

Can inherited thrombophilia modulate the clinical phenotype of patients with antiphospholipid syndrome?

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Abstract

Objective

The current case-control study was aimed to determine the prevalence and the clinical significance of inherited thrombophilia – factor V Leiden and G20210A prothrombin polymorphisms – in patients with antiphospholipid syndrome (APS).

Methods

100 patients with APS (77 with primary APS and 23 with systemic lupus erythematosus [SLE]-APS), and 100 patients with first lower extremity deep venous thrombosis (DVT), and 200 healthy individuals as a control groups were analysed. Patients and control group were tested for factor V Leiden and prothrombin G20210A gene polymorphism.

Results

Factor V Leiden variant was found in 1% of APS patients, in 3% of healthy individuals ($p=0.49$), and 16% of patients with first DVT ($p<0.0005$). Prothrombin gene polymorphism was found in 6% of APS patients and in 2.5% of healthy subjects ($p=0.21$), and 13% of patients with DVT ($p=0.14$). In primary APS patients, factor V Leiden was present in 1.3% (1/77) and prothrombin gene polymorphism in 6.5% (5/77). No patient with SLE-APS had factor V Leiden and prothrombin gene variant was present in only one patient (4.3%). Patients with prothrombin polymorphism had higher prevalence of venous thrombosis, with no statistical significance (80% vs. 47.9%, $p=0.35$). There were no differences in the prevalence of recurrent thrombosis before or after APS diagnosis in patients with or without prothrombin gene polymorphism.

Conclusion

Factor V Leiden and G20210A prothrombin variant seem to play no role in either the development of APS or in the type of involved vessel, with no increased risk of re-thrombosis during follow-up.

Key words

antiphospholipid syndrome, inherited thrombophilia, factor V Leiden, prothrombin polymorphism, recurrent thrombosis

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Introduction

The antiphospholipid syndrome (APS) is characterised by the presence of venous and/or arterial thrombosis and/or pregnancy morbidity in combination with the positive persistence of medium to high titers of antiphospholipid antibodies (aPL): lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and/or anti- β 2glycoprotein I antibodies (anti- β 2GPI) (1).

The prevalence of aPL is between 2–12% for healthy population (2, 3) and 30–50% for patients with systemic lupus erythematosus (SLE) (4, 5). Regarding the relationship between thrombosis and aPL, in a recent study, 141 (24%) of 597 patients with a first episode of venous thrombosis had aPL (6). Moreover, 20% of patients less than 50 years of age with stroke had aPL (7).

Patients with aPL and thrombosis frequently have one or several additional cardiovascular risk factors such as hypertension, smoking, hypercholesterolemia or oestrogen use (8, 9). In this sense, hypertension and LA were significantly predictive of thrombosis in a series of 258 aPL carriers (10). In fact, the last classification criteria recommended that APS patients should be stratified in APS trials according to the presence or absence of other additional factors contributing thrombosis (1).

Some important questions in the field of APS remain unanswered. The first is what is the reason by which some patients having aPL never present with thrombosis; the second is why some APS patients develop thrombosis in the venous vasculature whereas others suffered from arterial thrombosis; finally, the factors related with the recurrence of thrombosis despite anticoagulation remain unknown. Inherited thrombophilia might explain, at least in part, these questions. In fact, it may be one of the thrombotic triggers but their influence in patients with APS is not clear. Single nucleotide polymorphism (SNP) G1691A in factor V gene, also called factor V Leiden occurs with a frequency of 12–30% and G20210A prothrombin gene with a frequency of 7–18% in cohorts of patients with thrombosis, and are the

most frequent genetic abnormalities in patients with a first venous thrombosis (11). In addition, heterozygous carriage of factor V Leiden or prothrombin polymorphism is associated with an increased risk of recurrent thromboembolism (12).

In the last decades, several studies investigating the role of hereditary thrombophilic risk factors in patients with APS have been made but they gave conflicting results (13). Regarding factor V Leiden variant, some authors have identified factor V Leiden as a possible contributor to the development of venous thrombosis associated with aPL (14–16). In this sense, LA and factor V Leiden were identified as independent risk factors for venous thrombosis in a series of 152 patients with LA (17) and factor V Leiden in combination with aPL increased the venous thrombosis risk of 30-fold as an independent risk factor in a cohort of 144 patients with SLE (18). Other authors, however, did not find an association between factor V Leiden and increased thrombotic risk in APS (19–25). In 157 aPL-positive patients (69 with previous thrombosis), LA, male sex, and hypertension were the strongest risk factors for developing thrombosis and no additional risk was conferred by factor V Leiden (26).

Considering prothrombin G20210A SNP, some studies have demonstrated an association between this polymorphism and the development of thrombosis in patients with aPL (20, 25), and, specifically, its role as an independent risk factor for venous thromboembolism when presented together with aPL in a cohort of 144 patients with SLE (18). Other studies, however, have shown no relationship between prothrombin G20210A polymorphism and thrombosis in APS (17, 21, 26–29).

The aim of the current case-control study was to determine the prevalence of inherited thrombophilia – factor V Leiden and G20210A prothrombin polymorphisms – in patients with APS. In addition, we compared the role of inherited thrombophilia in patients with primary and SLE associated APS (SLE-APS) and their role in the recurrence of thrombosis.

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Competing interests: none declared.

Materials and methods

Patients

Patients with APS and history of thrombosis who attended in the Department of Autoimmune Diseases of the Hospital Clinic, Barcelona, Spain, from 1985 to March 2011 were screened. They were included in the study if they fulfilled the latest classification criteria for APS (1) with at least one thrombotic event (venous or arterial). Associated SLE was diagnosed by revised criteria of the American College of Rheumatology (30). Women with obstetric APS with no history of thrombosis were excluded.

Data were summarised using a standardised data form, including age, gender, primary APS or SLE associated, type of thrombotic events, recurrent thrombosis before and after APS diagnosis and immunologic features. In addition, cardiovascular risk factors were assessed as follows: hypertension was defined as systolic blood pressure greater than 140 mmHg and/or diastolic blood pressure greater than 90 mmHg on two or more prospective visits or prior antihypertensive medication. Smoking was defined as current consumption of more than one cigarette per day. Diabetes was defined as a physician diagnosis of diabetes requiring insulin or hypoglycaemic agents. Dyslipidaemia was defined in the presence of hypercholesterolaemia (total serum cholesterol level greater than 6.39 mmol/l on two or more prospective visits or current use of lipid lowering agents) and/or hypertriglyceridaemia (serum triglyceride level greater than 1.69 mmol/l on two or more prospective visits).

Two age-, sex- and ethnicity-matched control groups were included, the former composed by 200 healthy individuals without autoimmune disease, bleeding disorders, thrombosis or history of pregnancy loss and the second by a high-risk group for thrombosis represented by 100 patients with lower-extremity deep venous thrombosis (DVT).

All the thrombotic events were assessed clinically and confirmed with objective methods. Diagnosis of deep venous thrombosis was confirmed by Doppler ultrasonographic scans or by

venography. Pulmonary embolism was diagnosed by ventilation / perfusion scanning or by pulmonary angiography. Cerebrovascular ischaemic episodes were confirmed by computed tomography scanning or by magnetic resonance imaging techniques. Myocardial infarctions were confirmed by electrocardiographic studies and by elevated levels of cardiac enzymes.

The study was approved by the Human Experimental Committee of the Hospital Clinic and was performed according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Laboratory

Factor V Leiden polymorphism was tested using a nucleic acid sequence-based amplification assay (Organon Teknika, Boxtel, The Netherlands). For the prothrombin gene G20210A polymorphism, DNA samples were analysed by polymerase chain reaction (PCR), as reported by Poort *et al.* (31).

LA was detected using activated partial thromboplastin time, dilute Russell's viper venom time, and the tissue thromboplastin inhibition test. Tests were also performed in mixtures with control plasmas or phospholipids following the guidelines of the Subcommittee for the Standardisation of Lupus Anticoagulants of the International Society of Thrombosis and Haemostasis (32). The aCL and anti- β 2GPI were measured using standardised ELISA (Cheshire Diagnostics, Cheshire, UK) and results were expressed in GPL and MPL units. Titers higher than 40 GPL or MPL were considered positive for aCL and higher than the 99th percentile for anti- β 2GPI. Of note, anti- β 2GPI were only determined in patients carrier of hereditary thrombophilia.

Statistical analysis

Results are shown as mean \pm standard deviation (SD). Comparisons were performed by χ^2 test or analysis of variance (ANOVA). Data were analysed with the SPSS version 18.0.

Independently of aPL, the risk of thrombosis for SLE patients is significantly higher than for the general population. In fact, due to the increased inci-

dence of traditional cardiovascular and nontraditional lupus-related thrombosis risk factors, SLE patients are at significantly increased risk of premature atherosclerosis and/or thrombosis (8). Given these data, we compared the role of inherited thrombophilia in patients with primary and SLE associated APS.

Results

We included 100 patients with APS, 68 (68%) were female with a mean age at diagnosis of APS of 42.2 \pm 17.0 years. Seventy-seven patients had primary APS and 23 SLE associated (SLE-APS). Two hundred healthy individuals (62% females) with a mean age of 48.7 \pm 12.6 years and 100 patients (59% females) with a first lower-extremity DVT with a mean age of 51.2 \pm 14.3 years were included as control groups. Fifty (50%) APS patients had venous thrombosis, 43% arterial thrombosis, and 7% both arterial and venous thrombosis. All patients had been treated with long-term anticoagulation since the APS diagnosis at international normalised ratio between 2.0 and 3.0.

Regarding the distribution of aPL, the most common was LA present in 87 (87%) APS patients followed by the isotype IgG of aCL, present in 62 (62%), and the isotype IgM of aCL present in 33 (33%) of them. Considering the aPL profile, 65 (65%) patients had the combination of LA plus aCL, 22 (22%) had isolated LA, and 13 (13%) isolated aCL.

Factor V Leiden polymorphism was found in 1% (1/100) of patients, in 3% (6/200) of healthy individuals (p =NS), and 16% (16/100) of patients with first DVT (p <0.0005). The G20210A prothrombin gene polymorphism was found in 6% (6/100) of APS patients, in 2.5% (5/200) of healthy group (p =0.21), and 13% (13/100) of patients with first lower extremity DVT (p =0.14). In all cases, patients were heterozygous for both mutations. The aPL profile of six patients with G20210A prothrombin gene polymorphism was the following: triple positivity (LA plus aCL IgG plus anti- β 2GPI IgG) in one patient, double positivity (LA plus aCL IgG and LA and aCL IgM) in two, isolated aCL IgG in one, isolated aCL IgM in one,

and isolated LA in the remaining. The patient with factor V Leiden polymorphism had LA plus aCL IgG.

Comparison between patients with primary APS and SLE-APS

There were no differences between the two groups in terms of distribution by gender. In addition, the presence of venous and arterial thrombotic manifestations and thrombotic relapse after and before the APS diagnosis was similar. Reference to cardiovascular risk factors, as expected, the group of patients with SLE-APS had a higher frequency of hypertension ($p=0.034$), taking into account that some patients had some degree of kidney involvement (Table I). Of note, patients with SLE-APS were younger than those with primary APS (35.8 ± 14.0 years vs. 44.1 ± 17.3 years, $p=0.038$) with a longer follow-up period (7.2 ± 4.8 years vs. 9.6 ± 3.9 , $p=0.035$). Heart valve disease (18.2% vs. 43.5% , $p=0.013$) and haemolytic anaemia was more frequent in SLE-APS patients (17.4% vs. 3.9% , $p=0.04$).

Regarding the aPL profile, LA was more prevalent in patients with SLE-APS (100% vs. 83.1% , $p=0.03$). The prevalence of inherited thrombophilia was similar in the two groups of patients. Factor V Leiden mutation was present in 1.3% ($1/77$) of patients with primary APS whereas none of those with SLE-APS had the mutation. Regarding G20210A prothrombin gene mutation, it was present in 6.5% ($5/77$) of patients with primary APS and in only one patient (4.3%) with SLE-APS (Table I).

Comparison between patients according to the presence of inherited thrombophilia

As we pointed out, factor V Leiden polymorphism was found in only one patient with primary APS and therefore, we could not assess the role of this mutation in the clinical phenotype of patients with APS. This patient was a 33 year-old woman who had no cardiovascular risk factor presenting with lower limb venous thrombosis at diagnosis. She has been treated with long-term anticoagulation without recurrent thrombosis during follow-up. Immunologically, she had the combination of aCL IgG plus LA.

Table I. Demographic, clinical characteristics and laboratory features of 100 patients with primary APS and SLE-APS.

Characteristics	Primary APS n=77 (%)	SLE-APS n=23 (%)	p-value
Age	44.1 ± 17.3	35.8 ± 14.0	0.038
Gender (female)	49 (64)	19 (83)	
Follow-up, age	7.2 ± 4.8	9.6 ± 3.9	0.035
Cardiovascular risk factors	31 (40.3)	12 (52.2)	
Smoking	18 (24.7)	8 (34.8)	
Hypertension	19 (24.7)	11 (47.8)	0.034
Diabetes Mellitus	3 (3.9)	0	
Dyslipidaemia	17 (22.1)	4 (17.4)	
Vein thrombosis at diagnosis	37 (48.1)	13 (56.5)	
Arterial thrombosis at diagnosis	33 (42.9)	10 (43.5)	
Lower limb vein thrombosis	32 (41.6)	12 (52.2)	
Pulmonary embolism	15 (19.5)	1 (4.3)	
Central nervous system manifestations	23 (29.9)	6 (26.1)	
Stroke	18 (23.4)	4 (17.4)	
Transient ischaemic attack	3 (3.9)	2 (8.7)	
Cardiac manifestations	10 (13)	4 (17.4)	
Ischaemic heart disease	3 (3.9)	0	
Acute myocardial infarction	4 (5.2)	2 (8.7)	
Valve disease	14 (18.2)	10 (43.5)	0.013
Renal manifestations	3 (3.9)	0	
Renal vein thrombosis	1 (1.3)	0	
Ocular manifestations	11 (14.3)	0	
Retina central vein thrombosis	3 (3.9)	0	
Retina central artery thrombosis	3 (3.9)	0	
Skin manifestations	6 (7.8)	3 (13)	
Livedo reticularis	5 (6.5)	3 (13)	
Skin ulcers	1 (1.3)	1 (4.3)	
Hematological manifestations	18 (23.4)	10 (43.5)	
Thrombocytopenia	19 (24.7)	11 (47.8)	
Haemolytic anaemia	3 (3.9)	4 (17.4)	0.04
Recurrent thrombosis prior to diagnosis	24 (31.2)	4 (17.4)	
Recurrent thrombosis after diagnosis	26 (33.8)	7 (30.4)	
Laboratory			
aCL	60 (77.9)	18 (78.3)	
aCL IgG	48 (62.3)	14 (60.9)	
aCL IgM	25 (32.4)	8 (34.8)	
LA	64 (83.1)	23 (100)	0.03
Inherited thrombophilia	6 (7.8)	1 (4.3)	
Factor V Leiden	1 (1.3)	0	
G20210A prothrombin gene mutation	5 (6.5)	1 (4.3)	

APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus; aCL: anticardiolipin antibodies; LA: lupus anticoagulant.

nologically, she had the combination of aCL IgG plus LA. Six patients were heterozygous for G20210A prothrombin polymorphism. To study the role of this inherited thrombophilia on the clinical phenotype of APS patients, we considered only the five patients with primary APS who were carriers of this mutation (Table II). We did not find significant dif-

ferences in demographic, clinical and laboratory features regardless the coexistence of inherited thrombophilia. Although the patients with prothrombin variant had higher prevalence of venous thrombosis compared with those without thrombophilic mutation, it did not reach statistical significance (80% vs. 47.9% , $p=0.35$). In the same sense, we did not find differences in the prev-

absence of recurrent thrombosis before or after APS diagnosis in patients with or without prothrombin polymorphism (Table II). In other words, in our series of patients with primary APS, inherited thrombophilia seems not modulate the clinical phenotype either at diagnosis or during the follow-up.

Discussion

Our study demonstrated that the prevalence of inherited thrombophilia was similar in APS patients and healthy controls. Of note, it seems not modulate the clinical presentation of patients with APS. In addition, it did not increase the risk or recurrent thrombosis during follow-up.

In the field of APS, some questions are unknown such as the reason by which some patients having aPL never present with thrombosis or why some patients develop thrombosis in venous vasculature whereas others suffer from arterial thrombosis. A possible answer may be that genetic background could modulate the type of involved vessel (33). Inherited thrombophilia may be the overriding factor by which aPL precipitate venous or arterial thrombosis. From the experimental point of view, Katzav *et al.* (34) demonstrated an increase in aPL levels and a number of behavioral/cognitive dysfunction and neurodegenerative changes associated with aPL in mice with induced experimental APS carrying the factor V Leiden. In fact, these effects were significantly more pronounced in homozygous for factor V Leiden than in heterozygous mice.

From the clinical point of view, factor V Leiden and prothrombin polymorphism combined with LA and/or aCL contributed to the risk of venous thrombosis in a cohort of 144 SLE patients (18). However, we could not demonstrate this attractive hypothesis. In the light of our data, factor V Leiden and G20210A prothrombin polymorphism do not play a role neither in the development of APS nor in the type of involved vessel. In fact, our study is in accordance with previous analysis that found similar prevalence of factor V Leiden when APS patients were compared with healthy people (20, 24,

Table II. Demographic, clinical characteristics and laboratory features of 76 patients with primary APS according to the presence of inherited thrombophilia.

	With G20210A n=5 (%)	Without G20210A n=71 (%)
Age	54.6 ± 24.4	43.5 ± 16.7
Gender (female)	5 (100)	43 (60.6)
Follow-up, age	5.8 ± 7.2	7.4 ± 4.7
Cardiovascular risk factors		
Smoking	1 (20)	30 (42.3)
Hypertension	0	33 (49.3)
Diabetes Mellitus	0	19 (26.8)
Dyslipidaemia	1 (20)	3 (4.2)
16 (22.5)		
Vein thrombosis at diagnosis	4 (80)	34 (47.9)
Arterial thrombosis at diagnosis	1 (20)	35 (49.3)
Lower limb vein thrombosis	4 (80)	27 (38)
Pulmonary embolism	2 (40)	13 (18.3)
Central nervous system manifestations	1 (20)	22 (31)
Stroke	1 (20)	17 (23.9)
Transient ischaemic attack	0	3 (4.2)
Cardiac manifestations	0	10 (14.1)
Ischaemic heart disease	0	3 (4.2)
Acute myocardial infarction	0	4 (5.6)
Renal manifestations	0	3 (4.2)
Renal vein thrombosis	0	1 (1.4)
Ocular manifestations	0	11 (15.5)
Retina central vein thrombosis	0	3 (4.2)
Retina central arterial thrombosis	0	3 (4.2)
Skin manifestations	1 (20)	5 (7)
Livedo reticularis	1 (20)	4 (5.6)
Skin ulcers	0	1 (1.4)
Haematological manifestations	0	18 (25.4)
Thrombocytopenia	0	19 (26.8)
Haemolytic anaemia	0	3 (4.2)
Recurrent thrombosis prior to diagnosis	2 (20)	22 (31)
Recurrent thrombosis after diagnosis	0	26 (36.6)
Laboratory		
aCL IgG	1 (20)	45 (63.4)
aCL IgM	3 (60)	21 (29.6)
LA	3 (60)	59 (83.1)

APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus; aCL: anticardiolipin antibodies; LA: lupus anticoagulant.

25, 35). In fact, in a cohort of 105 Hungarian patients with SLE, the presence of inherited thrombophilia did not increase the relative risk of thrombosis [21]. In a cohort of 69 APS patients with thrombosis, the strongest risk factors for developing thrombosis were LA, male sex and hypertension but no additional risk was conferred by factor V Leiden and prothrombin gene polymorphism [26]. In a recent review of 590 patients with APS with thrombosis, (326 with venous thrombosis and 157 with arterial thrombosis), the prevalence of factor V Leiden polymor-

phism was 6.4% in patients with arterial thrombosis and 6.9% in patients with venous thrombosis (36). Similar results were described for G20210A prothrombin polymorphism, with a prevalence of 2.1% in patients with arterial thrombosis and 2.8% in those with venous thrombosis (36). In other words, routine screening for these additional hereditary risk factors seems to be unwarranted in APS patients. Nevertheless, the results are contradictory. In fact, in a cohort of 39 APS patients the incidence of factor V Leiden variant was significantly elevated

in those with venous thrombosis (15). Venous thrombosis was associated with hereditary thrombophilia assessed as factor V Leiden in a cohort of 76 patients with APS with a 7.3-fold increase (9). Galli *et al.* (17) achieved similar conclusion in a cohort of 152 patients with LA, 96 of them with a history of thrombosis. Five patients were heterozygous for the mutation in the factor V gene (3%) and all of them (100%) suffered from venous thrombosis compared with 68 out of the 147 cases without the mutation (46%) ($p=0.047$). These data suggest that in patients with aPL the factor V Leiden may play a major role in the occurrence of venous thrombosis. Conversely, in the same study, 50% of the patients that were heterozygous for the G20210A SNP in the prothrombin gene experienced venous thrombosis compared with 65 out of the 137 (47%) patients without the polymorphism. In our study, the prevalence of venous thrombosis tended to be higher in patients with prothrombin gene polymorphism although it did not reach statistical significance (80% vs. 47.9%, $p=0.35$).

The reason for the controversy in the results among the different studies may have its origin in methodological differences with different methods of ELISA to detect aPL and the different aCL titer reported. It is possible that some patients did not fulfill strictly the laboratory criteria for APS.

To prevent recurrent thrombosis is the main objective of the secondary thromboprophylaxis in patients with APS. The potential role of inherited thrombophilia as a risk factor for recurrent thrombosis in APS patients was assessed in only two studies (36, 37). In the former, the frequency of factor V Leiden A allele was higher in APS patients with recurrent arterial and venous thrombosis. Unfortunately, this group of patients was compared to aPL patients without thrombosis but not with APS patients without recurrent thrombotic event (36). In the second study that evaluated paediatric APS patients, the prevalence of factor V Leiden and prothrombin gene polymorphism was similar in children who presented with a single thrombosis (4/15; 26.6%) and

in those who experienced recurrences (1/7; 14.3%). In other words, hereditary thrombophilia did not predict recurrent thrombosis in paediatric patients with APS (37). Otherwise, Ames *et al.* (38) found a higher re-thrombosis rate (13-fold more) in patients with primary APS compared with those with hereditary thrombophilia.

The most prominent shortcoming of the present study is its sample size and the low number of patients with thrombotic SNP. This fact prevented to find differences between the two groups of patients. However, the patients included had LA and/or aCL at high titers representing a homogeneous sample of patients with APS. An important point is the fact that in the present study 87% of APS patients had a "high-risk" aPL profile, represented by the combination of LA plus aCL and isolated LA (39). It is possible that in this group of patients with double or triple aPL positivity (40) the impact of inherited thrombophilia is minimised, since aPL can be *per se* the major determinant of thrombosis. Only one of the seven APS patients with hereditary thrombophilia had anti- β 2GPI. This patient was a 40 year-old woman who had no cardiovascular risk factor presenting with stroke at diagnosis, triple aPL positivity (LA plus aCL IgG and anti- β 2GPI IgG) and G20210A prothrombin polymorphism. In conclusion, in our series the prevalence of inherited thrombophilia in both primary and secondary APS patients was similar to the healthy population. In this study, factor V Leiden and G20210A prothrombin polymorphism seem to play no statistically significant role neither in the development of APS nor in the type of involved vessel, with no increased risk of re-thrombosis during follow-up. However, these results should be confirmed in studies including larger number of APS patients.

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