REVIEW

Ethnic and geographical variation in antiphospholipid (Hughes) syndrome

I Uthman, M Khamashta

Ann Rheum Dis 2005;64:1671–1676. doi: 10.1136/ard.2005.038448

Investigation of the clinical epidemiology of the antiphospholipid syndrome (APS) is in its early stages. During the past 20 years, studies of antiphospholipid antibodies (aPL) and APS have been made in many countries and ethno-geographical groups. aPL appear to occur in all populations studied, with some variations noted in their frequency and in the clinical complications. Environmental and genetic factors contribute to ethnic variation and susceptibility to APS and thus interethnic differences in disease patterns may be due to environmental or genetic factors, or both.

Antiphospholipid antibodies (aPL) are recognised as a group of antibodies whose specificity is directed not only towards phospholipids such as cardiolipin but also towards phospholipid binding proteins such as ((β_2 -glycoprotein I (β_2 GPI) and prothrombin (PT)) or the complexes of phospholipids and phospholipid binding proteins.^{1 2} The presence of aPL is associated with arterial/venous thrombosis, recurrent fetal loss, neurological disorders, pulmonary hypertension, and thrombocytopenia. The term “antiphospholipid syndrome” (APS) or “Hughes syndrome” was coined to link these clinical manifestations with the persistence of aPL, which are now recognised as one of the most common causes of acquired thrombophilia.^{3 4}

Investigation of the clinical epidemiology of APS is in its early stages. During the past 20 years, studies of aPL and APS have been made in many countries and ethno-geographical groups.^{5–17} aPL appear to occur in all populations studied, with some variations noted in their frequency and in the clinical complications.^{5 13 14 16 18} Environmental and genetic factors contribute to ethnic variation and susceptibility to APS and thus interethnic differences in disease patterns may be due to environmental or genetic factors, or both.^{5 19} In this review we examine the features of APS in various epidemiological studies.

ANTIPHOSPHOLIPID ANTIBODIES (aPL)

Routine screening for aPL now occurs in systemic lupus erythematosus (SLE) clinics because of the strong experimental and clinical evidence of the procoagulant nature of aPL and the demonstrations that anticoagulation provides effective secondary prophylaxis of thrombosis or pregnancy loss in patients with aPL.

Table 1 summarises the prevalence and isotype distribution of anticardiolipin antibodies (aCL) and lupus anticoagulant (LA) in different populations of patients with primary APS and patients with SLE, mainly providing a point prevalence of these antibodies in various populations. It is evident from this that these antibodies occur in all populations of patients with SLE and primary APS studied, but with highly variable point prevalence.

A relative paucity of IgG (2%) and IgM (2%) aCL in Afro-Caribbean patients with SLE was noted and warrants further study.¹⁸ SLE in African-Americans and Afro-Caribbean patients with SLE is characterised by a generally worse outcome and a higher prevalence of autoantibodies than in other ethnic or geographical groups, and it would be of interest to determine if aPL are an exception to this pattern in SLE. In a largely Afro-American obstetric prenatal clinic population,²⁰ the prevalence of IgG aCL was 1.25% which approximates the frequency of IgG aCL found in other unselected prenatal clinic populations.²¹ In general, most studies from various countries report a mixture of aCL isotypes in individual patients, with IgG aCL being the commonest and most closely associated with thromboses and fetal losses.

“IgG aCL are the commonest isotypes in most patients with SLE”

IgA aCL are rarely present alone, except in Afro-Caribbean patients with SLE. In African-American patients with SLE, IgA aCL are also common, but often coexist with other isotypes. The value of IgA aCL and their relationship with thrombotic events is still controversial.^{22–26} Some experimental work suggests that IgA aCL are as prothrombotic as the IgG or IgM isotypes.²⁶ Although some reports showed that testing for IgA aCL was of additional benefit in patients with APS, especially in certain ethnic groups,^{17 18 23} other authors could not support these data.^{24 25}

Gharavi *et al* were the first to determine the distribution of immunoglobulin isotypes and phospholipid specificities of aCL in 40 patients with one or more of the following “aPL associated clinical complications”—namely, thrombosis, fetal loss, and thrombocytopenia.²⁴ They found IgA aCL in 52% of their population.²⁴

Abbreviations: aCL, anticardiolipin antibodies; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; aPT, anti-prothrombin antibodies; β_2 GPI, β_2 -glycoprotein I; LA, lupus anticoagulant; PT, prothrombin; SLE, systemic lupus erythematosus

See end of article for authors' affiliations

Correspondence to:
Professor I Uthman,
American University of
Beirut, Medical Centre, PO
Box 113-6044, Beirut,
Lebanon;
iuthman@aub.edu.lb

Accepted 19 May 2005
Published Online First
26 May 2005

Only one patient had IgA aCL as the sole aPL; thus it was concluded that this test is useful to identify occasional patients with APS.²⁴ Molina *et al* studied 152 African-American, 136 Afro-Caribbean (Jamaican), and 163 Hispanic (Colombian) unselected patients with SLE.¹⁸ The major finding of their study was the higher prevalence of IgA aCL in the Afro-Caribbean population (21%), IgA aCL being the sole isotype, detected in 82% of these positive patients. This isotype was usually detected at low titres and did not seem to be associated with clinical features of APS. However, in 1999 Diri *et al* reported eight Afro-American female patients with the APS, in whom IgA aCL were present in seven, co-occurring with IgG or IgM isotype in four of them.¹⁷ In the same study they also found IgA anti- β_2 GPI in 4/8 patients, co-occurring with IgM isotype in three of them.

In a cross sectional study to determine the prevalence of IgA aCL and anti- β_2 GPI and study their clinical significance in a cohort of 134 patients with SLE, we found a low prevalence (13%) of IgA aCL in patients with SLE.²⁷ It is not clear whether the African origin or ancestry of these populations correlates with the autoantibody profile.²⁸ It would seem likely that methodological, and possibly, environmental factors underlie the variations that occur in the aCL isotypes among these populations. Whether IgA aCL might contribute to a more comprehensive identification of APS in some SLE populations is still controversial.²²

HLA ASSOCIATIONS

The aetiology of the APS is linked to genetic predisposition, which may be accounted for, at least in part, by genes of the major histocompatibility complex (HLA system). The association between HLA class II genes and aPL production has been reported in a number of studies from different areas of the world,^{11 29–58} summarised in table 2.

The association of HLA-DRB1*04, DRB1*07(0701), DRB1*1302, DR53, DQB1*0301 (DQ7), *0302, and *0303, HLA-DR4, -DR7, DR5, -DRw53, DRB10901, DPB11501, DPB1-2301, HLA-DPB10301, DPB11901, and DQB106, with aCL has been demonstrated in APS. In addition, the various aPL show similar HLA association, again independent of the clinical context (primary APS or SLE), and across various ethnic groups.^{41 44–58}

Arnett *et al*³² reported the correlation between HLA-DQ7 and LA in 20 patients with a group of connective tissue diseases, and suggested that the risk factor for aPL was an HLA-DQB1 sequence comprising seven consecutive amino acid residues (71–77, TRAE LDT) in the third hypervariable region of the DQB1 outer domain.

We found a number of possible HLA alleles and haplotypes associated with APS. The major association seen was between the DQB1*0604/5/6/7/9-DQA1* 0102-DRB1*1302 haplotype and APS.^{37 39} The frequency of this haplotype was further increased when we analysed a more clinically homogeneous

Table 1 Prevalence and isotype distribution of aCL and LA in different populations of patients with primary APS and SLE

Reference	Ethnicity or country (n)	Any aCL isotype (%)	IgG aCL (%)	IgM aCL (%)	IgA aCL (%)	LA (%)	Correlation with thrombosis and/or fetal loss
<i>European</i>							
Sturfelt <i>et al</i> (1987) ⁷⁷	Sweden (59)	54	47	13	NA	NA	No
Sebastiani <i>et al</i> (1991) ⁷⁸	Italy (64)	44	44	9	NA	NA	Yes
Gourley <i>et al</i> (1996) ¹⁵	Ireland (95)	44	31	28	NA	NA	Yes
Sebastiani <i>et al</i> (1999) ⁷⁹	European†(574)	NA	23	14	14	NA	Yes
Cervera <i>et al</i> (2002) ⁸⁰	European (1000)	87.9	43.6	12.2	NA	53.6	Yes
<i>American (North, South)</i>							
Wilson <i>et al</i> (1988) ⁷	Black American (44)	27	100	NA	NA	NA	Yes
Alarcon-Segovia <i>et al</i> (1989) ⁸¹	Mexico (500)	53	39	33	16	NA	Yes
Chahade <i>et al</i> (1989) ⁸²	Brazilian (54)	20	20	5.5	NA	17	NA
Molina <i>et al</i> (1997) ¹⁸	Afro-Caribbean‡ (136)	21	2	2	21	NA	No
Alarcon <i>et al</i> (1999) ⁸³	Hispanic§ (70)	7*	NA	NA	NA	NA	NA
Alarcon, <i>et al</i> (1999) ⁸³	African-American§ (88)	11*	NA	NA	NA	NA	NA
Alarcon <i>et al</i> (1999) ⁸³	Caucasian§ (71)	5*	NA	NA	NA	NA	NA
Diri <i>et al</i> (1999) ¹⁷	African American (8)	100	NA	75	87.5	NA	Yes
Cucurull <i>et al</i> (1999) ⁸⁴	African American‡ (100)	33	18	7	24	NA	Yes
Cucurull <i>et al</i> (1999) ¹⁶	Colombian‡ (160)	25	18	13	15	NA	Yes
Cucurull <i>et al</i> (1999) ¹⁶	Spaniards‡ (160)	34	27	15	16	NA	Yes
Aguirre <i>et al</i> (2001) ⁸⁵	Chilean‡ (129)	30	16	14	8	NA	Yes
<i>Asian</i>							
Saluja <i>et al</i> (1990) ¹²	India¶ (76)	27	27	1	NA	NA	Yes
Jones <i>et al</i> (1991) ⁸	Malaysia** (200)	16.5	13	2.5	NA	NA	No
Wong <i>et al</i> (1991) ⁸⁶	China (91)	46	44	1	4	11	No
Wong <i>et al</i> (1991) ⁸⁶	China (91)	46	44	1	4	11	No
Ninomiya <i>et al</i> (1992) ⁸⁷	Japan (349)	35	28	NA	NA	27	Yes
Saxena <i>et al</i> (1994) ⁸⁸	India¶ (70)	19	NA	NA	NA	16	yes
Tsutsumi <i>et al</i> (1996) ⁸⁹	Japan (308)	NA	12	4	NA	8	Yes
Shrivastava <i>et al</i> (2001) ⁹⁰	India (76)	51	51	7	5	NA	No
<i>Middle-east Africa</i>							
Malaviya <i>et al</i> (1996) ⁹¹	Kuwaiti, Middle-Eastern and North-African Arabs (29)	75	NA	NA	NA	NA	Yes
Al Maini <i>et al</i> (2002) ⁹²	Gulf Arabs and Arabs of Persian descent (83)	NA	17.3	14.2	NA	NA	Yes
Houman <i>et al</i> (2004) ⁹³	Tunisian (North Africans) (100)	66	NA	NA	NA	NA	Yes

*IgG and IgM aCL or LA, or both; †patients from seven European countries: 97.7% white, 2.3% other races; ‡in-house ELISA test done at Louisiana State University Health Sciences Center in New Orleans; §LUMINA Study Group: LUPus in MInority populations: NAture v nurture. From University of Alabama at Birmingham, University of Texas-Houston Health Science Center, and University of Texas Medical Branch at Galveston; ¶both studies from All India Institute of Medical Sciences, New Delhi; **population comprised 164 Chinese, 26 Malay, and 10 Indian. No differences were found in the prevalence of raised aCL between the three ethnic groups. NA, data not available.

Table 2 HLA associations in different populations of patients with APS

Reference	Patients (n)	Ethnicity	Antibodies
<i>European</i> Savi <i>et al</i> (1988) ⁴⁸	80	Northern Italian	A highly significant ($p=6.17 \times 10^{-7}$) association was found between anticardiolipin antibodies and DR7
Trabace <i>et al</i> (1991) ⁵⁷	49	Italian	HLA-DR7 frequency was 40% in aCL positive patients v 8.3% in aCL negative patients ($p=0.011$)
Hartung <i>et al</i> (1992) ³³	314	Central European	Both HLA-DR4 and DR7 were increased in aCL positive patients, and aCL were significantly associated with DRw53
Colucci <i>et al</i> (1992) ⁴⁴	82	Italian	HLA-B8, DR3 positive young women have significantly higher levels of aPL than HLA-B8, DR3 negative women
Camps <i>et al</i> (1995) ⁴⁰	19	South of Spain	HLA-DQ7 antigen showed the highest relative risk for primary APS, followed by DRw53
Panzer <i>et al</i> (1997) ⁵⁶	27	Austria	An increased frequency of HLA-DQB1*06
Christiansen <i>et al</i> (1998) ⁵⁸	123	Danish and Czech women	HLA-DR3 phenotypes seem to predispose to the formation of aCL antibodies and antinuclear antibodies
Bertolaccini <i>et al</i> (2000) ³⁹	82	British caucasoid	IgG antiphosphatidylserine/prothrombin antibodies (aPS/PT) were present in 41/82 (50%) patients
Caliz <i>et al</i> (2001) ³⁷	83	British caucasoid	The frequencies of DQB1*0301/4, DQB1*0604/5/6/7/9, and DRB1*1302 alleles were increased in patients with aPS/PT compared with controls
Domenico Sebastiani <i>et al</i> (2003) ⁴¹	–	Italian	DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302 and DQB1*0303-DQA1*0201-DRB1*0701 haplotypes showed significantly positive correlations with the APS
			The association of HLA-DR4, -DR7, -DRw53, and -DQB1*0302 with aCL that has been demonstrated in primary APS can also be found in SLE
<i>American (North, South)</i> Vargas-Alarcon <i>et al</i> (1995) ⁴⁹	–	Mexican	HLA-DR5 (possibly DRB1*1201) with the primary APS in Mexican patients
Goldstein <i>et al</i> (1996) ¹¹	91 SLE and 16 primary APS	White	The strongest association is with the HLA-DR53 haplotypes, some of which include the DQ7 allele
Granados <i>et al</i> (1997) ⁵²	80	Mexican	Patients with SLE with aCL had statistically significantly increased corrected frequencies of HLA-DR3; DR7 and DQ2 antigens
Ioannidis <i>et al</i> (1999) ²⁹	67 Greek, 74 others	Greek, white, African-American, Mexican-American	The major alleles associated with anti- β_2 GPI response are HLA-DQA1*03 (in particular, *0301) and the HLA-DRB1*1302-
Arnett <i>et al</i> (1999) ³⁸	262	Mexican, American whites, blacks	HLA-DQB1*0302, as well as HLA-DQB1*03 alleles overall (DQB1*0301, *0302, and *0303), were strongly correlated with anti- β_2 GPI antibodies in all ethnic groups
			The HLA-DR6 (DR13) haplotype DRB1*1302; DQB1*0604/5 was also significantly increased, primarily in blacks
			HLA-DR7 was not significantly increased in any of these three ethnic groups
			HLA-DR53 (DRB4*0101) was increased in Mexican Americans only
			Compared with controls, patients with primary APS exhibited a non-significantly increased frequency of DR53 associated alleles, and patients with secondary APS presented an increased frequency of HLA-DRB1*03 alleles
			A trend towards an increase in the frequency of the DQB1*0604 allele and of the DQB1*0302 allele was seen in secondary APS
<i>Freitas <i>et al</i> (2004)⁴²</i>	123 patients and 166 controls	Brazilian	
<i>Asian</i> Hashimoto <i>et al</i> (1998) ⁵³	145	Japanese	Patients with SLE with β_2 GPI dependent aCL were significantly associated with DRB1*0901

group; its frequency was increased more in primary APS than in secondary APS, the association being even stronger in anti- β_2 GPI positive primary APS.³⁷ Accordingly, it is suggested that this (DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302) haplotype predisposes to anti- β_2 GPI, which is one of the most specific markers of APS.^{59–61} HLA-DR and DQ molecules function by binding specific peptides with subsequent presentation by antigen presenting cells to regulatory or effector T cells.^{62–63} Our data may be viewed in this context and suggest that a molecule encoded by the DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302 haplotype may preferentially present peptides derived from β_2 GPI or associated molecules.³⁷ Consequently, people bearing this haplotype may be prone to generate anti- β_2 GPI, after taking other genetic and environmental variables into account.³⁷

In addition to these classic class II molecules, it has recently been shown that HLA-DM molecules have crucial roles in HLA class II restricted antigen presentation, by studies of cell lines lacking HLA-DM, which are defective in class II restricted antigen processing.^{64–66} The presence of polymorphisms in DM genes^{67–68} raised the possibility of their involvement in the development of HLA class II associated

diseases, although the relation between these polymorphisms and the function of DM molecules has not yet been clarified. A study of SLE in a Japanese population did not find any significant association between the HLA-DM polymorphisms and the development of SLE and specific manifestations, but the existence of aPL was not considered.⁶⁹

We have recently examined the susceptibility of these polymorphisms to aPL production in a white British population, and observed the skewed distribution of DMA alleles including an increase of DMA*0102 in patients with aPL, which is the first report on HLA-DM and aPL/APS. The minor effect of the DMA polymorphisms on the presence of anti-prothrombin antibodies (aPT), especially of IgG class, suggested the importance of another genetic predisposition in aPT production. More than half of aPL positive patients did not have DMA*0102, indicating the involvement of other genetic risk factors for aPL production separate from the DMA*0102 or DMA*0102 containing haplotype. Furthermore, the presence of a considerable number of patients with DMA*0102 but without aPL suggests the requirement of a coexistence of other genetic or environmental (for example, infections) factors for aPL production.⁴⁶

Table 3 Polymorphisms of target antigens and coagulation factors other than HLA in different populations of patients with APS

Reference	Patients (n)	Ethnicity	Polymorphisms
Delrieu <i>et al</i> (1999) ⁴⁵	171 SLE, 88 primary APS, 193 controls	French whites	Poly(ADP-ribose) polymerase alleles do not influence susceptibility to SLE or primary APS in French white subjects
Hirose <i>et al</i> (1999) ³¹	149	Whites, African American, Asians	In Asian patients with APS, expression of a Val at position 247, especially in the homozygous state, is significantly associated with the presence of anti- β_2 GPI antibodies
Atsumi <i>et al</i> (1999) ⁷⁰	88	Caucasoid	β_2 GPI polymorphism, Val/Leu 247, is correlated with anti- β_2 GPI antibody production in patients with primary APS, and Val 247 may be important in the formation of β_2 GPI antigenicity
Ruiz-Arguelles <i>et al</i> (1999) ⁴³	14	Mexican mestizo	The G20210A polymorphism (the G→A mutation at nucleotide position 20210) in the 3'-untranslated region of the PT gene in Mexican mestizo patients with APS does not seem to be related to the thrombophilia seen in these patients
Yasuda <i>et al</i> (2002) ⁹⁴	77 Japanese and 82 British patients with aPL	Japanese and British	Polymorphisms of the tissue plasminogen activator and plasminogen activator inhibitor-1 genes probably do not significantly influence the risk of arterial thrombosis, venous thrombosis, or pregnancy morbidity in patients with aPL
Prieto <i>et al</i> (2003) ⁷¹	39 primary APS, 106 healthy controls	Mexican	Anti- β_2 GPI positive patients had significantly higher frequencies of the Val/Val genotype and Val allele expression than control subjects and the β_2 GPI negative patients
Camilleri <i>et al</i> (2003) ³⁰	230	White	The Val/Val genotype at position 247 of the β_2 GPI gene may have a role in the generation of anti- β_2 GPI antibodies and perhaps in the expression of arterial thrombosis in primary APS Significantly decreased prevalence of the Ser316 allele in aPL negative women (n = 98) in comparison with female normal control subjects

"Different HLA polymorphisms associated with APS may lead to development of different aspects of the disease"

We conclude that several HLA class II gene polymorphisms are associated with APS, probably along with other genetic factors, and may determine the development of different aspects of the disease.³⁷ These polymorphisms may be correlated with the immune response against thrombosis related autoantigens, such as phospholipid binding proteins and phospholipids.³⁷ It is also possible that some undefined polymorphisms in linkage disequilibrium with the HLA region are responsible for the induction of anti- β_2 GPI antibodies.³⁷

POLYMORPHISMS OF TARGET ANTIGENS AND COAGULATION FACTORS

Polymorphisms of target antigens and coagulation factors are reported (table 3) to be associated with aPL induction and the development of thrombosis.

The human β_2 GPI gene is located on chromosome 17 and so far four common single nucleotide polymorphisms in protein coding region have been identified.¹⁹ The polymorphisms Ser/Asn 88, Val/Leu 247, Cys/Gly306, and Trp/Ser 316 are located in exons 3, 7, 7, and 8 of the β_2 GPI gene, respectively. The Val/Leu polymorphism at codon 247 has been extensively studied among these polymorphisms.¹⁹ Hirose *et al* reported that Val 247 alleles were found more frequently in Asian patients with APS than among controls matched for ethnicity, and Val 247 was significantly associated with the presence of anti- β_2 GPI.³¹ They found no significant differences in allele frequencies in comparisons of the white or the black patients with APS with appropriate controls, although Val 247 alleles were more common in these ethnic groups than in Asians. Atsumi *et al* analysed the Val/Leu 247 polymorphism in a cohort of 88 British patients with APS and found that Val 247 correlated with anti- β_2 GPI production in patients with primary APS, and Val 247 might be important in the formation of β_2 GPI antigenicity.⁷⁰ Prieto *et al* suggested that the Val/Val genotype at codon 247 played a part in the generation of anti- β_2 GPI and in the expression of arterial thrombosis in primary APS in Mexican patients.⁷¹ More recently, Yasuda *et al* in a study of 65 Japanese patients

with APS and /or SLE compared with 61 controls found that the Val 247 β_2 GPI allele, compared with the Leu 247 β_2 -GPI allele, was associated with both a high frequency of anti- β_2 GPI antibodies and stronger reactivity with anti- β_2 GPI antibodies. This suggests that the Val(247) β_2 GPI allele may be one of the genetic risk factors for development of APS.⁷² On the other hand, Camilleri *et al* found no association Val/Leu 247 polymorphism and the presence of anti- β_2 GPI in a white population.³⁰

LIMITATIONS OF GENETIC STUDIES IN APS

Interpretation of epidemiological studies in various ethnic groups is quite difficult for the following reasons:

- Although the enzyme linked immunosorbent assay (ELISA) for aCL antibodies and LA testing has been extensively standardised, significant variation between laboratories in the results of testing still remains. The precise cut off points for positive/negative results vary among laboratories.⁷³
- Clinical heterogeneity: the clinical definition of APS has varied among studies⁴. Some patients with APS also manifest SLE, and constitute a heterogeneous population, making it difficult to analyse the role of a single factor. With the publication of the Sapporo criteria for the preliminary classification criteria for definite APS⁷⁴ this problem will be solved with studies done on more uniform patient groups.
- Interethnic variation in the associations of aPL with thrombosis or pregnancy loss must also take into account the multiple risk factors that exist in most populations for these complications. Possibly, variation in such collateral risk factors—for example, drug use or genetic risk factors for thrombosis, may influence complication rates associated with aPL in various populations. For instance, in Lebanon, a high prevalence of prothrombin G20210A and factor V Leiden mutations exists.^{75–76} These factors will increase the thrombotic risk, especially in patients with aPL.
- Disease activity: The level of disease activity is an important factor to control for in future studies. In early studies in the African-American clinic population in New

Orleans it was found that IgG aCL were present in 27% of patients with SLE during periods of disease activity, compared with only 5% of patients with SLE during periods of less active SLE.⁷

- Geographical migration: with the current increasing geographical migration and intermingling across geographical and ethnic groups, it is important to consider these variables in the interpretation of future studies.

CONCLUSIONS

Genetic susceptibility related to aPL and APS has been extensively examined in past years. However, it has been difficult to determine genetic risk factors for aPL and APS because of the heterogeneity in the antigen specificity, and pathogenesis of the clinical manifestations of APS. It is clear from the above that the study of the clinical epidemiology of aPL is still in its infancy. Most studies have reported data on only one ethnic and/or geographical group, and comparisons between these studies are confounded by methodological variations or patient selection. The publication in 1999 of international consensus criteria for APS⁷⁴ should facilitate future studies. Genome-wide linkage analysis and multi-centre international collaboration would be useful to obtain a better understanding of the genetic predisposition which produces aPL and leads to the development of the clinical features of APS.

Authors' affiliations

I Uthman, Division of Rheumatology, Faculty of Medicine, American University of Beirut, Beirut, Lebanon
M Khamashta, Lupus Research Unit, The Rayne Institute, King's College London School of Medicine, St Thomas' Hospital, London, UK

REFERENCES

- Roubey RA. Autoantibodies to phospholipid-binding plasma proteins: a new view of lupus anticoagulants and other "antiphospholipid" autoantibodies. *Blood* 1994;**84**:2854–67.
- Roubey RA. Immunology of the antiphospholipid antibody syndrome. *Arthritis Rheum* 1996;**39**:1444–54.
- Hughes GR. The antiphospholipid syndrome: ten years on. *Lancet* 1993;**342**:341–4.
- Khamashta MA, Hughes GR. Antiphospholipid antibodies and antiphospholipid syndrome. *Curr Opin Rheumatol* 1995;**7**:389–94.
- Wilson WA. Ethnicity and APS. *J Autoimmun* 2000;**15**:153–5.
- Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young CG, Loizou S, et al. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* 1983;**2**:1211–14.
- Wilson WA, Perez MC, Michalski JP, Armatas PE. Cardiolipin antibodies and null alleles of C4 in black Americans with systemic lupus erythematosus. *J Rheumatol* 1988;**15**:1768–72.
- Jones HW, Ireland R, Senaldi G, Wang F, Khamashta M, Bellingham AJ, et al. Anticardiolipin antibodies in patients from Malaysia with systemic lupus erythematosus. *Ann Rheum Dis* 1991;**50**:173–5.
- Molina JF, Molina J, Garcia C, Gharavi AE, Wilson WA, Espinoza LR. Ethnic differences in the clinical expression of systemic lupus erythematosus: a comparative study between African-Americans and Latin Americans. *Lupus* 1997;**6**:63–7.
- Font J, Lopez-Soto A, Cervera R, Balasch J, Pallares L, Navarro M, et al. The 'primary' antiphospholipid syndrome: antiphospholipid antibody pattern and clinical features of a series of 23 patients. *Autoimmunity* 1991;**9**:69–75.
- 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.
- Saluja S, Kumar A, Khamashta M, Hughes GR, Malaviya AN. Prevalence and clinical associations of anticardiolipin antibodies in patients with systemic lupus erythematosus in India. *Indian J Med Res* 1990;**92**:224–7.
- Malaviya AN, Chandrasekaran AN, Kumar A, Shamar PN. Systemic lupus erythematosus in India. *Lupus* 1997;**6**:690–700.
- Abu-Shakra M, Gladman DD, Urowitz MB, Farewell V. Anticardiolipin antibodies in systemic lupus erythematosus: clinical and laboratory correlations. *Am J Med* 1995;**99**:624–8.
- Gourley IS, McMillan SA, Bell AL. Clinical features associated with a positive anticardiolipin antibody in Irish patients with systemic lupus erythematosus. *Clin Rheumatol* 1996;**15**:457–60.
- Cucurull E, Espinoza LR, Mendez E, Molina JF, Molina J, Ordi-Ros J, et al. Anticardiolipin and anti-beta2glycoprotein-I antibodies in patients with systemic lupus erythematosus: comparison between Colombians and Spaniards. *Lupus* 1999;**8**:134–41.
- Diri E, Cucurull E, Gharavi AE, Kapoor D, Mendez EA, Scopelitis E, et al. Antiphospholipid (Hughes') syndrome in African-Americans: IgA aCL and beta2 glycoprotein-I is the most frequent isotype. *Lupus* 1999;**8**:263–8.
- Molina JF, Gutierrez-Urena S, Molina J, Uribe O, Richards S, De Ceulaer C, et al. Variability of anticardiolipin antibody isotype distribution in 3 geographic populations of patients with systemic lupus erythematosus. *J Rheumatol* 1997;**24**:291–6.
- Horita T, Merrill JT. Genetics of antiphospholipid syndrome. *Curr Rheumatol Rep* 2004;**6**:458–62.
- Perez MC, Wilson WA, Brown HL, Scopelitis E. Anticardiolipin antibodies in unselected pregnant women. Relationship to fetal outcome. *J Perinatol* 1991;**11**:33–6.
- Harris EN, Spinnato JA. Should anticardiolipin tests be performed in otherwise healthy pregnant women? *Am J Obstet Gynecol* 1991;**165**(Pt 1):1272–7.
- Wilson WA, Faghiri Z, Taheri F, Gharavi AE. Significance of IgA antiphospholipid antibodies. *Lupus* 1998;**7**(suppl 2):S110–13.
- Lopez LR, Santos ME, Espinoza LR, La Rosa FG. Clinical significance of immunoglobulin A versus immunoglobulins G and M anti-cardiolipin antibodies in patients with systemic lupus erythematosus. Correlation with thrombosis, thrombocytopenia, and recurrent abortion. *Am J Clin Pathol* 1992;**98**:449–54.
- Gharavi AE, Harris EN, Asherson RA, Hughes GR. Anticardiolipin antibodies: isotype distribution and phospholipid specificity. *Ann Rheum Dis* 1987;**46**:1–6.
- Loizou S, Cofner C, Weetman AP, Walport MJ. Immunoglobulin class and IgG subclass distribution of anticardiolipin antibodies in patients with systemic lupus erythematosus and associated disorders. *Clin Exp Immunol* 1992;**90**:434–9.
- Pierangeli SS, Liu XW, Barker JH, Anderson G, Harris EN. Induction of thrombosis in a mouse model by IgG, IgM and IgA immunoglobulins from patients with the antiphospholipid syndrome. *Thromb Haemost* 1995;**74**:1361–7.
- Bertolaccini ML, Atsumi T, Escudero Contreras A, Khamashta MA, Hughes GR. The value of IgA antiphospholipid testing for diagnosis of antiphospholipid (Hughes) syndrome in systemic lupus erythematosus. *J Rheumatol* 2001;**28**:2637–43.
- Hammond M, Du Toit E, Sanchez-Mazas A. HLA in Sub-Saharan Africa: 12th International Histocompatibility Workshop SSAF report. In: D C, eds. *HLA*. Paris: EDK, 1997:345–53.
- Ioannidis JP, Tektonidou MG, Vlachoyiannopoulos PG, Stavropoulos-Giokas C, Spyropoulou-Vlachou M, Reveille JD, et al. HLA associations of anti-beta2 glycoprotein I response in a Greek cohort with antiphospholipid syndrome and meta-analysis of four ethnic groups. *Hum Immunol* 1999;**60**:1274–80.
- Camilleri RS, Mackie IJ, Humphries SE, Machin SJ, Cohen H. Lack of association of beta2-glycoprotein I polymorphisms Val247Leu and Trp316Ser with antiphospholipid antibodies in patients with thrombosis and pregnancy complications. *Br J Haematol* 2003;**120**:1066–72.
- Hirose N, Williams R, Alberts AR, Furie RA, Chartash EK, Jain RI, et al. A role for the polymorphism at position 247 of the beta2-glycoprotein I gene in the generation of anti-beta2-glycoprotein I antibodies in the antiphospholipid syndrome. *Arthritis Rheum* 1999;**42**:1655–61.
- Arnett FC, Olsen ML, Anderson KL, Reveille JD. Molecular analysis of major histocompatibility complex alleles associated with the lupus anticoagulant. *J Clin Invest* 1991;**87**:1490–5.
- Hartung K, Coldevey R, Corvetta A, Deicher H, Kalden JR, Krapf F, 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.
- Asherson RA, Doherty DG, Vergani D, Khamashta MA, Hughes GR. Major histocompatibility complex associations with primary antiphospholipid syndrome. *Arthritis Rheum* 1992;**35**:124–5.
- Wilson WA, Scopelitis E, Michalski JP, Pierangeli SS, Silveira LH, Elston RC, et al. Familial anticardiolipin antibodies and C4 deficiency genotypes that coexist with MHC DQB1 risk factors. *J Rheumatol* 1995;**22**:227–35.
- Wilson WA. Histocompatibility genes in antiphospholipid antibody syndrome. *Lupus* 1996;**5**:259–62.
- Caliz R, Atsumi T, Kondeatis E, Amengual O, Khamashta MA, Vaughan RW, et al. HLA class II gene polymorphisms in antiphospholipid syndrome: haplotype analysis in 83 Caucasoid patients. *Rheumatology (Oxford)* 2001;**40**:31–6.
- 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.
- Bertolaccini ML, Atsumi T, Caliz AR, Amengual O, Khamashta MA, Hughes GR, et al. Association of antiphosphatidylserine/prothrombin autoantibodies with HLA class II genes. *Arthritis Rheum* 2000;**43**:683–8.
- Camps MT, Cuadrado MJ, Ocon P, Alonso A, Gutierrez A, Guil M, et al. Association between HLA class II antigens and primary antiphospholipid syndrome from the south of Spain. *Lupus* 1995;**4**:51–5.
- Domenico Sebastiani G, Minisola G, Galeazzi M. HLA class II alleles and genetic predisposition to the antiphospholipid syndrome. *Autoimmun Rev* 2003;**2**:387–94.
- Freitas MV, da Silva LM, Deghaide NH, Donadi EA, Louzada-Junior P. Is HLA class II susceptibility to primary antiphospholipid syndrome different from susceptibility to secondary antiphospholipid syndrome? *Lupus* 2004;**13**:125–31.

- 43 Ruiz-Arguelles GJ, Garces-Eisele J, Ruiz-Delgado GJ, Alarcon-Segovia D. The G20210A polymorphism in the 3'-untranslated region of the prothrombin gene in Mexican mestizo patients with primary antiphospholipid syndrome. *Clin Appl Thromb Hemost* 1999;5:158-60.
- 44 Colucci AT, Di Lorenzo G, Ingrassia A, Crescimanno G, Modica MA, Candore G, et al. Blood antiphospholipid antibody levels are influenced by age, sex and HLA-B8, DR3 phenotype. *Exp Clin Immunogenet* 1992;9:72-9.
- 45 Delrieu O, Michel M, Frances C, Meyer O, Michel C, Witte F, et al. Poly(ADP-ribose) polymerase alleles in French Caucasians are associated neither with lupus nor with primary antiphospholipid syndrome. GRAID Research Group. Group for Research on Auto-Immune Disorders. *Arthritis Rheum* 1999;42:2194-7.
- 46 Sanchez ML, Katsumata K, Atsumi T, Romero FI, Bertolaccini ML, Funke A, et al. Association of HLA-DM polymorphism with the production of antiphospholipid antibodies. *Ann Rheum Dis* 2004;63:1645-8.
- 47 Wilson WA, Gharavi AE. Genetic risk factors for aPL syndrome. *Lupus* 1996;5:398-403.
- 48 Savi M, Ferraccioli GF, Neri TM, Zanelli P, Dall'Aglia PP, Tincani A, et al. HLA-DR antigens and anticardiolipin antibodies in northern Italian systemic lupus erythematosus patients. *Arthritis Rheum* 1988;31:1568-70.
- 49 Vargas-Alarcon G, Granados J, Bekker C, Alcocer-Varela J, Alarcon-Segovia D. Association of HLA-DR5 (possibly DRB1*1201) with the primary antiphospholipid syndrome in Mexican patients. *Arthritis Rheum* 1995;38:1340-1.
- 50 Doherty DG, Vaughan RW, Donaldson PT, Mowat AP. HLA DQA, DQB, and DRB genotyping by oligonucleotide analysis: distribution of alleles and haplotypes in British caucasoids. *Hum Immunol* 1992;34:53-63.
- 51 Lanchbury JS, Jaeger EE, Sansom DM, Hall MA, Wordsworth P, Stedeford J, et al. Strong primary selection for the Dw4 subtype of DR4 accounts for the HLA-DQw7 association with Felty's syndrome. *Hum Immunol* 1991;32:56-64.
- 52 Granados J, Vargas-Alarcon G, Drenkard C, Andrade F, Melin-Aldana H, Alcocer-Varela J, et al. Relationship of anticardiolipin antibodies and antiphospholipid syndrome to HLA-DR7 in Mexican patients with systemic lupus erythematosus (SLE). *Lupus* 1997;6:57-62.
- 53 Hashimoto H, Yamanaka K, Tokano Y, Iida N, Takasaki Y, Kabasawa K, et al. HLA-DRB1 alleles and beta 2 glycoprotein I-dependent anticardiolipin antibodies in Japanese patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 1998;16:423-7.
- 54 Galeazzi M, Sebastiani GD, Tincani A, Piette JC, Allegri F, Morozzi G, 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.
- 55 Sebastiani GD, Galeazzi M, Tincani A, Scorza R, Mathieu A, Passiu G, et al. HLA-DPB1 alleles association of anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. *Lupus* 2003;12:560-3.
- 56 Panzer S, Pabinger I, Gschwandtner ME, Mayr WR, Hutter D. Lupus anticoagulants: strong association with the major histocompatibility complex class II and platelet antibodies. *Br J Haematol* 1997;98:342-5.
- 57 Trabace S, Nicotra M, Cappellacci S, Morellini M, Mutinelli C, Sbracia M, et al. HLA-DR and DQ antigens and anticardiolipin antibodies in women with recurrent spontaneous abortions. *Am J Reprod Immunol* 1991;26:147-9.
- 58 Christiansen OB, Ulcova-Gallova Z, Mohapeloa H, Kraus V. Studies on associations between human leukocyte antigen (HLA) class II alleles and antiphospholipid antibodies in Danish and Czech women with recurrent miscarriages. *Hum Reprod* 1998;13:3326-31.
- 59 Amengual O, Atsumi T, Khamashta MA, Hughes GR. Clinical significance of anti-beta 2-glycoprotein I antibodies. *Ann Med Interne (Paris)* 1996;147(suppl 1):15-17.
- 60 Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Suzuki T, Sumida T, et al. Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. *J Immunol* 1992;148:3885-91.
- 61 Galli M, Finazzi G, Bevers EM, Barbui T. Kaolin clotting time and dilute Russell's viper venom time distinguish between prothrombin-dependent and beta 2-glycoprotein I-dependent antiphospholipid antibodies. *Blood* 1995;86:617-23.
- 62 O'Sullivan D, Sidney J, Appella E, Walker L, Phillips L, Colon SM, et al. Characterization of the specificity of peptide binding to four DR haplotypes. *J Immunol* 1990;145:1799-808.
- 63 Kwok WW, Nepom GT, Raymond FC. HLA-DQ polymorphisms are highly selective for peptide binding interactions. *J Immunol* 1995;155:2468-76.
- 64 Kelly AP, Monaco JJ, Cho SG, Trowsdale J. A new human HLA class II-related locus, DM. *Nature* 1991;353:571-3.
- 65 Mellins E, Smith L, Arp B, Cotner T, Celis E, Pious D. Defective processing and presentation of exogenous antigens in mutants with normal HLA class II genes. *Nature* 1990;343:71-4.
- 66 Vogt AB, Krapshofer H, Hammerling GJ. How HLA-DM affects the peptide repertoire bound to HLA-DR molecules. *Hum Immunol* 1997;54:170-9.
- 67 Carrington M, Yeager M, Mann D. Characterization of HLA-DMB polymorphism. *Immunogenetics* 1993;38:446-9.
- 68 Carrington M, Harding A. Sequence analysis of two novel HLA-DMA alleles. *Immunogenetics* 1994;40:165.
- 69 Takeuchi F, Nabeta H, Hong GH, Kuwata S, Tanimoto K, Ito K. Polymorphisms of DMA and DMB genes in Japanese systemic lupus erythematosus. *Br J Rheumatol* 1998;37:95-7.
- 70 Atsumi T, Tsutsumi A, Amengual O, Khamashta MA, Hughes GR, Miyoshi Y, et al. Correlation between beta2-glycoprotein I valine/leucine247 polymorphism and anti-beta2-glycoprotein I antibodies in patients with primary antiphospholipid syndrome. *Rheumatology (Oxford)* 1999;38:721-3.
- 71 Prieto GA, Cabral AR, Zapata-Zuniga M, Simon AJ, Villa AR, Alarcon-Segovia D, et al. Valine/valine genotype at position 247 of the beta2-glycoprotein I gene in Mexican patients with primary antiphospholipid syndrome: association with anti-beta2-glycoprotein I antibodies. *Arthritis Rheum* 2003;48:471-4.
- 72 Yasuda S, Atsumi T, Matsuura E, Kaihara K, Yamamoto D, Ichikawa K, et al. Significance of valine/leucine247 polymorphism of beta2-glycoprotein I in antiphospholipid syndrome: increased reactivity of anti-beta2-glycoprotein I autoantibodies to the valine247 beta2-glycoprotein I variant. *Arthritis Rheum* 2005;52:212-18.
- 73 Gharavi AE, Pierangeli SS, Wilson WA. Anticardiolipin antibodies: importance, controversies, discrepancies; the need for guidelines and calibrators. *Clin Exp Rheumatol* 2001;19:237-9.
- 74 Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum* 1999;42:1309-11.
- 75 Taher A, Khalil I, Abou-Merhi R, Shamseddine A, Bazarbachi A. High prevalence of prothrombin G20210A mutation among patients with deep venous thrombosis in Lebanon. *Thromb Haemostasis* 2003;89:945-6.
- 76 Taher A, Khalil I, Shamseddine A, El-Ahdab F, Bazarbachi A. High prevalence of factor V Leiden mutation among healthy individuals and patients with deep venous thrombosis in Lebanon: is the eastern Mediterranean region the area of origin of this mutation? *Thromb Haemostasis* 2001;86:723-4.
- 77 Sturfelt G, Nived O, Norberg R, Thorstenson R, Krook K. Anticardiolipin antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum* 1987;30:382-8.
- 78 Sebastiani GD, Passiu G, Galeazzi M, Porzio F, Carcassi U. Prevalence and clinical associations of anticardiolipin antibodies in systemic lupus erythematosus: a prospective study. *Clin Rheumatol* 1991;10:289-93.
- 79 Sebastiani GD, Galeazzi M, Tincani A, Piette JC, Font J, Allegri F, et al. Anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. Prevalence and clinical associations. European Concerted Action on the Immunogenetics of SLE. *Scand J Rheumatol* 1999;28:344-51.
- 80 Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum* 2002;46:1019-27.
- 81 Alarcon-Segovia D, Deleze M, Oria CV, Sanchez-Guerrero J, Gomez-Pacheco L, Cabiedes J, et al. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. *Medicine (Baltimore)* 1989;68:353-65.
- 82 Chahade WH, Staub HL, Khamashta M, Hughes GR. Antiphospholipid antibodies in a Brazilian population with systemic lupus erythematosus. *Clin Exp Rheumatol* 1989;7:446-7.
- 83 Alarcon GS, Friedman AW, Straaton KV, Moulds JM, Lisse J, Bastian HM, et al. Systemic lupus erythematosus in three ethnic groups: III. A comparison of characteristics early in the natural history of the LUMINA cohort. Lupus in Minority populations: NATURE vs. nurture. *Lupus* 1999;8:179-209.
- 84 Cucurull E, Gharavi AE, Diri E, Mendez E, Kapoor D, Espinoza LR. IgA anticardiolipin and anti-beta2-glycoprotein I are the most prevalent isotypes in African American patients with systemic lupus erythematosus. *Am J Med Sci* 1999;318:55-60.
- 85 Aguirre V, Cuchacovich R, Barria L, Aris H, Trejo C, Massardo L, et al. Prevalence and isotype distribution of antiphospholipid antibodies in Chilean patients with systemic lupus erythematosus (SLE). *Lupus* 2001;10:75-80.
- 86 Wong KL, Liu HW, Ho K, Chan K, Wong R. Anticardiolipin antibodies and lupus anticoagulant in Chinese patients with systemic lupus erythematosus. *J Rheumatol* 1991;18:1187-92.
- 87 Ninomiya C, Taniguchi O, Kato T, Hirano T, Hashimoto H, Hirose S. Distribution and clinical significance of lupus anticoagulant and anticardiolipin antibody in 349 patients with systemic lupus erythematosus. *Intern Med* 1992;31:194-9.
- 88 Saxena R, Saraya AK, Dhot PS, Singh Y, Malaviya AN. Lupus anticoagulant and anticardiolipin antibodies in systemic lupus erythematosus. *Natl Med J India* 1994;7:163-5.
- 89 Tsutsumi A, Matsuura E, Ichikawa K, Fujisaku A, Mukai M, Kobayashi S, et al. Antibodies to beta 2-glycoprotein I and clinical manifestations in patients with systemic lupus erythematosus. *Arthritis Rheum* 1996;39:1466-74.
- 90 Shrivastava A, Dwivedi S, Aggarwal A, Misra R. Anti-cardiolipin and anti-beta2 glycoprotein I antibodies in Indian patients with systemic lupus erythematosus: association with the presence of seizures. *Lupus* 2001;10:45-50.
- 91 Malaviya AN, Marouf R, Al-Jarallah K, Al-Awadi A, Al-Saied K, Al-Gaurer S, et al. Hughes syndrome: a common problem in Kuwait hospitals. *Br J Rheumatol* 1996;35:1132-6.
- 92 Al Maini MH, El-Ageel N, Al-Shukaily AK, Al-Wahaibi S, Richens ER. Tribal and geographical variations of lupus in the Sultanate of Oman: a hospital-based study. *Rheumatol Int* 2002;21:141-5.
- 93 Houman MH, Smiri-Khanfir M, Ben Ghorbell I, Miled M. Systemic lupus erythematosus in Tunisia: demographic and clinical analysis of 100 patients. *Lupus* 2004;13:204-11.
- 94 Yasuda S, Tsutsumi A, Atsumi T, Bertolaccini ML, Ichikawa K, Khamashta MA, et al. Gene polymorphisms of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with antiphospholipid antibodies. *J Rheumatol* 2002;29:1192-7.