REVIEW

Ethnic and geographical variation in antiphospholipid (Hughes) syndrome

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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_2GPI) 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_2GPI, β_2-glycoprotein I; LA, lupus anticoagulant; PT, prothrombin; SLE, systemic lupus erythematosus

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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_2GPI 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_2GPI 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, TRAELDT) 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
European							
Sturfelt *et al* (1987)[77]	Sweden (59)	54	47	13	NA	NA	No
Sebastiani *et al* (1991)[78]	Italy (64)	44	44	9	NA	NA	Yes
Gourley *et al* (1996)[15]	Ireland (95)	44	31	28	NA	NA	Yes
Sebastiani *et al* (1999)[79]	European†(574)	NA	23	14	14	NA	Yes
Cervera *et al* (2002)[80]	European (1000)	87.9	43.6	12.2	NA	53.6	Yes
American (North, South)							
Wilson *et al* (1988)[7]	Black American (44)	27	100	NA	NA	NA	Yes
Alarcon-Segovia *et al* (1989)[81]	Mexico (500)	53	39	33	16	NA	Yes
Chahade *et al* (1989)[82]	Brazilian (54)	20	20	5.5	NA	17	NA
Molina *et al* (1997)[18]	Afro-Caribbean‡ (136)	21	2	2	21	NA	No
Alarcon *et al* (1999)[83]	Hispanic§(70)	7*	NA	NA	NA	NA	NA
Alarcon, *et al* (1999)[83]	African-American§ (88)	11*	NA	NA	NA	NA	NA
Alarcon *et al* (1999)[83]	Caucasian§ (71)	5*	NA	NA	NA	NA	NA
Diri *et al* (1999)[17]	African American (8)	100	NA	75	87.5	NA	Yes
Cucurull *et al* (1999)[84]	African American‡ (100)	33	18	7	24	NA	Yes
Cucurull *et al* (1999)[16]	Colombian‡ (160)	25	18	13	15	NA	Yes
Cucurull *et al* (1999)[16]	Spaniards‡ (160)	34	27	15	16	NA	Yes
Aguirre *et al* (2001)[85]	Chilean‡(129)	30	16	14	8	NA	Yes
Asian							
Saluja *et al* (1990)[12]	India¶ (76)	27	27	1	NA	NA	Yes
Jones *et al* (1991)[8]	Malaysia**(200)	16.5	13	2.5	NA	NA	No
Wong *et al* (1991)[86]	China (91)	46	44	1	4	11	No
Wong *et al* (1991)[86]	China (91)	46	44	1	4	11	No
Ninomiya *et al* (1992)[87]	Japan (349)	35	28	NA	NA	27	Yes
Saxena *et al* (1994)[88]	India¶ (70)	19	NA	NA	NA	16	yes
Tsutsumi *et al* (1996)[89]	Japan (308)	NA	12	4	NA	8	Yes
Shrivastava *et al* (2001)[90]	India (76)	51	51	7	5	NA	No
Middle-east Africa							
Malaviya *et al* (1996)[91]	Kuwaiti, Middle-Eastern and North-African Arabs (29)	75	NA	NA	NA	NA	Yes
Al Maini *et al* (2002)[92]	Gulf Arabs and Arabs of Persian descent (83)	NA	17.3	14.2	NA	NA	Yes
Houman *et al* (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
European			
Savi *et al* (1988)[48]	80	Northern Italian	A highly significant ($p=6.17\times10^{-7}$ association was found between anticardiolipin antibodies and DR7
Trabace *et al* (1991)[57]	49	Italian	HLA-DR7 frequency was 40% in aCL positive patients *v* 8.3% in aCL negative patients ($p=0.011$)
Hartung *et al* (1992)[33]	314	Central European	Both HLA-DR4 and DR7 were increased in aCL positive patients, and aCL were significantly associated with DRw53
Colucci *et al* (1992)[44]	82	Italian	HLA-B8, DR3 positive young women have significantly higher levels of aPL than HLA-B8, DR3 negative women
Camps *et al* (1995)[40]	19	South of Spain	HLA-DQ7 antigen showed the highest relative risk for primary APS, followed by DRw53
Panzer *et al* (1997)[56]	27	Austria	An increased frequency of HLA-DQB1*06
Christiansen *et al* (1998)[58]	123	Danish and Czech women	HLA-DR3 phenotypes seem to predispose to the formation of aCL antibodies and antinuclear antibodies
Bertolaccini *et al* (2000)[39]	82	British caucasoid	IgG antiphosphatidylserine/prothrombin antibodies (aPS/PT) were present in 41/82 (50%) patients 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
Caliz *et al* (2001)[37]	83	British caucasoid	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
Domenico Sebastiani *et al* (2003)[41]	–	Italian	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
American (North, South)			
Vargas-Alarcon *et al* (1995)[49]	–	Mexican	HLA-DR5 (possibly DRB1*1201) with the primary APS in Mexican patients
Goldstein *et al* (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 *et al* (1997)[52]	80	Mexican	Patients with SLE with aCL had statistically significantly increased corrected frequencies of HLA-DR3; DR7 and DQ2 antigens
Ioannidis *et al* (1999)[29]	67 Greek, 74 others	Greek, white, African-American, Mexican-American	The major alleles associated with anti-β_2GPI response are HLA-DQA1*03 (in particular, *0301) and the HLA-DRB1*1302-
Arnett *et al* (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_2GPI 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
Freitas *et al* (2004)[42]	123 patients and 166 controls	Brazilian	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
Asian			
Hashimoto *et al* (1998)[53]	145	Japanese	Patients with SLE with β_2GPI 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_2GPI 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_2GPI, 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_2GPI or associated molecules.[37] Consequently, people bearing this haplotype may be prone to generate anti-β_2GPI, 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 *et al* (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 *et al* (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_2GPI antibodies
Atsumi *et al* (1999)[70]	88	Caucasoid	β_2GPI polymorphism, Val/Leu 247, is correlated with anti-β_2GPI antibody production in patients with primary APS, and Val 247 may be important in the formation of β_2GPI antigenicity
Ruiz-Arguelles *et al* (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 *et al* (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 *et al* (2003)[71]	39 primary APS, 106 healthy controls	Mexican	Anti-β_2GPI positive patients had significantly higher frequencies of the Val/Val genotype and Val allele expression than control subjects and the β_2GPI negative patients The Val/Val genotype at position 247 of the β_2GPI gene may have a role in the generation of anti-β_2GPI antibodies and perhaps in the expression of arterial thrombosis in primaryAPS
Camilleri *et al* (2003)[30]	230	White	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_2GPI 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_2GPI 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_2GPI 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_2GPI.[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_2GPI production in patients with primary APS, and Val 247 might be important in the formation of β_2GPI antigenicity.[70] Prieto *et al* suggested that the Val/Val genotype at codon 247 played a part in the generation of anti-β_2GPI 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_2GPI allele, compared with the Leu 247 β_2-GPI allele, was associated with both a high frequency of anti-β_2GPI antibodies and stronger reactivity with anti-β_2GPI antibodies. This suggests that the Val(247) β_2GPI 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_2GPI 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 studies[4]. 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 APS[74] 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 APS[74] should facilitate future studies. Genome-wide linkage analysis and multicentre 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.

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