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Ethnic and Geographic Variation in Antiphospholipid (Hughes) Syndrome.

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Abstract:

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 done in many countries and ethno-geographic groups. aPL appear to occur in all populations studied, with some variations noted in frequency of occurrence as well as in the clinical complications. Environmental as well as 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. In this review we will examine the genetic features of APS in various epidemiologic studies.

Introduction:

Antiphospholipid antibodies (aPL) are recognized as a group of antibodies whose specificity is directed not only towards phospholipids such as cardiolipin but also towards phospholipid binding proteins such as (($ß_2$-glycoprotein I ($ß_2$-GPI) and prothrombin (PT))or the complexes of phospholipids and phospholipids 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 is now recognized 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 done in many countries and ethno-geographic groups ***(5-17)***. aPL appear to occur in all populations studied, with some variations noted in frequency of occurrence as well as in the clinical complications ***(5, 13, 14, 16, 18).*** Environmental as well as 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 will examine the features of APS in various epidemiologic 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 pro-coagulant nature of aPL and the demonstrations that anticoagulation provides effective secondary prophylaxis of thrombosis or pregnancy loss in patients with aPL.

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

A relative paucity of IgG (2%) and IgM (2%) aCL in Afro-Caribbean SLE patients was noted and warrants further study ***(18)***. SLE in African-Americans and Afro-Caribbean SLE patients is characterized by a generally worse outcome and a higher prevalence of autoantibodies than in other ethnic or geographic groups, and it would be of interest 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 pre-natal 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.

IgA aCL is rarely present alone, except in Afro-Caribbean SLE patients. In African-American SLE patients, IgA aCL is also frequent, but often coexists with other

isotypes. The value of IgA aCL antibodies and their relationship with thrombotic events is still a controversial issue ***(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* ***(24)*** 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. They found IgA aCL in 52% of their population ***(24)***. Only one patient had IgA aCL as the sole aPL; thus this test was concluded to be useful to identify occasional patients with APS ***(24)***. Molina, *et al* ***(18)*** studied 152 African-American, 136 Afro-Caribbean (Jamaican), and 163 Hispanic (Colombian) unselected patients with SLE. The major finding of this 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 titers and did not seem to be associated with clinical features of APS. However, in 1999 Diri, *et al* ***(17)*** reported 8 Afro-American female patients with the APS, in which IgA aCL were present in 7, co-occurring with IgG or IgM isotype in 4 of them. In the same study they also found IgA anti-ß$_2$-GPI in 4/8 patients, co-occurring with IgM isotype in 3 of them. In a cross sectional study to determine the prevalence of IgA aCL and anti-ß$_2$-GPI and study their clinical significance in a cohort of 134 SLE patients, 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 etiology of the APS is linked to genetic predisposition, which may be accounted for, at least in part, by genes of 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)*** summarized 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 have been demonstrated in APS. In addition, the various aPL show similar HLA association, again independent of the clinical context (PAPS 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 in the *DQB1* outer domain. We found a number of possible APS-associated HLA alleles and haplotypes. The major association observed 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 analyzed a more clinically homogeneous group; its frequency was increased more in PAPS than in secondary APS (SAPS), the association being even stronger in anti-ß2GPI-positive PAPS ***(37)***. Accordingly, it is suggested that this (*DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302*) haplotype predisposes to anti-ß2GPI, 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 ß2GPI or associated molecules ***(37)***. Consequently, individuals bearing this haplotype may be prone to generate anti-ß2GPI, after taking other genetic and environmental variables into account ***(37)***.

In addition to these classical class II molecules, it has recently been revealed that HLA-DM molecules play 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. The study on SLE, in a Japanese population, did not find any significant association of the HLA-DM polymorphisms with 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 the 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 implication of other genetic risk factors for aPL production separate from DMA*0102 or DMA*0102 containing haplotype. Furthermore, the presence of a considerable number of individuals 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).***

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-ß2GPI 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.

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)***. Eighty-eight Ser/Asn, 247 Val/Leu, 306 Cys/Gly, and 316 Trp/Ser polymorphisms are located in

exon 3, 7, 7, and 8 of β_2-GPI gene, respectively. Val/Leu polymorphism at codon 247 has been extensively studied among these polymorphisms ***(19)***. Hirose *et al*. ***(31)*** reported that 247 Val alleles were found more frequently in Asian APS patients than among ethnicity matched controls and 247 Val was significantly associated with the presence of anti-β_2-GPI. They found no significant differences in allele frequencies in comparisons of the white or the black APS patients with appropriate controls although 247 Val alleles were more frequent in these ethnic groups than in Asians. Atsumi *et al* ***(70)*** analyzed 247 Val/Leu polymorphism in a cohort of 88 British APS patients and found Val 247 was correlated with anti-β_2-GPI production in patients with primary APS, and 247 Val might be important in the formation of β_2-GPI antigenicity. Prieto *et al*. ***(71)*** suggested that Val/Val genotype at codon 247 played a role in the generation of anti-β_2-GPI and in the expression of arterial thrombosis in Mexican primary APS. More recently Yasuda *et al.* ***(72)*** in a study on 65 Japanese patients with APS and /or SLE compared to 61 controls found that the Val(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 compared with the Leu(247) β_2-GPI allele, suggesting that the Val(247) β_2-GPI allele may be one of the genetic risk factors for development of APS. On the other hand, Camilleri *et al*. ***(30)*** found no association 247 Val/Leu polymorphism and the presence of anti-β_2-GPI in the white population.

The Limitations of Genetic Studies in APS:

The interpretation of epidemiologic studies in various ethnic groups is quite difficult for the following reasons:

1. Although the enzyme linked immuno sorbent assay (ELISA) for aCL antibodies and LA testing has been extensively standardized, there remains significant inter-laboratory variation in the results of testing. The precise levels of cutoff for positive/negative vary among laboratories ***(73)***.
2. Clinical heterogeneity: the clinical definition of APS has varied among studies ***(4)***. Some APS patients also manifest SLE, and constitute a heterogeneous population, making it difficult to analyze 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 patients' groups.
3. 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. It is possible that variation in such collateral risk factors, e.g. medication use or genetic risk factors for thrombosis could influence complication rates associated with aPL in various populations. For instance, in Lebanon, a high prevalence of prothrombin G20210A and factor V Leiden mutations ***(75, 76).*** These factors will increase the thrombotic risk especially in patients with aPL.
4. Disease activity: The level of disease activity would be 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 was present in 27% of SLE patients during periods of disease activity, compared with only 5% of SLE patients during periods of less active SLE ***(7)***.

5. Geographic migration: with the current status of increasing geographic migration and intermingling across geographic 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 geographic group, and comparisons between these studies are confounded by methodologic variations or patient selection. The publication in 1999 of international consensus criteria for APS ***(74)*** should facilitate future studies. Genome-wide linkage analysis and multicenter international collaboration would be useful in better understanding of genetic predisposition to produce aPL and to develop the clinical features of APS.

Table 1. Prevalence and isotype distribution of aCL and LA in different populations of PAPS & SLE patients.

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 GD, et al, (1991) ***(78)***	Italy (64)	44	44	9	NA	NA	Yes
Gourley IS, et al, (1996) ***(15).***	Ireland (95)	44	31	28	NA	NA	Yes
Sebastiani GD, et al, (1999) ***(79)***	European[a] (574)	NA	23	14	14	NA	Yes
Cervera R, et al, (2002) ***(80)*** .	European (1000)	87.9	43.6	12.2	NA	53.6	Yes
American (North, South)							
Wilson WA,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 JF, et al, (1997) ***(18)***	Afro-Caribbean[b] (136)	21	2	2	21	NA	No
Alarcon et	Hispanic[c]	7*	NA	NA	NA	NA	NA

al ***(83)***	(70)						
Alarcon et al ***(83)***	African-American[c] (88)	11*	NA	NA	NA	NA	NA
Alarcon et al ***(83)***	Caucasian[c] (71)	5*	NA	NA	NA	NA	NA
Diri E, et al, (1999) ***(17)***	African American (8)	100	NA	75	100	NA	Yes
Cucurull E, et al, (1999) ***(84)***	African American[b] (100)	33	18	7	24	NA	Yes
Cucurull E, et al, (1999) ***(16).***	Colombian[b] (160)	25	18	13	15	NA	Yes
Cucurull E, et al, (1999) ***(16).***	Spaniards[b] (160)	34	27	15	16	NA	Yes
Aguirre V, et al (2001) ***(85).***	Chilean[b] (129)	30	16	14	8	NA	Yes
Asian							
Saluja S, et al, (1990) ***(12)***	India[d](76)	27	27	1	NA	NA	Yes
Jones HW, et al, (1991) ***(8) .***	Malaysia[e] (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[d] (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 AN, et al, (1996) ***(91).***	Kuwaiti, Middle-Eastern and North-African Arabs (29)	75	NA	NA	NA	NA	Yes
Al Maini MH, et al, (2002) ***(92).***	Gulf Arabs & Arabs of Persian descent (83).	NA	17.3	14.2	NA	NA	Yes
Houman MH, et al, (2004) ***(93).***	Tunisian (North Africans) (100)	66	NA	NA	NA	NA	Yes

[a]Patients from 7 European countries: 97.7% Caucasians, 3.3% other races.
[b]In-house ELISA test done at Louisiana State University Health Sciences Center in New Orleans.
[c]LUMINA Study Group: LUpus in MInority populations: NAture vs Nurture. From University of Alabama at Birmingham, University of Texas-Houston Health Science Center, and University of Texas Medical Branch at Galveston. * IgG and IgM aCL and/or LA.
[d]Both studies from All India Institute of Medical Sciences, New Delhi.
[e]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 APS patients' populations.

Authors (year)	***# of Patients***	***Ethnicity***	***Antibodies***
European			
Savi M,et al, (1988) ***(48).***	80	Northern Italian	A highly significant (P = 6.17 x 10(-7) association was observed between anticardiolipin antibodies and DR7.
Trabace S, et al, (1991) ***(57).***	49	Italian	HLA-DR7 frequency was 40% in ACL positive patients vs. 8.3% in ACL negative patients (P = 0.011).
Hartung K, 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 AT, et al, (1992) ***(44).***	82	Italian	HLA-B8,DR3-positive young females display significantly higher levels of APA than HLA-B8, DR3-negative ones.
Camps MT, et al, (1995) ***(40).***	19	South of Spain.	HLA-DQ7 antigen showed the highest relative risk for PAPS, followed by DRw53.
Panzer S, et al, (1997), ***(56).***	27	Austria	An increased frequency of HLA-DQB1*06.
Christiansen OB, et al, (1998), ***(58)***.	123	Danish & Czech women	The HLA-DR3 phenotypes seem to predispose to the formation of ACL antibodies and ANA.
Bertolaccini ML, et al (2000) ***(39)***.	82	British caucasoid	IgG antiphosphatidylserine/prothrombin antibodies aPS/PT were present in 41 of 82 patients (50%). 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 R, et al, 2001 ***(37)***	83	Caucasoid British	DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302 and DQB1*0303-DQA1*0201-DRB1*0701 haplotypes showed significantly positive correlations with APS.
Domenico Sebastiani G, et al,	-	Italian	The association of HLA-DR4, -DR7, -DRw53 and -DQB1*0302

2003, ***(41)***.			with aCL that has been demonstrated in primary APS can also be found in SLE.
American (North, South)			
Vargas-Alarcon G, et al, (1995) ***(49).***	-	Mexican	HLA-DR5 (possibly DRB1*1201) with the PAPS in Mexican patients
Goldstein R, et al, 1996 ***(11) .***	91 SLE & 16 PAPS	Caucasian	The strongest association is with the HLA-DR53 haplotypes, some of which include the DQ7 allele.
Granados J, et al, (1997) ***(52)***	80	Mexican	SLE patients with aCL had statistically significant increased corrected frequencies of HLA-DR3; DR7 and DQ2 antigens.
Ioannidis JP, et al (1999) ***(29).***	67 Greek 74 others	Greek, white, African-American, Mexican-American	The major alleles associated with anti-beta2GPI response are HLA-DQA1*03 (in particular *0301) and the HLA-DRB1*1302-
Arnett FC, 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-beta2GPI 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 3 ethnic groups. HLA-DR53 (DRB4*0101) was increased in Mexican Americans only.
Freitas MV, et al, (2004) ***(42).***	123 patients & 166 controls	Brazilian	Compared to controls, PAPS patients exhibited a nonsignificantly increased frequency of DR53-associated alleles, and SAPS patients

			presented an increased frequency of HLA-DRB1*03 alleles. A trend to an increase in the frequency of the DQB1*0604 allele and of the DQB1*0302 allele was observed in SAPS.
Asian			
Hashimoto H, et al, (1998)(53)	145	Japanese	SLE patients with β2 GPI-dependent aCL were significantly associated with DRB1*0901.

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

Delrieu O, et al, (1999), ***(45)***	171 SLE, 88 primary APS, 193 controls.	French Caucasians	Poly(ADP-ribose) polymerase (PARP) alleles do not influence susceptibility to SLE or primary APS in French Caucasians
Hirose N, et al, (1999) ***(31)***	149	Caucasians, African Americans, Asians.	In Asian patients with APS, expression of a V at position 247, especially in the homozygous state, is significantly associated with the presence of anti-beta2GPI antibodies.
Atsumi T, et, (1999) ***(70)***	88	Caucasoid	β2GPI polymorphism, valine/leucine247, is correlated with anti-β2GPI antibody production in patients with PAPS, and valine247 may be important in the formation of β2GPI antigenicity.
Ruiz-Arguelles GJ, 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 prothrombin gene in Mexican mestizo patients with APS does not seem to be related to the thrombophilia observed in these patients.
Yasuda S, et al, (2002) ***(94).***	77 Japanese and 82 British patients with aPL	Japanese and British	Polymorphisms of the tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) genes probably do not significantly influence the risk of arterial thrombosis, venous thrombosis, or pregnancy morbidity in patients with aPL
Prieto GA, *et al*, (2003) ***(71)***	39 PAPS, 106 healthy controls.	Mexican	Anti-β2GPI-positive patients had significantly higher frequencies of the VV genotype and V allele expression than the control subjects and the β2GPI-negative patients. The VV genotype at position 247

			of the β2GPI gene may play a role in the generation of anti- β2GPI antibodies and perhaps in the expression of arterial thrombosis in PAPS
Camilleri RS, et al (2003) ***(30).***	230	Caucasian	There was a significantly decreased prevalence of the Ser316 allele in aPL-negative women (n = 98) when compared with female normal control subjects.

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