RHEUMATOLOGY

Rheumatology 2023;62:924–933
https://doi.org/10.1093/rheumatology/keac327
Advance access publication 1 June 2022

Original article

Anti-phosphatidylserine/prothrombin antibodies and thrombosis associate positively with HLA-DRB1*13 and negatively with HLA-DRB1*03 in SLE

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Abstract

Objectives. Emerging evidence demonstrates that aPS-PT associate with thrombotic events. Genetic predisposition, including *HLA-DRB1* alleles, is known to contribute to the occurrence of conventional aPL [anti-$\beta_2$glycoprotein-I (anti-β_2GPI) and aCL]. We investigated associations between aPS-PT and *HLA-DRB1** alleles and thrombosis in SLE. Conventional aPL were included for comparison.

Methods. We included 341 consecutive SLE patients, with information on general cardiovascular risk factors, including blood lipids, LA and thrombotic events. aPS/PT, anti-β_2GPI and aCL of IgA/G/M isotypes and LA were quantified.

Results. aPS/PT antibodies associated positively with *HLA-DRB1**13 [odds ratio (OR) 2.7, $P = 0.002$], whereas anti-β_2GPI and aCL antibodies associated primarily with *HLA-DRB1*04* (OR 2.5, $P = 0.0005$). These associations remained after adjustment for age, gender and other *HLA-DRB1** alleles. *HLA-DRB1*13*, but not *DRB1*04*, remained as an independent risk factor for thrombosis and APS after adjustment for aPL and cardiovascular risk factors. The association between *DRB1*13* and thrombosis was mediated by aPS-PT positivity. *HLA-DRB1*03*, on the other hand, associated negatively with thrombotic events as well as all aPL using both uni- and multivariate analyses. *HLA-DRB1*03* had a thrombo-protective effect in aPL-positive patients. Additionally, *HLA-DRB1*03* was associated with a favourable lipid profile regarding high-density lipoprotein and triglycerides.

Conclusions. *HLA-DRB1*13* confers risk for both aPS-PT and thrombotic events in lupus. The association between *HLA-DRB1*13* and thrombosis is largely, but not totally, mediated through aPS-PT. *HLA-DRB1*03* was negatively associated with aPL and positively with favourable lipid levels. Thus, *HLA-DRB1*03* seems to identify a subgroup of SLE patients with reduced vascular risk.

Key words: antiphosphatidylserine/prothrombin, aPL, HLA, SLE, thrombosis

BASIC SCIENCE

Rheumatology key messages

- *HLA-DRB1*13* alleles independently associate with aPS-PT and vascular events in SLE patients.
- *HLA-DRB1*13* association with vascular events is, to a large extent, mediated by aPS-PT antibodies.
- *HLA-DRB1*03* associate negatively with thrombosis and aPL, and favourably with lipid profile, suggesting a thrombo-protective role.

Introduction

aPL are directed against negatively charged phospholipids and/or plasma proteins and represent the immunological hallmark of primary and secondary APS. Genetic predisposition contributes to the occurrence of aPL and to the development of APS, with the human

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Submitted 19 February 2022; accepted 20 May 2022

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leucocyte antigens (*HLA*) gene loci having a documented contributive role [1, 2].

Both family and population-based studies demonstrated that *HLA-DRB1*04* and *-DRB1*07* are more frequent in APS patients with or without SLE, as compared with healthy controls [3–9]. aCL and anti-β_2 glycoprotein I (anti-β_2GPI) have associated with a number of *HLA-DRB1* alleles, most consistently with *HLA-DRB1*04* [2, 6, 8, 10, 11]. Among the *HLA-DRB1*04* alleles, *HLA-DRB1*0402* confers high risk for the presence of both antibodies [8]. In addition to *HLA-DRB1*04*, several studies have reported that anti-β_2GPI antibodies also associate with *HLA-DRB1*13* alleles [9, 12, 13].

Venous and arterial vascular events associated with *HLA-DRB1*13*, and ischaemic cerebrovascular disease with *HLA-DRB1*04* also after adjustment for traditional risk factors [14]. Associations between *HLA-DRB1*13* and clinical events/aPL remained significant when excluding *HLA-DRB1*04*-positive patients, indicating an *HLA-DRB1*04*-independent risk. Other studies have also described *HLA-DRB1*07* as a risk factor for vascular events [9, 15].

Most previous immuno-genetic studies on APS only investigated aCL and anti-β_2GPI associations, i.e. the conventional 'criteria' aPL of IgM/G isotypes. More recently, positivity for aPS-PT has emerged as a risk factor for the presence of LA and vascular events [16, 17]. In a recent publication we reported that aPS-PT antibodies associated more strongly with venous thromboembolism (VTE) and arterial thrombosis than did the conventional aPL [18]. In the current study we investigated a well-characterized Swedish SLE cohort regarding the relationship between *HLA-DRB1* alleles, aPS-PT antibodies (IgG, IgM and IgA) and vascular events. Conventional aPL were included for comparison.

Methods

Participants and clinical characteristics

Consecutive SLE patients from the Karolinska lupus cohort ($n = 341$) fulfilling the 1982 revised SLE criteria [19] were included. A detailed description of the cohort was reported in our previous publications [20, 21]. All patients were subjected to a thorough clinical investigation by a rheumatologist at inclusion and medical files were reviewed. APS was diagnosed according to the revised Sydney classification criteria for APS and history of any thrombotic event (VTE: including deep venous thrombosis or pulmonary embolism. Arterial thrombosis: cerebrovascular events, myocardial infarction or ischaemic peripheral tissue loss) [22]. Arterial hypertension was defined as systolic and/or diastolic blood pressure $\geq$140/90 mmHg or use of antihypertensive medications. Diagnostic methods to establish clinical diagnoses were specified in our previous publication [18]. The Global APS Score (GAPSS) was calculated for all patients [23]. BMI and lipid measurements including low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG) and total cholesterol levels were also included in the analyses, with hyperlipidaemia defined as LDL $>$3 mmol/l or treatment with lipid-lowering agents.

All participants gave written informed consent and the study conformed to the guidelines of the Declaration of Helsinki. Karolinska University Hospital approved the study [03-556 (16 December 2003)].

aPL testing

Quantification of aPS-PT, aCL and anti-β_2GPI IgA/G/M was performed using the Aptiva system based on a particle-based multi-analyte technology (PMAT; research use only, Inova Diagnostics, San Diego, CA, USA [24]). Clinical associations for all aPL antibodies among patients were previously published in comparative studies between Sudan and Sweden, using a slightly lower number of Swedish patients ($n = 332$) [18, 21]. The 99th percentile values of 162 age- and sex-matched population controls were used as cut-offs [22]. Information about LA positivity determined by the modified dRVVT (Bioclot LA; Biopool, Umeå, Sweden) was available for 329 patients.

Genotyping

HLA typing was performed using sequence-specific primer PCR assay (SSP-PCR) (DR 2 digit-resolution kit; Olerup SSP, Saltsjöbaden, Sweden). The studied *HLA-DRB1* alleles were **01*, **03*, **04*, **07*, **08*, **09*, **10*, **11*, **12*, **13*, **14*, **15* and **16*.

Statistical analyses

For comparisons between categorical variables, Chi^2-test was used with Fisher's exact test applied when appropriate, and for quantitative differences between groups, Mann–Whitney's *U* test was used. To estimate the individual impact of independent variables we conducted multivariable logistic regression including *HLADRB1* alleles with significant associations to the specific outcomes in univariate analyses. Linear regression was also used to adjust for confounders. We used stepwise forward regression analyses when logistic regression models were unfeasible due to number of independent variables. We performed a series of regression analyses to estimate whether aPS-PT positivity acts as a mediator between the relationship of *HLA-DR*13* and thrombosis.

All statistical analyses were conducted using JMP statistical software (SAS institute, Cary, NC, USA), except for the mediation analysis where the packages tidyverse [25], lavaan [26] and mediation [27] for R software (version 4.1.1 Kick Things) were used. *P*-values <0.05 were considered significant.

Results

Seventy-eight SLE patients (23.0%) had presented with at least one vascular event, and 31 (9.6%) were confirmed as secondary APS [22]. Among all SLE patients,

50 (15.0%) tested positive for aPS-PT antibodies of any isotype, 100 (30.2%) were positive for anti-β_2GPI and/or aCL antibodies and LA was positive in 60 patients (18.2%). Patient demographics, history of thrombotic events, cardiovascular risk factors and antibody prevalence are shown in supplementary Table S1, available at *Rheumatology* online.

HLA-DRB1 genotype associations with aPS-PT antibodies

HLA-DRB1 allele frequencies among all patients are presented in Table 1. aPS/PT-positive patients had higher frequency of *HLA-DRB1*13* allele compared with aPS-PT-negative patients [odds ratio (OR) 2.7 (95% CI 1.4, 5.2), $P = 0.002$]. This association remained significant after exclusion of patients positive for any anti-β_2GPI and aCL isotypes (IgG/M/A): 8.7% *vs* 1.2% in DRB1*13 carriers *vs* non-carriers [OR 7.8 (95% CI 1.4, 44.3), $P = 0.02$]. No other positive association was observed with aPS-PT. Occurrence of anti-β_2GPI and/or aCL associated also with *HLA-DRB*13* [OR 2.0 (95% CI 1.2, 3.4), $P = 0.01$] but even more strongly with *HLA-DRB1*04* [OR 2.5 (95% CI 1.5, 4.1), $P = 0.0005$]. Negative associations were found between *HLA-DRB1*03* and all aPL including LA, and also between aPS-PT and anti-β_2GI/aCL with *HLA-DRB1*15*. Using multiple logistic regression, where the *HLA-DRB1*04*, **13*, **03* and **15* were independent variables and status for aPS-PT, anti-β_2GPI/aCL and LA as the outcome, similar results were obtained except that the negative associations with *HLA-DRB1*15* were lost (Fig. 1A–C). When we used age, gender, HCQ treatment and the *HLA-DRB1*04*, **13*, **03* and **15* in a forward step-wise regression model, associations remained significant as shown in Fig. 1A–C, except for the negative association between anti-β_2GPI/aCL and *HLA-DRB1*03* that was lost.

Associations between *HLA-DRB1* alleles and the different aPL isotypes are shown in Fig. 2. IgA and IgM, but not IgG, aPS-PT associated with *HLA-DRB1*13*, and all aPS-PT isotypes associated negatively with *HLA-DRB1*03*, whereas no isotype showed association with *HLA-DRB1*04*. On the contrary, all isotypes of anti-β_2GPI and aCL associated with *HLA-DRB1*04*, with the highest ORs observed for IgM. Only aCL IgG among the conventional aPL associated with *HLA-DRB1*13*.

HLA-DRB1 genotype associations with vascular events

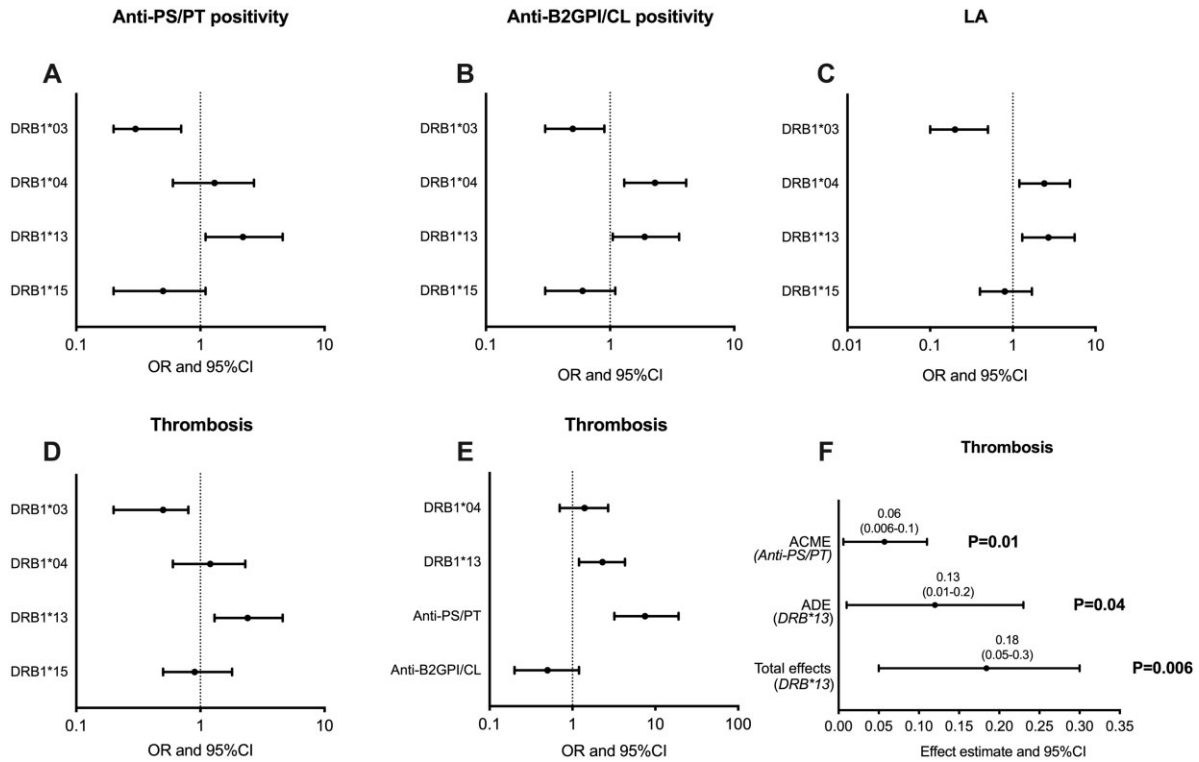
In univariate analyses, *HLA-DRB1*13* was more frequent in patients with a history of any thrombosis, VTE or APS diagnosis, compared with patients without (Table 2). No clinical association with *HLA-DRB1*04* was observed, except for higher GAPSS. In multivariate analysis including *HLA-DRB1*03*, **04*, **13* and **15* alleles as independent factors and thrombosis as outcome, only *HLA-DRB1*13* and *HLA-DRB1*03* showed significant

Table 1 Frequency of individual *HLA DRB1* alleles among SLE patients and associations with antibody phenotypes

HLA-DRB1	*HLA-DRB1*, *n* (%) total patients	Anti-PS/PT positive (any isotype) $n = 48$, *n* (%)	OR (95% CI); *P*	Anti-β_2GPI or aCL positive (any isotype) $n = 96$, *n* (%)	OR (95% CI); *P*	LA $n = 57$, *n* (%)	OR (95% CI); *P*
**01*	41 (12.9)	4 (8.3)	0.6 (0.2, 1.7); 0.3	11 (11.4)	0.8 (0.4, 1.7); 0.6	6 (10.5)	0.8 (0.3, 1.9); 0.6
**03*	147 (46.5)	13 (27.1)	0.4 (0.2, 0.7); 0.004	33 (34.4)	0.5 (0.3, 0.8); 0.006	11 (19.2)	0.2 (0.1, 0.4); <0.0001
**04*	94 (29.7)	18 (37.5)	1.6 (0.8, 2.9); 0.2	41 (42.7)	2.5 (1.5, 4.1); 0.0005	27 (47.4)	2.4 (1.4, 4.4); 0.002
**07*	28 (8.9)	6 (12.5)	1.5 (0.6, 4); 0.4	9 (9.4)	1 (0.4, 2.4); 0.9	7 (12.2)	1.6 (0.6, 4.0); 0.3
**08*	28 (8.9)	6 (12.5)	1.6 (0.6, 4.3); 0.3	9 (9.4)	1.2 (0.5, 2.7); 0.7	5 (8.8)	0.9 (0.3, 2.6); 0.9
**09*	9 (2.8)	1 (2.1)	0.7 (0.1, 5.5); 0.7	2 (2.1)	0.6 (0.1, 3.0); 0.5	1 (1.7)	0.7 (0.1, 5.1); 0.6
**10*	7 (2.2)	0 0	NA	2 (2.1)	0.9 (0.2, 4.6); 0.9	0 0	NA
**11*	27 (8.5)	6 (12.5)	1.6 (0.6, 4.3); 0.3	8 (8.3)	0.9 (0.4, 2.2); 0.8	6 (10.5)	1.3 (0.5, 3.3); 0.6
**12*	7 (2.2)	0 0	NA	1 (1.0)	0.4 (0.05, 3.7); 0.4	1 (1.7)	0.7 (0.1, 6.1); 0.8
**13*	79 (25)	21 (43.7)	2.7 (1.4, 5.2); 0.002	33(34.3)	2 (1.2, 3.4); 0.01	23 (40.3)	2.4 (1.3, 4.5); 0.003
**14*	6 (1.9)	2 (4.2)	3.7 (0.6, 23); 0.1	2 (2.1)	1.4 (0.2, 8.9); 0.8	2 (3.5)	2.2 (0.4, 12.5); 0.3
**15*	118 (37.3)	12 (25.0)	0.5 (0.2, 0.9); 0.045	27 (28.1)	0.5 (0.3, 0.9); 0.01	18 (31.6)	0.7 (0.4, 1.4); 0.4
**16*	8 (2.5)	1 (2.1)	0.8 (0.1, 6.4); 0.8	4 (4.2)	2.2 (0.5, 9.2); 0.2	2 (3.5)	1.5 (0.3, 7.5); 0.6

Chi^2 tests were used to compare aPL positive and aPL negative patients concerning the risk of carrying individual HLA-DRB1 alleles, and presented as odds ratios (OR) and CIs were calculated using Chi^2 tests. Data are expressed as number (%) of *HLA-DRB1*-positive and aPL-positive patients among total number of aPL-positive patients. Significant associations are underlined. Anti-β_2GPI: anti-β_2 glycoprotein I. NA: not applicable.

FIG. 1 Associations of *HLA-DRB1* alleles with anti-phospholipid phenotypes and thrombosis in 341 SLE patients



Odds ratios (OR) with CIs of the independent variables (*DRB1*03*, **04*, **13*, **15*) are illustrated for the outcomes (**A**) occurrence of any isotype of anti-phosphatidylserine/prothrombin, (**B**) any isotype of anti-β_2GPI or aCL, (**C**) LA and (**D**) thrombosis. In (**E**), thrombosis as outcome is related to aPL, *HLA-DRB1*04* and **13*. Multiple logistic regression models were used to calculate OR (central bullets) and CI (brackets). In (**F**), using mediation analysis, *HLA-DRB*13* and aPS-PT effect estimates with CI and *P*-values are shown for the outcome thrombosis. Anti-β_2GPI/aCL: anti-β_2 glycoprotein I and/or anti-cardiolipin; ACME: average causal mediation effects; ADE: average direct effect of *DRB*13*.

associations (Fig. 1D). After adjustment for HCQ/prednisolone use and cardiovascular thrombotic risk factors (i.e. age, gender, hypertension, smoking, BMI and hyperlipidaemia) using one full forward stepwise regression model as well as several follow-up step-wise models excluding, one at a time, the independent variables that had significant associations with each other (supplementary Table S2, available at *Rheumatology* online), the associations to *HLA-DRB1*13 and HLA-DRB1*03* remained significant, with age, hyperlipidemia and steroids use also showing positive associations.

In a separate logistic regression model including anti-β_2GPI/aCL, aPS-PT, *HLA-DRB*13* and **04* as predictors, occurrence of thrombosis associated only with *HLA-DRB*13* and aPS-PT positivity (Fig. 1E). The effect of *HLA-DRB1*13* on thrombosis [β (95% CI): 0.18 (0.05, 0.3)] was partially mediated by aPS-PT positivity [β (95% CI): 0.06 (0.006, 0.1)] (Fig. 1F), implying that aPS-PT positivity accounts for about one-third (31.4%) of the total effect of *HLA-DRB1*13* on thrombosis. In SLE patients without secondary APS, only *HLA-DRB*13*, among HLA-DRB* alleles, associated with thrombosis [OR 1.2 (CI 1.1–3.9), $P = 0.04$] and cerebrovascular events [OR 3.1 (CI 1.1–9.6), $P = 0.03$].

HLA-DRB1*03 negative associations

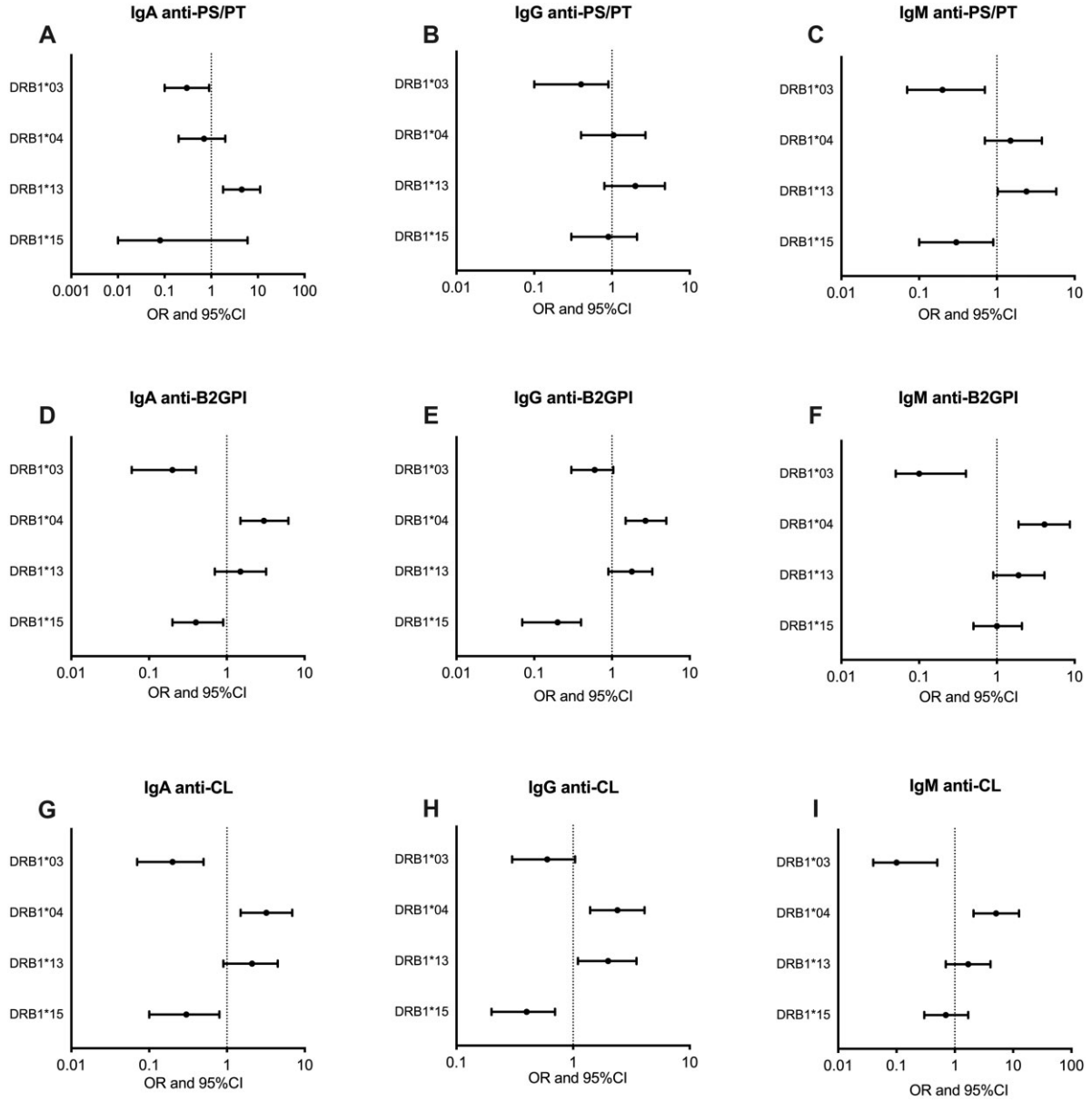
*HLA-DRB1*03* associated negatively with any thrombosis, VTE or APS diagnosis (Table 2). Associations between aPL positivity and VTE were significant, but after stratification for *HLA-DRB1*03* status, these associations were lost in the *HLA-DRB1*03* carrier patient group, for both aPS-PT and conventional aPL (Table 3). In addition, *HLA-DRB1*03* carriers had significantly lower levels of aPL compared with non-carriers (supplementary Table S3, available at *Rheumatology* online).

*HLA-DRB1*03* associated with higher levels of HDL (median 1.4 *vs* 1.2 mmol/l, $P = 0.02$) and lower levels of TG (median 0.9 *vs* 1.1 mmol/l, $P = 0.04$), Fig. 3. When correcting for age, gender, BMI and treatment with lipid-lowering agents, these associations remained, for HDL (standardized $\beta = 0.11$, $P = 0.04$) as well as TG (standardized $\beta = 0.12$, $P = 0.03$; data not shown). No other *HLA-DRB1* alleles associated with TG, HDL, LDL or total cholesterol (data not shown).

Discussion

Risk factors, such as HLA alleles as well as autoantibody profiles, have the potential to predict thrombotic events

FIG. 2 Associations of *HLA-DRB1*03*, **04*, **13*, **15* with the occurrence of individual anti-phospholipid isotypes in SLE patients



Odds ratios (OR) with CIs of the illustrated *HLA-DRB1* alleles for the occurrence of IgA, IgG, IgM antibodies against phosphatidylserine/prothrombin (A, B, C), β_2 glycoprotein I (D, E, F), cardiolipin (G, H, I) are demonstrated. Chi2 tests were used to calculate OR (central bullets) and CI (brackets). Anti-β_2GPI: anti-β_2 glycoprotein I.

in patients with APS. Therefore, understanding the association between these groups of risk factors and clinical variables is instrumental in the understanding and management of APS. Here we demonstrate that *HLA-DRB1*13* is a risk factor for expression of aPS-PT autoantibodies in SLE patients, while in contrast to conventional aPL, no association between aPS-PT and *HLA-DRB1*04* was observed. In addition, we demonstrate that aPS-PT antibodies mediate the association between *HLA-DRB1*13* and vascular events. Interestingly, *HLA-DRB1*03* associated negatively with aPL and thrombotic events, and positively with a favourable lipid profile.

To our best knowledge, only Bertolaccini *et al.* have previously investigated the associations between *HLA* and aPS-PT antibodies [28]. Also, they reported a higher prevalence of *HLA-DRB1*13* alleles among 82 patients from the UK with primary and secondary APS and positivity for aPS-PT as compared with healthy controls. However, this association was lost when anti-β_2GPI-positive patients were excluded [28]. We here confirm

Table 2 Associations of the various HLA DRB1 genotypes with APS-associated features

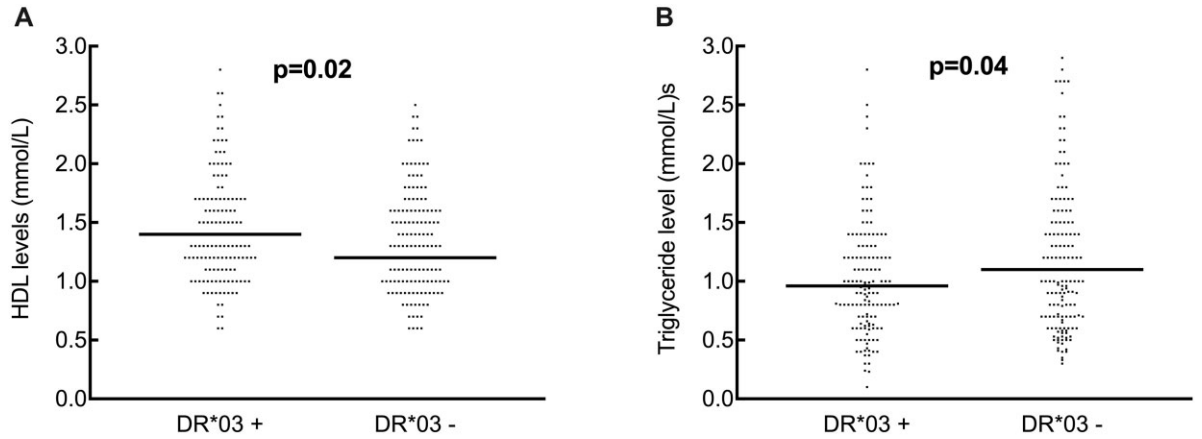
HLA-DRB1	Any thrombosis $n = 73$, *n* (%)	OR (95% CI); *P*	VTE $n = 48$, *n* (%)	OR (95% CI); *P*	AT $n = 36$, *n* (%)	OR (95% CI); *P*	APS diagnosis $n = 30$, *n* (%)	OR (95% CI); *P*	GAPSS median (mean) in DRB1 (pos/neg)	*P*
**01*	8 (10.9)	0.9 (0.3, 1.8); 0.6	3 (6.2)	0.4 (0.1, 1.3); 0.1	6 (17.1)	1.4 (0.5, 3.7); 0.5	5 (16.7)	1.5 (0.5, 4.2) 0.4	3 (5.8)/4 (5.8)	0.6
**03*	23 (31.5)	0.4 (0.2, 0.8); 0.004	14 (29.2)	0.4 (0.2, 0.8); 0.01	11 (31.4)	0.5 (0.2, 1.1); 0.06	6 (20.0)	0.2 (0.09, 0.6); 0.002	3 (4.3)/4 (7.1)	0.001
**04*	24 (32.9)	1.2 (0.7, 2.1); 0.5	18 (37.5)	1.5 (0.8, 2.8); 0.2	11 (31.4)	1.1 (0.5, 2.3); 0.8	12 (40.0)	1.6 (0.7, 3.4); 0.2	4 (7.7)/3 (4.9)	0.003
**07*	6 (8.2)	0.9 (0.3, 2.2); 0.8	2 (4.2)	0.4 (0.09, 1.7); 0.2	4 (11.4)	1.3 (0.4, 4.2); 0.6	2 (6.7)	0.7 (0.1, 3.2); 0.7	4 (7.3)/4 (5.7)	0.2
**08*	6 (8.2)	0.9 (0.3, 2.2); 0.8	4 (8.3)	0.9 (0.3, 2.7); 0.9	3 (8.6)	0.9 (0.3, 3.3); 0.9	3 (10.0)	1.1 (0.3, 4.0); 0.8	3 (5.7)/4 (5.8)	0.6
**09*	2 (2.7)	1.3 (0.2, 6.9); 0.7	2 (4.2)	2.2 (0.4, 11.9); 0.3	2 (5.7)	3.3 (0.6, 17.6); 0.1	1 (3.3)	1.3 (0.1, 10.9); 0.8	3 (3.7)/4 (5.9)	0.5
**10*	1 (1.3)	0.5 (0.1, 4.5); 0.5	0 0	NA	1 (2.9)	1.3 (0.1, 11.3); 0.8	0 0	NA	4 (3.2)/4 (5.9)	0.7
**11*	5 (6.8)	0.7 (0.3-2.1); 0.6	5 (10.4)	1.3 (0.5, 3.7); 0.6	2 (5.7)	0.6 (0.1, 2.8); 0.5	1 (3.3)	0.3 (0.04, 2.6); 0.3	4 (5.1)/4 (5.9)	0.9
**12*	3 (4.1)	2.5 (0.5, 11.4); 0.2	2 (4.2)	2.2 (0.4, 11.9); 0.3	1 (2.9)	1.3 (0.1, 11.3); 0.8	1 (3.3)	1.8 (0.2, 16.2); 0.6	2 (4.2)/4 (5.8)	0.3
**13*	29 (39.7)	2.5 (1.4, 4.4); 0.001	19 (39.6)	2.3 (1.2, 4.3); 0.01	12 (34.3)	1.7 (0.8, 3.5); 0.2	14 (46.7)	2.9 (1.4, 6.4); 0.004	4.5 (7.7)/3 (5.1)	0.007
**14*	3 (4.1)	3.3 (0.6, 16.9); 0.1	3 (6.2)	5.8 (1.1, 29.5); 0.049	0 0	NA	0 0	NA	9.5 (9.5)/4 (5.7)	0.6
**15*	25 (34.3)	0.8 (0.5, 1.4); 0.5	15 (31.2)	0.7 (0.4, 1.4); 0.3	13 (37.1)	0.9 (0.5, 2.0); 0.9	10 (33.3)	0.8 (0.4, 1.8); 0.6	3 (4.8)/4(6.4)	0.1
**16*	1 (1.3)	0.4 (0.05, 3.8); 0.4	0 0	NA	1 (2.9)	1.1 (0.1, 9.5); 0.9	1 (3.3)	1.5 (0.2, 13.1); 0.7	6.5 (7.7)/4 (5.8)	0.2

Table 3 Associations of aPL with thrombotic events among patients, stratified for the occurrence of *HLA-DRB1*03* alleles

	Any thrombosis antibody pos, *n/N* (%)	No thrombosis antibody pos, *n/N* (%)	OR (95% CI); *P*	VTE antibody pos, *n/N* (%)	No VTE antibody pos, *n/N* (%)	OR (95% CI); *P*
aPS/PT (any isotype)						
All patients	28/76 (36.8)	22/248 (8.9)	6 (3.1, 11.3); <0.0001	23/50 (46)	27/275 (9.8)	7.8 (3.9, 15.5); <0.0001
*HLA-DRB1*03* carriers	4/23 (17.4)	9/115 (7.8)	2.5 (0.7, 8.9); 0.1	3/14 (21.4)	10/124 (8.1)	3.1 (0.7, 13); 0.1
*HLA-DRB1*03* non-carriers	23/49 (46.9)	12/116 (10.3)	7.7 (3.4, 17.4); <0.0001	19/33 (57.7)	16/133 (12)	9.9 (4.2, 23.6); <0.0001
Anti-β_2GPI/aCL (any isotype)						
All patients	30/76 (39.5)	70/246 (28.4)	1.6 (0.1, 2.8); 0.07	25/50 (50)	75/273 (27.4)	2.6 (1.4, 4.9); 0.001
*HLA-DRB1*03* carriers	3/23 (13)	30/114 (26.3)	0.4 (0.1, 1.5); 0.2	3/14 (21.4)	30/123 (24.3)	0.8 (0.2, 3.2); 0.8
*HLA-DRB1*03* non-carriers	27/49 (55.1)	36/115 (31.3)	2.7 (1.3, 5.3); 0.004	22/33 (66.7)	41/132 (31.1)	4.4 (1.9, 10); 0.0002

Odds ratios (OR) and CIs were calculated using Chi^2 tests and data are expressed as fractions of antibody positive individuals among patients with and without thrombotic events, respectively. Significant associations are underlined. Anti-β_2GPI/aCL: anti-β_2 glycoprotein I or aCL; VTE: venous thromboembolism.

FIG. 3 *HLA-DRB1*03* allele association with HDL and LDL levels



Distribution of (**A**) HDL levels and (**B**) triglyceride levels among SLE patients stratified by carriage of *HLA-DRB1*03* alleles. HDL and TG data were available from 307 patients. HDL were outside the y axis limits for one patient and TG data for 13 patients; statistics and median bars represent data from all patients. HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglycerides.

associations between *HLA-DRB1*13* and aPS-PT, but in our larger cohort this association remain significant after exclusion of patients with anti-β_2GPI/aCL.

Among the conventional aPL markers, only aCL IgG associated with *HLA-DRB1*13*. Instead, there were strong associations between all isotypes of anti-β_2GPI and aCL and *HLA-DRB1*04*, in agreement with previous studies [12–14, 29]. In the study conducted by Lundström *et al.* on 665 Caucasian SLE patients, where parts of the current cohort were included, both *HLA-DRB1*13* and **04* associated with conventional aPL, although the *DRB1*13* allele associated only with anti-β_2GPI/aCL IgG, while *HLA-DRB1*04* associated with all conventional aPL measures [14]. Arnett *et al.* studied *HLA* risk haplotypes in a multiethnic North American cohort with primary and secondary APS [12]. They also documented a significant association between *HLA-DRB1*04* and anti-β_2GPI in patients with Caucasian ancestry, without association with *HLA-DRB1*13*. In that study, *HLA-DRB1*13* was associated with anti-β_2GP1 among patients with African genetic ancestry. We observed, in a previous publication, positive associations between *HLA-DRB1*13* and antiphospholipid-associated microvascular damage in kidney biopsies from patients with SLE [30]. None of these studies analysed associations for aPS-PT, which, as shown in the present study, differ from a main *HLA-DRB1*04* association present for conventional aPL in SLE. We could not confirm the previous association between aPL and *HLA-DRB1*07* [6, 7].

In our study, *HLA-DRB1*13* but not **04* increased the risk for thrombosis using both univariate and multivariate analyses. Lundström *et al.* reported that *HLA-DRB1*13* associated with any vascular events, while *HLA-DRB1*04* associated with cerebrovascular events. This *HLA-DRB1*04* association disappeared when excluding *HLA-DRB1*13* carrier patients. On the other hand, associations between *HLA-DRB1*13* and thrombosis remained significant after exclusion of *HLA-DRB1*04* carrier patients, indicating that *HLA-DRB1*13* confers an independent contribution to thrombosis [14]. In a study by Caliz *et al.*, both *HLA-DRB1*04* and *DRB1*13* were more frequent in primary and secondary APS as compared with controls, but only *HLA-DRB1*13* associated with arterial and venous thromboses [9]. Similarly, Tkachenko *et al.* showed that *HLA-DRB1*04* associated with anti-β_2GPI carriage but not with APS diagnosis, whereas *HLA-DRB1*13* associated with APS [13]. Collectively these data and our results indicate that *HLA-DRB1*13* is a stronger risk factor for thrombosis than *HLA-DRB1*04* in SLE patients. It is noteworthy that *HLA-DRB1*13* alleles were shown previously to be more frequent in SLE patients with aPL-associated nephritis compared with LN, without *HLA-DRB1*04* showing such associations [30].

Our study adds an understanding that the *HLA-DRB1*13* contribution to thrombosis risk is, to a large extent, mediated via aPS-PT. It is intriguing to note that besides being associated with aPS-PT, we find that *HLA-DRB1*13* is also independently associated with vascular events. This might point to a separate contributive role in vascular disease, perhaps linked to another not yet defined group of aPL, or to other factors.

The lower prevalence of *HLA-DRB1*03* in SLE patients with aPL and/or thrombotic events was significant also after adjustment for the presence of *HLA-DRB*04, *13* and **15*, and other possible confounding factors. Asherson *et al.* previously reported total absence of *HLA-DRB1*03* among 13 patients with primary APS [29]. In another study, *HLA-DRB1*03* was rare in APS patients compared with non-APS SLE patients and healthy controls [31]. Murali *et al.* also described lower prevalence of *HLA-DRB1*03* in patients with ischaemic stroke compared with healthy controls [15]. In contrast, other studies reported increased *HLA-DRB1*03* in APS, when comparing APS secondary to lupus with healthy controls [13, 32]. We suspect that their findings are

due to the previously well-known association between *DRB1*03* and SLE, and not to an association with aPL [33]. Therefore, the interpretation of results is complicated not only by the fact that *HLA-DRB1*03* is common in SLE, but also that this genotype is specifically associated with anti-SSA/anti-SSB autoantibodies [34–36]. We have recently demonstrated that an unsupervised cluster analysis based on 13 SLE-associated autoantibodies, not including aPS-PT, identified four SLE subgroups that differ regarding *HLA-DRB1* associations, clinical and immunological presentations. The *HLA-DRB1*03* genotype is accumulated in a separate subgroup of SLE patients, which is commonly positive for anti-SSA/anti-SSB autoantibodies [37].

When we stratified SLE patients into *HLA-DRB1*03* carriers and non-carriers, the association between aPS-PT and thrombotic events among carriers was lost among carriers, but became stronger among non-carriers; this was also evident for conventional aPL. Likewise, *HLA-DRB1*03* carriers had lower levels of aPL compared with non-carriers, perhaps explaining the less likely risk of thrombosis observed. In line with these favourable effects, our results also demonstrate that only *HLA-DRB1*03* carrier state, and no other studied *HLA-DRB1* allele, had a beneficial effect on lipid levels. To our knowledge there is only one paper describing a thrombo-protective effect of *DRB1*03*, but no study other than the current one has related a protective role of *DRB1*03* to lower aPL levels or the favourable lipid levels [15]. We have only found one study relating *HLA-DRB* alleles to lipid levels. A study by Giger *et al.* on postmenopausal African American women reported a strong association between *DRB1*07* and low HDL levels, and a weaker association between *HLA-DRB1*13* and high HDL levels, but no association with *HLA-DRB1*03* [38]. An older study by Kovalev *et al.* evaluated HLA class I alleles in relation to atherosclerotic disease and also reported relation to HDL levels. No association was found to *HLA-B8*, a finding that could imply that there is also no association with *HLA-DRB1*03* as both belong to the common ancestral *HLA-A1/B8/DR3* haplotype [39].

In univariate analysis we found a negative association between *HLA-DRB1*15* and aPL, which has also been reported by others [12, 13]. These associations were lost in multivariate analyses, and were not further analysed.

Our findings that individual *HLA-DRB1* alleles not only associate with specific aPL but also constitute independent risk factors for thrombosis imply that occurrence of *HLA-DRB1*03*, **04* and **13* might be evaluated as components in future APS risk scores, as today's scoring systems do not take HLA into account [23].

The fact that *HLA-DRB1*04* and *HLA-DRB1*13* associate with distinct groups of aPL suggests that the emergence of these groups of autoantibodies is driven by different initiating T lymphocytes. There is currently a vast literature on anti-β_2GPI-reactive T cells in APS [40, 41]. These T lymphocytes recognize β_2GPI peptides in the context of DR53 [42], an HLA-DR paralogue in linkage disequilibrium with *HLA-DRB1*04* but not with *HLA-DRB1*13* [43], in agreement with our findings. The literature concerning PS/PT-reactive T cells is essentially lacking; we found only one short communication dealing with prothrombin-reactive T cells in APS [44].

Limitations to our current investigation are the retrospective inclusion of thrombotic events and that the aPL assay we used was a prototype test not yet in clinical use at the time of analysis.

To conclude, our study is the first to confirm that *HLA-DRB1*13* is a risk factor for aPS-PT, and that *HLA-DRB1*13* is a stronger risk factor than *HLA-DRB1*04* for thrombosis in SLE patients. In contrast, *HLA-DRB1*03* seems to confer a protection for vascular events, being associated with lower levels of aPL and favourable lipid profiles. Our data give further support to the notion that different *HLA-DRB1* genotypes associate with different SLE subsets. Prospective large studies are required to examine this narrative.

Acknowledgements

We thank Silvia Casas at Werfen who provided laboratory reagents, access to assay equipment and manpower for autoantibody analyses.

Funding: This work was supported by the Swedish Rheumatism Association (R-932093, to J.R., R-840401 to E.S.); King Gustav Vth 80-year foundation (FAI-2019-0577, to J.R., FAI-2019-0628 to E.S.); the Agnes and Mac Rudberg Foundation; the Signe and Reinhold Sund's Foundation for Rheumatological Research; the Swedish Society of Medicine and the Ingegerd Johansson Donation (SLS-713911 to E.S.); the Swedish Research Council (2019-01632, to J.R., 2018-02535 to E.S.); the Uppsala County Council (ALF); the Stockholm County Council (ALF) and the Swedish Heart-Lung Foundation (20170257 and 20190510 to E.S.).

Disclosure statement: M.M. is employee of Werfen, and the company provided the aPL analyses used in this paper free of charge. The other authors have declared no conflicts of interest.

Data availability statement

The data underlying this article will be shared at reasonable request to the corresponding author.

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Willis R, Pierangeli SS. Pathophysiology of the antiphospholipid antibody syndrome. Auto Immun Highlights 2011;2:35–52.

2 Iuliano A, Galeazzi M, Sebastiani GD. Antiphospholipid syndrome's genetic and epigenetic aspects. Autoimmun Rev 2019;18:102352.

3 Dagenais P, Urowitz MB, Gladman DD, Norman CS. A family study of the antiphospholipid syndrome associated with other autoimmune diseases. J Rheumatol 1992;19:1393–6.

4 May KP, West SG, Moulds J, Kotzin BL. Different manifestations of the antiphospholipid antibody syndrome in a family with systemic lupus erythematosus. Arthritis Rheum 1993;36:528–33.

5 Bhattacharya S, Kendra J, Schiach C. A case study of familial anti-phospholipid syndrome. Clin Lab Haematol 2002;24:313–6.

6 Hartung K, Coldewey R, Corvetta A *et al.* MHC gene products and anticardiolipin antibodies in systemic lupus erythematosus results of a multicenter study. SLE Study Group. Autoimmunity 1992;13:95–9.

7 Goldstein R, Moulds JM, Smith CD, Sengar DP. MHC studies of the primary antiphospholipid antibody syndrome and of antiphospholipid antibodies in systemic lupus erythematosus. J Rheumatol 1996;23:1173–9.

8 Galeazzi M, Sebastiani GD, Tincani A *et al.* HLA class II alleles associations of anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. Lupus 2000;9:47–55.

9 Caliz R, Atsumi T, Kondeatis E *et al.* HLA class II gene polymorphisms in antiphospholipid syndrome: haplotype analysis in 83 Caucasoid patients. Rheumatology (Oxford) 2001;40:31–6.

10 McHugh NJ, Maddison PJ. HLA-DR antigens and anticardiolipin antibodies in patients with systemic lupus erythematosus. Arthritis Rheum 1989;32:1623–4.

11 McNeil HP, Gavaghan TP, Krilis SA, Geczy AF, Chesterman CN. HLA-DR antigens and anticardiolipin antibodies. Clin Exp Rheumatol 1990;8:425–6.

12 Arnett FC, Thiagarajan P, Ahn C, Reveille JD. Associations of anti-beta2-glycoprotein I autoantibodies with HLA class II alleles in three ethnic groups. Arthritis Rheum 1999;42:268–74.

13 Tkachenko O, Lapin S, Maslyansky A *et al.* Influence of HLA-DRB1 susceptibility alleles on the autoantibodies spectrum of systemic lupus erythematosus in European part of Russia. Autoimmun Rev 2019;18:558–60.

14 Lundström E, Gustafsson JT, Jönsen A *et al.* HLA-DRB104/13 alleles are associated with vascular disease and antiphospholipid antibodies in systemic lupus erythematosus. Ann Rheum Dis 2013;72:1018–25.

15 Murali V, Rathika C, Ramgopal S *et al.* Susceptible and protective associations of HLA DRB1/DQB1 alleles and haplotypes with ischaemic stroke. Int J Immunogenet 2016;43:159–65.

16 Sciascia S, Sanna G, Murru V *et al.* Anti-prothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome. A systematic review. Thromb Haemost 2014;111:354–64.

17 Radin M, Foddai SG, Cecchi I *et al.* Antiphosphatidylserine/prothrombin antibodies: an update on their association with clinical manifestations of antiphospholipid syndrome. Thromb Haemost 2020;120:592–8.

18 Elbagir S, Grosso G, Mohammed NA *et al.* Associations with thrombosis are stronger for antiphosphatidylserine/prothrombin antibodies than for the Sydney criteria antiphospholipid antibody tests in SLE. Lupus 2021;30:1289–99.

19 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.

20 Elbagir S, Elshafie AI, Elagib EM *et al.* Sudanese and Swedish patients with systemic lupus erythematosus: immunological and clinical comparisons. Rheumatology (Oxford) 2020;59:968–78.

21 Elbagir S, Elshafie AI, Elagib EM *et al.* High IgA antiphospholipid autoantibodies in healthy Sudanese explain the increased prevalence among Sudanese compared to Swedish systemic lupus erythematosus patients. Lupus 2020;29:1412–22.

22 Miyakis S, Lockshin MD, Atsumi T *et al.* International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4:295–306.

23 Sciascia S, Sanna G, Murru V *et al.* GAPSS: the Global Anti-Phospholipid Syndrome Score. Rheumatology (Oxford) 2013;52:1397–403.

24 Sciascia S, Radin M, Ramirez C *et al.* Evaluation of novel assays for the detection of autoantibodies in antiphospholipid syndrome. Autoimmun Rev 2020;19:102641.

25 Hadley Wickham MA, Bryan J, Chang W *et al.* Welcome to the tidyverse. Open Source Softw 2019;4:1686.

26 Rosseel Y. lavaan: an {R} package for structural equation modeling. J Stat Softw 2012;48:1–36.

27 Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. mediation: R package for causal mediation analysis. J Stat Softw 2014;59:1–38.

28 Bertolaccini ML, Atsumi T, Caliz AR *et al.* Association of antiphosphatidylserine/prothrombin autoantibodies with HLA class II genes. Arthritis Rheum 2000;43:683–8.

29 Asherson RA, Doherty DG, Vergani D, Khamashta MA, Hughes GR. Major histocompatibility complex associations with primary antiphospholipid syndrome. Arthritis Rheum 1992;35:124–5.

30 Gerhardsson J, Sundelin B, Zickert A *et al.* Histological antiphospholipid-associated nephropathy versus lupus nephritis in patients with systemic lupus erythematosus: an observational cross-sectional study with longitudinal follow-up. Arthritis Res Ther 2015;17:109.

31 Kapitany A, Tarr T, Gyetvai A *et al.* Human leukocyte antigen-DRB1 and -DQB1 genotyping in lupus patients with and without antiphospholipid syndrome. Ann N Y Acad Sci 2009;1173:545–51.

32 Freitas MV, da Silva LM, Deghaide NH, Donadi EA, Louzada-Júnior P. Is HLA class II susceptibility to primary antiphospholipid syndrome different from susceptibility to secondary antiphospholipid syndrome? Lupus 2004;13:125–31.

33 Reveille JD, Moulds JM, Ahn C *et al.*; the Lumina Study Group. Systemic lupus erythematosus in three ethnic groups: I. The effects of HLA class II, C4, and CR1

alleles, socioeconomic factors, and ethnicity at disease onset. LUMINA Study Group. Lupus in minority populations, nature versus nurture. Arthritis Rheum 1998; 41:1161–72.

34 Hamilton RG, Harley JB, Bias WB *et al.* Two Ro (SS-A) autoantibody responses in systemic lupus erythematosus. Correlation of HLA-DR/DQ specificities with quantitative expression of Ro (SS-A) autoantibody. Arthritis Rheum 1988;31:496–505.

35 Dudek NL, Maier S, Chen ZJ *et al.* T cell epitopes of the La/SSB autoantigen in humanized transgenic mice expressing the HLA class II haplotype DRB10301/ DQB10201. Arthritis Rheum 2007;56:3387–98.

36 Hom G, Graham RR, Modrek B *et al.* Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. N Engl J Med 2008;358:900–9.

37 Diaz-Gallo LM, Oke V, Lundström E *et al.* Four systemic lupus erythematosus subgroups, defined by autoantibodies status, differ regarding HLA-DRB1 genotype associations and immunological and clinical manifestations. ACR Open Rheumatol 2022;4:27–39.

38 Giger JN, Strickland OL, Weaver M, Taylor H, Acton RT. Genetic predictors of coronary heart disease risk factors in premenopausal African-American women. Ethn Dis 2005;15:221–32.

39 Kovalev Yu P, Dzeranova N, Serova LD *et al.* Comparison of HLA antigen spectrum in atherosclerosis of various localization. Cor Vasa 1990;32:118–25.

40 Hattori N, Kuwana M, Kaburaki J *et al.* T cells that are autoreactive to beta2-glycoprotein I in patients with antiphospholipid syndrome and healthy individuals. Arthritis Rheum 2000;43:65–75.

41 Arai T, Yoshida K, Kaburaki J *et al.* Autoreactive CD4(+) T-cell clones to beta2-glycoprotein I in patients with antiphospholipid syndrome: preferential recognition of the major phospholipid-binding site. Blood 2001;98:1889–96.

42 Kuwana M. Beta2-glycoprotein I: antiphospholipid syndrome and T-cell reactivity. Thromb Res 2004;114: 347–55.

43 Sebastiani GD, Galeazzi M, Morozzi G, Marcolongo R. The immunogenetics of the antiphospholipid syndrome, anticardiolipin antibodies, and lupus anticoagulant. Semin Arthritis Rheum 1996;25:414–20.

44 Yoshida K, Tsutsumi A, Ohnishi Y *et al.* T cell epitopes of prothrombin in patients with antiphospholipid syndrome. Ann Rheum Dis 2003;62:905–6.