	0.001	
	*04	*13	13/13	6.79 (2.95 to 15.63)	5.0×10^{-7}	0.34 (−0.21 to 0.89)
aCL IgG§	No*04	No*13	50/277	1.0 (ref)		
	*04	No*13	48/120	2.22 (1.41 to 3.48)		
	No*04	*13	35/105	1.85 (1.13 to 3.00)	0.01	
	*04	*13	12/14	4.75 (2.08 to 10.87)	6.9×10^{-5}	0.355 (−0.19 to 0.90)
aCL IgM§	No*04	No*13	58/269	1.0 (ref)		
	*04	No*13	50/118	1.97 (1.27 to 3.04)		
	No*04	*13	34/106	1.49 (0.92 to 2.40)		
	*04	*13	9/17	2.46 (1.04 to 5.78)	0.03	0.001(−0.89 to 0.89)
aPT IgG‡	No*04	No*13	27/261	1.0 (ref)		
	*04	No*13	25/127	1.90 (1.06 to 3.41)		
	No*04	*13	21/108	1.88 (1.02 to 3.47)	0.04	
	*04	*13	7/17	3.98 (1.51 to 10.45)	0.003	0.30 (−0.39 to 1.0)
>2aPL§	No*04	No*13	46/281	1.0 (ref)		
	*04	No*13	51/117	2.66 (1.69 to 4.19)		
	No*04	*13	33/107	1.88 (1.14 to 3.10)	0.01	
	*04	*13	12/14	5.23 (2.27 to 12.03)	2.1×10^{-5}	0.32 (−0.25 to 0.89)
Lupus anticoagulant¶	No*04	No*13	24/153	1.0 (ref)		
	*04	No*13	28/66	2.71 (1.46 to 5.01)		
	No*04	*13	17/58	1.87 (0.94 to 3.73)	0.07	
	*04	*13	9/8	7.17 (2.52 to 20.39)	3.7×10^{-5}	0.50 (−0.02 to 1.0)

†Significant interaction.
‡Number investigated (N)=593.
§N=661.
¶Only analysed in the Stockholm group, N=363.
a, anti; AP, attributable proportion due to interaction; ≥2aPL, at least two positive tests of aβ_2GP-1, aCL IgG and aCL IgM; β_2GP1, β_2-glycoprotein1; CL, cardolipin; Ig, immunoglobulin; PT, prothrombin; +/−, positive/negative.

of CVD, as used in this and in many other genetic studies, may systematically underestimate the occurrence of IHD. Longitudinal studies are needed to reliably evaluate the genetic impact on IHD in SLE.

The positive associations between HLA-DRB1*04/*13 and aPL suggest that aPL is one underlying mechanism, which contributes to vascular vulnerability among carriers of these genotypes. In RA the SE has been associated with the occurrence of both anti-citrullinated protein antibodies and rheumatoid factor,[36 37] erosive disease and extra-articular manifestations,[38] suggesting an association with a more pro-inflammatory phenotype. It remains to ascertain whether this is also the case in SLE.

Among SLE patients aPL are well-known risk factors for stroke.[17 39] In the general population several studies,[40 41] but not all,[40] report a positive association between stroke and aPL, in particular among younger stroke patients.[41 42] Early studies reported that the occurrence of aPL was linked to certain HLA genotypes. Several of these found that HLA-DRB1*04[18 19 28 43] and/or HLA-DRB1*13[18] were associated with aPL, both among SLE patients and among patients with the PAPS.[28 44] We confirm the association between the HLA-DRB1*04 allele and aCL/aβ_2GP1 antibodies reported in a large multicentre study by Galeazzi *et al*.[20] We also corroborate the association between HLA-DRB1*04 and aPT reported by Bertolaccini *et al* and Sebastiani *et al*.[19 21] The association with aCL IgM and with

Table 5 Associations between antiphospholipid antibodies (aPL) and HLA-DRB1*04 and HLA-DRB1*13 among patients with systemic lupus erythematosus

	Meta-analyses		
	N (%)	OR (95% CI)	p Value
DRB1*04			
aβ$_2$GP1 IgG	67 (46.5)	2.66 (1.81 to 3.91)	<0.00001
aCL IgG	60 (41.4)	1.99 (1.35 to 2.93)	0.0005
aCL IgM	59 (39.1)	1.76 (1.20 to 2.58)	0.004
aPT IgG†	32 (40.0)	1.69 (1.04 to 2.76)	0.03
≥2aPL‡	63 (44.4)	2.33 (1.58 to 3.44)	<0.00001
LAC§	40 (47.1)	2.57 (1.53 to 4.32)	0.0003
DRB1*13			
aβ$_2$GP1 IgG	48 (33.3)	1.69 (1.13 to 2.52)	0.01
aCL IgG	47 (32.4)	1.60 (1.07 to 2.40)	0.02
aCL IgM	43 (28.5)	1.25 (0.83 to 1.88)	0.28
aPT IgG†	28 (35.0)	1.66 (1.01 to 2.75)	0.05
≥2aPL‡	45 (31.7)	1.51 (1.00 to 2.28)	0.05
LAC§	28 (32.9)	1.65 (0.96 to 2.86)	0.06

N represents number of cases positive for the particular DRB1 allele and positive for the particular antibody test; (%) represents percentage of the total number positive for the antibody test (eg, for the first line, 46.5), how many patients out of all who have had aβ$_2$GP1 IgG antibodies are DRB1*04 positive).
†N=593.
‡N=661.
§Only analysed in the Stockholm group, N=363.
a, anti; ≥2aPL, at least two positive tests of aβ$_2$GP-1, aCL IgG and aCL IgM; β$_2$GP1, β$_2$-glycoprotein 1; CL, cardolipin; Ig, immunoglobulin; LAC, lupus anticoagulant; PT, prothrombin.

positivity in the LAC test is an extension of previous reports. The functional LAC test is generally considered to have higher clinical significance than the specific aPLs.[41 45] Taken together we demonstrate consistent associations between HLA-DRB1*04 and all aPL specificities investigated and a slightly weaker association between aPL and HLA-DRB1*13, preferentially with antibodies of the IgG isotype targeting CL and protein co-factors β$_2$GP1 and PT. The association between HLA-DRB1*07 and aPL[20] was not confirmed in our study (data not shown).

In multivariable analyses, which adjusted for aCL IgG and available traditional risk factors, both HLA-DRB1*04 and *STAT4* remained significantly associated with ICVD. Age and hypertension were also predictive as expected. Disease activity, hyperlipidaemia and body mass index have previously been associated with ICVD,[42 46] but these data were not available in our patients.

The association between SLE and HLA-DRB1*03 and HLA-DRB1*15 is well established.[10 29 47–49] In the investigated Swedish SLE patients, these associations were robust and of similar strength as previously reported. These SLE risk alleles did however not correlate with the occurrence of vascular events; rather there was a negative association between HLA-DRB1*15 and previous IPVD, but this figure should be interpreted with caution due to few cases.

Strength of this study is that all vascular events were confirmed clinically and all aPL were measured in one laboratory. The Stockholm and Uppsala cohorts are similar (table 1). The majority of included patients from Lund belong to a longitudinal cohort from a defined catchment area but consecutive patients from adjacent regions were also included. Disparities in collection procedures have previously been described.[4 50] Despite minor cohort differences our results remained significant in meta-analyses and they were even stronger in the patient group with the combined risk alleles, the HLA-DRB1*04/*13 genotype. Limitations are that aPL measurements were only performed once, LAC was only determined in the Stockholm cohort, aβ$_2$-GP1 IgM were not measured and we did not evaluate obstetric manifestations of antiphospholipid syndrome (APS). Consequently, we could not assess how many patients formally fulfilled APS criteria.[51] As the initial hypothesis was to investigate the association between vascular events and a few candidate HLA-DRB1 alleles we did not correct for multiple comparisons. The investigated patients were all of European Caucasian origin. Thus our results cannot be generalised to patients of other ethnicities.

To conclude, we demonstrate that SLE patients carrying HLA-DRB1*04 and/or HLA-DRB1*13 alleles have an increased risk for vascular events. Presence of the combined risk alleles, HLA-DRB1*04/*13, was associated with interaction regarding

Table 6 Previous ischaemic cerebrovascular disease in relation to the presence of known cardiovascular risk factors and occurrence of HLA-DRB1*04 and STAT4 risk alleles

	Univariable		Multivariable (N=547) R^2=0.17, whole model p=5.3×10^{-12}	
Risk factor	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value**
Age at study inclusion, year	1.04 (1.03 to 1.06)	3.1×10^{-7}	1.04 (1.03 to 1.07)	3.2×10^{-6}
Female (yes)	1.1 (0.6 to 2.7)		1.9 (0.8 to 5.2)	NS
Smoking ever (yes)	1.2 (0.7 to 1.9)		NE	
Hypertension treatment (yes)	3.2 (2.0 to 5.2)	9.6×10^{-7}	2.8 (1.6 to 5.1)	0.0004
Diabetes (yes)	1.3 (0.4 to 3.6)		NE	
HLA-DRB1*04	1.9 (1.2 to 3.1)	0.01	2.1 (1.1 to 3.7)	0.02
STAT4 risk alleles: 1 or 2 vs 0 risk alleles†	2.3 (1.3 to 4.2)	0.004	2.6 (1.4 to 5.0)	0.002
aCL IgG (N=661)	2.1 (1.2 to 3.4)	0.006	2.6 (1.3 to 5.0)	0.005
aCL IgM (N=661)	1.0 (0.6 to 1.7)		NE	
aβ$_2$GP1 (N=661)	2.1 (1.2 to 3.4)	0.006	NE	
aPT (N=593)	2.2 (1.2 to 4.0)	0.02	NE	
≥2 aPL	2.0 (1.2–3.3)	0.01	NE	

p values ≤0.05 are presented.
a, anti; β$_2$GP1, β$_2$-glycoprotein 1; CL, cardiolipin; Ig, immunoglobulin; NE, not entered; PT, prothrombin; ≥2aPL, at least two positive tests of aβ2GP-1, aCL IgG and aCL IgM; STAT4, signal transducer and activator of transcription factor 4, rs10181656(G), 1 or 2 vs 0 risk alleles.
†Determined in 547 patients.

risk of a previous vascular event. Both risk alleles were furthermore associated with an aPL positive immune phenotype. The HLA-DRB1*04 allele remained as an independent risk factor for prior ICVD, even after adjustment for aPL. Our results link genotype to both immune and clinical phenotypes. They illustrate that when investigating genetic susceptibility in complex diseases it is important not only to analyse genetic frequencies in the present diagnostic entities, but also to look in more detail at clinical symptoms and subgroups of patients.

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