

# Factor V Leiden, Prothrombin Gene Mutation, and Thrombosis Risk in Patients with Antiphospholipid Antibodies

NIKHIL CHOPRA, SHARON KOREN, WENDA L. GREER, PAUL R. FORTIN, JOYCE RAUCH, ISABELLE FORTIN, JEAN-LUC SENÉCAL, PETER DOCHERTY, and JOHN G. HANLY

**ABSTRACT. Objective.** To determine if the prevalence of 2 prothrombotic genetic factors, factor V Leiden and prothrombin gene mutation, is increased in patients with antiphospholipid (aPL) antibodies with a history of venous/arterial thrombosis compared to patients with aPL antibodies with no history of thrombosis.

**Methods.** One hundred fifty-seven patients with aPL antibodies were studied. The occurrence of venous and arterial thrombotic events since the time of antibody detection was determined retrospectively, using appropriate clinical and diagnostic criteria. Clinical risk factors for thrombosis were documented and included hypertension, hyperlipidemia, cigarette smoking, diabetes, positive family history, use of oral contraceptive, pregnancy, trauma, hospitalization, varicose veins, and malignancy. Genomic DNA was extracted from blood cells for determination of factor V Leiden mutation  $G_{1691} \rightarrow A$  and prothrombin mutation  $G_{20210} \rightarrow A$  by polymerase chain reaction and restriction fragment length polymorphism analysis.

**Results.** Of 157 patients, 69 had a history of thrombosis (venous 37, arterial 32); 147 (94%) patients had anticardiolipin (aCL) antibodies; 69 (45%) had lupus anticoagulant (LAC). The prevalence of factor V Leiden in patients with thrombosis was 13% compared to 4.6% in patients without thrombosis (OR 3.11, CI 0.92–10.6). In patients with aCL antibodies, 15% of patients with arterial thrombosis had factor V mutation compared to 3.5% of patients without thrombosis (OR 4.9, CI 1.2–19.3). The prothrombin gene mutation was identified in 5 patients, none of whom had thrombosis. Stepwise logistic regression analysis indicated that LAC ( $p = 0.005$ ), male sex ( $p = 0.04$ ), and hypertension ( $p = 0.03$ ) were the strongest risk factors for developing thrombosis and that no additional risk was conferred by factor V Leiden ( $p = 0.13$ ) and prothrombin gene mutation.

**Conclusion.** Although the prevalence of factor V Leiden is modestly increased in patients with autoimmune aPL antibodies and thrombosis, these results suggest that its detection does not significantly increase the risk of a thrombotic event, once other clinical risk factors have been considered. Prothrombin gene mutation is not associated with thrombosis in patients with aPL antibodies. (J Rheumatol 2002;29:1683–8)

## Key Indexing Terms:

ANTIPHOSPHOLIPID ANTIBODIES

FACTOR V LEIDEN

PROTHROMBIN GENE MUTATION

From the Division of Rheumatology, Dalhousie University, Halifax, Nova Scotia; Division of Rheumatology, McGill University, Montreal, Quebec; Division of Rheumatology, University of Toronto, Toronto, Ontario; and Division of Rheumatology, Université de Montréal, Montreal, Quebec, Canada.

Supported in part by grants from the Canadian Institutes of Health Research (Dr. Rauch), The Arthritis Society (Dr. Rauch, Dr. Fortin), Lupus Quebec (Dr. Fortin), and the Queen Elizabeth II Health Sciences Centre (Dr. Chopra, Dr. Hanly). Dr. Fortin is a Senior Research Scholar of The Arthritis Society and the Director of Clinical Research, Arthritis Center of Excellence, University of Toronto.

N. Chopra, MD, FRCPC, Rheumatology Fellow; W.L. Greer, PhD, FCCMG, Professor of Pathology; P. Docherty, MD, FRCPC, Rheumatologist; J.G. Hanly, MD, MRCPI, FRCPC, Professor of Medicine, Dalhousie University; S. Koren, MD, FRCPC, Rheumatology Fellow; J. Rauch, PhD, Associate Professor of Medicine, McGill University; P.R. Fortin, MD, MPH, FRCPC, Associate Professor of Medicine, University of Toronto; I. Fortin, MD, Rheumatology Fellow; J-L. Senecal, MD, FRCPC, Professor of Medicine, Université de Montréal.

Address reprint requests to Dr. J.G. Hanly, Division of Rheumatology, Department of Medicine, Suite 310, Bethune Building, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia, Canada B2H 2Y9. E-mail: jhanly@is.dal.ca

Submitted September 10, 2001; revision accepted January 15, 2002.

Antiphospholipid (aPL) antibodies are a heterogeneous group of circulating autoantibodies directed against negatively charged phospholipids and phospholipid-binding proteins such as  $\beta_2$ -glycoprotein I ( $\beta_2$ -GPI) and prothrombin<sup>1-4</sup>. Indeed, these proteins are the true antigenic targets of autoimmune aPL antibodies, rather than phospholipids per se<sup>1-4</sup>. The antiphospholipid syndrome (APS) is characterized by the presence of aPL antibodies and at least one clinical manifestation, such as venous or arterial thrombosis or recurrent fetal loss<sup>5,6</sup>. However, as thrombosis does not occur in all patients with aPL antibodies, it is likely that an additional factor(s) must be present for clinical manifestations of APS to occur.

Much interest has recently been generated in the inherited thrombophilic disorders. Inherited resistance to protein C is due primarily to a single mutation in factor V, known as factor V Leiden<sup>7-10</sup>. Activated protein C resistance as a result of the factor V Leiden mutation is the most common inherited condition leading to a hypercoagulable state. It is found

in all European populations, at a frequency of 3–8%, and in about 15–20% of patients who present with an initial deep venous thrombosis. A separate mutation in the prothrombin gene, described in 1996, has a prevalence of 2–5% in the general population and is an additional independent risk factor for venous thrombosis<sup>7–11</sup>. The clinical importance, if any, of these common genetic thrombophilic disorders in patients with autoimmune aPL antibodies remains unclear<sup>12–17</sup>.

We examined the association between thrombosis and the presence of factor V Leiden or the prothrombin gene mutation in patients with aPL antibodies. Additional risk factors for thrombosis were also studied. A priori, we hypothesized that the presence of either factor V Leiden or prothrombin gene mutation would be significantly increased in patients with aPL antibodies who had a history of venous and/or arterial thrombosis compared to patients with aPL antibodies without clinical sequelae.

## MATERIALS AND METHODS

**Patients.** This was a multicenter study in which patients were recruited from 3 tertiary care referral centers (Dalhousie University, Halifax, Nova Scotia; McGill University and Université de Montréal, Montreal, Quebec) and one community based practice (Moncton, New Brunswick). All patients with known aPL antibodies, identified through the lupus and general rheumatology clinics at all sites and through the immunopathology and hematology laboratories at the Queen Elizabeth II Health Sciences Centre (QEII HSC) in Halifax, Nova Scotia, were invited to participate. Patients had to have at least one positive test for aPL antibodies, as indicated by either anticardiolipin (aCL) antibodies of any isotype (IgG, IgM, or IgA) determined by ELISA, or lupus anticoagulant (LAC), as defined by Brandt, *et al*<sup>18</sup>. Patients were either not receiving anticoagulants at the time of testing or the coagulation assays used were not affected by anticoagulant use. All patients with connective tissue diseases were routinely screened for aPL antibodies in each of the participating centers. The study protocol was approved by the Research Ethics Committee at the QEII HSC and other participating institutions. All patients were offered appropriate counseling in the event of a positive result on genetic testing.

**Risk factors for thrombosis.** Patients recruited at Dalhousie University and Moncton, New Brunswick, were interviewed and their charts reviewed. Data were collected by chart review only for patients recruited at McGill University and Université de Montréal. Demographic data included current age, sex, thrombosis history, associated connective tissue disease [i.e., systemic lupus erythematosus (SLE), rheumatoid arthritis, or other connective tissue disease], and atherosclerotic risk factors (i.e., hypertension, diabetes, hypercholesterolemia, smoking, and family history). Hypertension was defined as blood pressure  $\geq 140/90$  on 2 occasions or self-report by the patient. Hypercholesterolemia was defined as low density lipoprotein level  $\geq 2.6$  mmol/l or self-report by the patient. Positive family history of atherosclerosis was defined as either the presence of a stroke or myocardial infarction in a first degree family member prior to the age of 60. Risk factors for venous thrombosis were also investigated and included concurrent or recent malignancy, pregnancy or postpartum state, use of oral contraceptives, varicose veins, trauma, surgery, and/or immobilization. Information regarding all risk factors was collected in relationship to whether thrombosis had or had not occurred. Thus, in patients with a history of thrombosis, risk factors were noted at the time of the first thrombotic event. If no thrombosis had occurred, risk factors were recorded if they had been present at any time since aPL antibodies were first detected. A history of thrombocytopenia (any platelet count below the lower limit of normal for the testing laboratory) and/or miscarriages, if applicable, was also recorded.

**Thrombosis.** A thrombotic event was defined as any of the following: nonhemorrhagic stroke (confirmed on computer tomography or magnetic resonance image), myocardial infarction (any 2 of typical acute cardiac chest pain, electrocardiograph changes, and/or elevated CPK-MB), peripheral vascular disease as manifested by arterial ulcers or acute vascular limb occlusion, deep venous thrombosis (observed on venography or duplex ultrasound), or pulmonary embolism (diagnosed by V/Q scanning or angiogram). Acute vascular occlusion, as recorded on the patient's chart (e.g., mesenteric infarction, retinal occlusion), was also accepted as a thrombotic event. Documentation of a specific diagnostic test was obtained in most cases, but in a minority of cases a thrombotic event was deemed to have occurred on clinical assessment by the patient's attending physician. Fetal loss, for the purposes of this study, was not included as a thrombotic event.

**Detection of gene mutations.** Roughly 30 ml of whole blood was obtained with informed consent from each patient recruited at Dalhousie University and Moncton, New Brunswick sites. Mononuclear cell pellets and frozen whole blood were available for each patient recruited from McGill University and Université de Montréal sites, respectively. Genomic DNA was extracted from peripheral white blood cells and analyzed by polymerase chain reaction (PCR) and restriction endonuclease digestion as described<sup>11,19</sup> for both the factor V Leiden and prothrombin gene mutation in the Hematology Laboratory, Department of Pathology, QEII HSC. The factor V Leiden mutation consists of a G<sub>1691</sub>  $\rightarrow$  A transition that converts Arg<sub>(506)</sub> to Gln. Since this change eliminates an MnlI restriction site, the mutation is readily detected using PCR to amplify across the mutation site, and MnlI digestion of amplicons. Undigested amplicons represent a mutated allele. The most common prothrombin gene mutation is a G<sub>20210</sub>  $\rightarrow$  A transition in the 3' untranslated region of the gene. Since this mutation generates a HindIII restriction site, it is readily detected by PCR amplification using primers that flank the mutation site, along with HindIII digestion of the amplicons. In this case, digested amplicons represent a mutated allele.

**Statistical analysis.** Individuals were divided into 2 groups based upon the presence or absence of thrombosis. Those without a history of thrombosis formed the control group. Sample size was calculated by conservatively estimating the risk of either or both of the genetic mutations (i.e., factor V Leiden or prothrombin gene mutation) to be increased 4-fold (5% vs 20%) in patients with aPL antibodies and thrombotic events, compared to patients with aPL antibodies without thrombosis<sup>9</sup>. Thus, a sample of 150 patients (75 in each group) would have 80% power to detect this difference at an alpha of 0.05. Statistical tests included chi-square analysis for comparison of proportions between groups, calculation of odds ratios, and logistic regression techniques. The predictor variables entered into the regression model included factor V Leiden, hypertension, smoking, diabetes mellitus, recent surgery, sex, malignancy, thrombocytopenia, aCL of any isotype, LAC, oral contraceptive use, pregnancy, and history of fetal loss.

## RESULTS

**Patient characteristics.** One hundred fifty-seven subjects were recruited (Table 1). The average age at time of study recruitment was 45 years (range 19–81). Eighty-three percent of the subjects were women. The number of individuals with primary and secondary APS was 18 (12%) and 51 (33%), respectively. One hundred thirty-three (85%) subjects had an associated connective tissue disease, which in the majority (124/133, 93%) was SLE.

Sixty-nine (44%) subjects had a history of thrombosis, while 88 (56%) did not. Thirty-seven (54%) of these presented initially with a venous thrombosis. Twenty-seven patients had recurrent thrombosis, defined as 2 or more

Table 1. Demographic features of the study population.

Patients, n (%)	157 (100)
Age, yrs, mean (range)	45 (19–81)
Followup, mo, mean (range)	58 (0–183)
Female, n (%)	130 (83)
SLE, n (%)	124 (80)
Anticardiolipin antibody (aCL) n, positive (%)	147 (94)
Lupus anticoagulant (LAC) n, positive (%)	69 (45)
aCL and LAC positive, n (%)	59 (38)
Thrombosis, n (%)	69 (44)
Initial venous thrombosis, n (%)	37 (24)
Initial arterial thrombosis, n (%)	32 (21)
Primary APS, n (%)	18 (12)
Secondary APS, n (%)	51 (33)

thrombotic events. Three of these patients had both recurrent arterial and venous thrombosis. Recurrent thrombotic events were generally of the same type (i.e., arterial or venous) as the original presentation (Table 2).

All patients had aPL antibodies, defined by the presence of either elevated aCL antibodies (147/157, 94%) or LAC (69/154, 45%). Fifty-nine (38%) patients had both elevated aCL antibodies and LAC. The mean ( $\pm$  SD) duration of followup since the first detection of aPL antibodies was  $58 \pm 37$  months, and this was not significantly different between patients with and without thrombosis ( $62 \pm 40$  vs  $55 \pm 35$ ;  $p = 0.24$ ).

*Factor V Leiden, prothrombin gene mutation, and thrombosis risk.* Thirteen patients were identified with factor V Leiden (all heterozygotes), 5 patients had the prothrombin gene mutation, and 2 patients had both. The prevalence of factor V Leiden was 9/69 (13%) in patients with thrombosis (venous, arterial, or both) compared to 4/87 (4.6%) in patients without thrombosis (OR 3.11, 95% CI 0.92–10.6)

(Table 3). In patients with isolated venous thrombosis, the prevalence of factor V Leiden was 5/38 (13.2%) compared to 8/118 (6.8%) in patients without thrombosis (OR 2.08, 95% CI 0.64–6.8). The prothrombin gene mutation was identified in 5 patients, 2 of whom also had factor V Leiden. None of these 5 patients had a history of thrombosis.

Subset analysis was performed to determine if the association between factor V Leiden and thrombosis varied with the method used to detect aPL antibodies. In patients with aCL antibodies, factor V Leiden was found in a significantly higher proportion of patients with arterial thrombosis compared to those without [5/33 (15%) vs 4/113 (3.5%); OR 4.9, 95% CI 1.2–19.3]. No such association was found for LAC or for venous thrombosis.

Additional risk factors for thrombosis were also examined by univariate analysis (Table 4). Male sex was associated with an increased risk of thrombosis (OR 3.1, 95% CI 1.29–7.43) in patients with aPL antibodies, regardless of the method used for antibody detection (i.e., either elevated aCL antibodies or presence of LAC). The association with male sex was present for patients who had either type of thrombosis or venous thrombosis only, but was not found in patients who had isolated arterial thrombosis. Other risk factors for arterial and venous thrombosis, including hypertension, hyperlipidemia, smoking history, diabetes, family history of atherosclerosis, trauma, recent surgery, varicose veins, malignancy, oral contraceptive use, and pregnancy/post-partum state were not associated with increased risk of thrombosis in patients with aPL antibodies, regardless of the method used for antibody detection. Thirty-six patients had thrombocytopenia, which was not associated with thrombosis in patients with aPL antibodies (OR 1.79, 95% CI 0.84–3.79).

Table 2. Pattern of recurrent thrombotic events.

Initial Thrombosis	Recurrent Arterial Thrombosis, n (%)	Recurrent Venous Thrombosis, n (%)	Recurrent Arterial and Venous Thrombosis, n (%)
Arterial	11 (84.6)	1 (7.7)	1 (7.7)
Venous	2 (14.3)	10 (71.4)	2 (14.3)

Table 3. Factor V Leiden and prothrombin gene mutation in patients with aPL antibodies.

Mutation	Thrombosis present, n (%)	Thrombosis Absent, n (%)	OR	95% CI
Factor V Leiden	9/69 (13.0)	4/87 (4.6)	3.11	0.92–10.6
Prothrombin	0/66 (0)	5/86 (5.8)	NA	NA

NA: Not available. The odds ratio and confidence interval could not be computed as no patient with thrombosis had the prothrombin gene mutation.

Table 4. Association with traditional risk factors for thrombosis.

Risk Factor	Venous or Arterial Thrombosis, OR (95% CI)	Venous Thrombosis Only, OR (95% CI)	Arterial Thrombosis Only, OR (95%CI)
Male Sex	3.10 (1.29–7.43)	2.75 (1.14–6.63)	1.47 (0.56–3.86)
Hypertension	1.12 (0.57–2.21)	0.58 (0.25–1.36)	2.10 (0.91–4.84)
Hyperlipidemia	0.96 (0.45–2.06)	0.73 (0.28–1.86)	1.30 (0.50–3.40)
Smoking	1.35 (0.71–2.55)	0.96 (0.45–2.02)	1.66 (0.75–3.67)
Diabetes	0.86 (0.23–3.18)	1.42 (0.35–5.78)	0.43 (0.05–3.56)
Positive family history	1.10 (0.51–2.36)	0.68 (0.28–1.65)	1.80 (0.72–4.55)
Trauma	0.63 (0.06–7.05)	1.56 (0.14–17.70)	NA
Recent surgery	0.23 (0.05–1.09)	0.61 (0.13–2.91)	NA
Varicose veins	NA	NA	NA
Malignancy	1.23 (0.17–8.95)	1.03 (0.10–10.19)	1.30 (0.13–12.95)
Use of oral contraceptive	1.52 (0.60–3.88)	1.00 (0.30–3.31)	1.81 (0.62–5.29)
Pregnancy/postpartum	0.38 (0.12–1.24)	0.42 (0.09–1.97)	0.50 (0.11–2.35)

NA: not available. Odds ratio and confidence interval could not be computed as no patient with thrombosis had the prothrombin gene mutation.

*LAC, aCL antibodies, and thrombotic risk.* The presence of LAC was associated with an increased risk for both an initial thrombosis (OR 2.96, 95% CI 1.53–5.74) and recurrent thrombosis (OR 3.65, 95% CI 1.45–9.17). However, aCL antibodies were not associated with an increased risk of isolated or recurrent thrombosis (OR 0.77, 95% CI 0.21–2.78, and OR 0.74, 95% CI 0.14–4.07, respectively) (Table 5). Thrombosis was noted in 64/147 (44%) patients with aCL antibodies. This prevalence rate did not differ significantly between aCL antibody isotype (43%, 41%, and 50% for IgG, IgM, and IgA aCL, respectively).

The prevalence of factor V Leiden in patients with recurrent thrombosis was 4/27 (14.8%) compared to 4/88 (4.6%) in patients without thrombosis (odds ratio = 3.65; CI = 0.85–15.74) (Table 6). Other risk factors were not predictive of recurrent thrombotic events.

*Multivariate analysis.* To determine which of the many clinical and laboratory variables were most strongly associated with thrombosis, multivariate analysis was performed with thrombosis as the dependent variable. Stepwise logistic

regression indicated that the presence of LAC ( $p = 0.003$ ) and male sex ( $p = 0.04$ ) were the strongest risk factors for developing thrombosis of any kind, and that no additional risk was conferred by either factor V Leiden or prothrombin gene mutation. When venous thrombosis was examined in isolation, similar associations with LAC ( $p = 0.03$ ) and male sex ( $p = 0.01$ ) were found. However, when the analysis was restricted to patients with arterial thrombosis the only statistically significant risk factor was hypertension ( $p = 0.03$ ).

## DISCUSSION

The prevalence of 2 prothrombotic genetic factors, factor V Leiden and prothrombin gene mutation, was examined to determine if they were more prevalent in patients with aPL antibodies and a history of venous/arterial thrombosis compared to patients with aPL antibodies with no history of thrombosis. Although our results show an increased prevalence of factor V Leiden in patients with aPL antibodies and a history of thrombosis, this was not statistically significant, regardless of whether patients had a single thrombotic event

Table 5. Thrombosis in patients with aPL antibodies: difference in risk between presence of aCL antibody and lupus anticoagulant (LAC).

Assay	Patients with Thrombosis, n (%)	Patients without Thrombosis, n (%)	OR	95% CI
aCL+	64/69 (92.8)	83/88 (94.3)	0.77	0.21-2.78
LAC+	40/67 (59.7)	29/87 (33.3)	2.96	1.53-5.74
	Recurrent Thrombosis Present, n (%)	Recurrent Thrombosis Absent, n (%)		
aCL+	25/27 (92.6)	84/89 (94.4)	0.74	0.14-4.07
LAC+	17/26 (65.4)	30/88 (34.1)	3.65	1.45-9.17

Table 6. Factor V Leiden and prothrombin gene mutation in patients with aPL antibodies with and without recurrent thrombosis.

Mutation	Recurrent Thrombosis Present, n (%)	No Thrombosis, n (%)	OR	95% CI
Factor V Leiden	4/27 (14.8)	4/88 (4.6)	3.65	0.85–15.74
Prothrombin	0/26 (0)	5/87 (5.8)	NA	NA

NA: Not available. Odds ratio and confidence interval could not be computed as no patient with recurrent thrombosis had the prothrombin gene mutation.

or recurrent thromboses. Some, but not all, previous studies have suggested that factor V Leiden confers a relatively small increase in the risk of thrombosis in patients with aPL antibodies<sup>14,16,20,21</sup>. Our findings support this view. Surprisingly, in subset analysis, factor V Leiden was significantly more common in patients with aCL antibodies and arterial thrombosis. Although factor V Leiden and the prothrombin gene mutation are generally linked with venous thromboembolism, recent studies have also suggested an association between these 2 genetic factors and arterial thrombosis<sup>22,23</sup>.

Only 5 (3.2%) of the patients with aPL antibodies had a prothrombin mutation and none of these patients had a history of thrombosis. This is in agreement with a study in which only one (1.4%) out of 74 patients with APS was heterozygous for this mutation<sup>24</sup>. Given these findings, it is interesting to speculate that this mutation may be “protective” against thrombosis in patients with aPL antibodies. It is recognized that prothrombin is one of the major antigenic targets of aPL antibodies; thus, a mutation at a critical antigenic site could modify antigen-antibody interactions and thereby downregulate the procoagulant consequences of aPL antibodies. Further studies are required to confirm this clinical observation and to explore the potential effects of the prothrombin gene mutation on the pathogenic effects of antiprothrombin antibodies.

There have been very few studies examining the role of clinical risk factors in patients with aPL antibodies. Reports have suggested an increased risk of thrombosis in patients with aPL antibodies taking oral contraceptives<sup>25,26</sup>. A retrospective study of 61 patients found male sex to be significantly correlated with venous, but not arterial, thrombosis. In the same study, hypertension, hyperlipidemia, and smoking were also found to be significantly associated with arterial thrombosis in patients with APS<sup>27</sup>. In our study, male sex was also associated with an increased risk of thrombosis. Use of the oral contraceptive was increased in patients with thrombosis, but this did not reach statistical significance. Analysis of other “traditional” risk factors for both arterial and venous thrombosis did not reveal any significant

relationships. This is surprising and may be due to the retrospective nature of this portion of the study protocol. Alternatively, our findings may indicate that in patients with aPL antibodies who are destined to develop thrombosis, the risk is attributable entirely to the autoantibody and is independent of concurrent thrombotic risk factors.

The presence of LAC appeared to confer a stronger risk of thrombosis than aCL antibodies of any isotype. This risk was increased in patients with recurrent thrombosis. Horbach, *et al* have reported<sup>28</sup> that although aCL antibodies and LAC were independently associated with venous and arterial thrombosis in their study population, multivariate analysis indicated that LAC was the strongest predictor and that no additional risk was conferred by the presence of aCL antibodies. In our study, the lack of a statistically significant association between aCL antibodies and thrombosis is due to the very high prevalence (94%) of aCL antibodies in the study population, which was highly selected to address the primary research question. Thus, this does not preclude an association between aCL antibodies and venous/arterial thrombosis in the general population, as reported<sup>29-31</sup>.

Although a potential shortcoming, the retrospective design of our study, was likely not a significant factor, in view of the major study endpoints, namely genetic mutations of coagulation factors and the presence or absence of thrombosis. These endpoints rely upon objective criteria for diagnosis. However, we did not examine the influence of variables such as hyperhomocysteinemia, aPL antibody titer, or duration of aPL antibody positivity on thrombosis risk. We attained our recruitment target for study enrollment, which was based upon the assumption of a 4-fold increase (20% vs 5%) in the prevalence of factor V Leiden and/or prothrombin gene mutation in the thrombosis group. The presence of either mutation in only 13% of the thrombosis group accounts for the failure of the analytical data to reach statistical significance, as the control group had the expected prevalence of  $\leq 5\%$ . A total of 201 patients per group would be required to reach a statistically significant difference, assuming a continuation of the same trend in mutation prevalence between groups. However, as indicated by stepwise regression, the presence of either mutation did not add to the thrombosis risk over and above that associated with male sex and LAC. On this basis, it is unlikely that the coagulation factor mutations studied here are major contributors to thrombosis risk in patients with aPL antibodies.

## REFERENCES

1. Roubey RA. Update on antiphospholipid antibodies. *Curr Opin Rheumatol* 2000;12:374-8.
2. Roubey RA. Antiphospholipid syndrome: antibodies and antigens. *Curr Opin Hematol* 2000;7:316-20.
3. Thiagarajan P, Shapiro SS. Lupus anticoagulants and antiphospholipid antibodies. *Hematol Oncol Clin North Am* 1998;12:1167-92.

4. Rauch J. Lupus anticoagulant antibodies: recognition of phospholipid-binding protein complexes. *Lupus* 1998;7:S29-31.
5. Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum* 1999;42:1309-11.
6. Lockshin MD, Sammaritano LR, Schwartzman S. Validation of the Sapporo criteria for antiphospholipid syndrome. *Arthritis Rheum* 2000;43:440-3.
7. Aznar J, Vaya A, Estelles A, et al. Risk of venous thrombosis in carriers of the prothrombin G20210A variant and factor V Leiden and their interaction with oral contraceptives. *Haematologica* 2000;85:1271-6.
8. Ordonez AJ, Carreira JM, Alvarez CR, Rodriguez JM, Alvarez MV, Coto E. Comparison of the risk of pulmonary embolism and deep vein thrombosis in the presence of factor V Leiden or prothrombin G20210A. *Thromb Haemost* 2000;83:352-4.
9. De Stefano V, Chiusolo P, Paciaroni K, Leone G. Epidemiology of factor V Leiden: Clinical implications. *Semin Thromb Hemost* 1998;24:367-79.
10. Vargas M, Soto I, Pinto CR, et al. The prothrombin 20210A allele and the factor V Leiden are associated with venous thrombosis but not with early coronary artery disease. *Blood Coagul Fibrinolysis* 1999;10:39-41.
11. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996;88:3698-703.
12. Regeczy N, Balogh I, Lakos G, et al. Hypercoagulability in various autoimmune diseases: no association with factor V Leiden mutation. *Haematologia* 2000;30:35-9.
13. Kenet G, Sadetzki S, Murad H, et al. Factor V Leiden and antiphospholipid antibodies are significant risk factors for ischemic stroke in children. *Stroke* 2000;31:1283-8.
14. Pablos JL, Caliz RA, Carreira PE, et al. Risk of thrombosis in patients with antiphospholipid antibodies and factor V Leiden mutation. *J Rheumatol* 1999;26:588-90.
15. Picillo U, De Lucia D, Palatiello E, et al. Association of primary antiphospholipid syndrome with inherited activated protein C resistance. *J Rheumatol* 1998;25:1232-4.
16. Simantov R, Lo SK, Salmon JE, Sammaritano LR, Silverstein RL. Factor V Leiden increases the risk of thrombosis in patients with antiphospholipid antibodies. *Thromb Res* 1996;84:361-5.
17. Fijnheer R, Horbach DA, Donders RC, et al. Factor V Leiden, antiphospholipid antibodies and thrombosis in systemic lupus erythematosus. *Thromb Haemost* 1996;76:514-7.
18. Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. *Thromb Haemost* 1995;74:1185-90.
19. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N Engl J Med* 1995;332:912-7.
20. Dizon-Townson D, Hutchison C, Silver R, Branch DW, Ward K. The factor V Leiden mutation which predisposes to thrombosis is not common in patients with antiphospholipid syndrome. *Thromb Haemost* 1995;74:1029-31.
21. Montaruli B, Borchiellini A, Tamponi G, et al. Factor V Arg506 → Gln mutation in patients with antiphospholipid antibodies. *Lupus* 1996;5:303-6.
22. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. *Blood* 1997;90:1747-50.
23. Rosendaal FR, Siscovick DS, Schwartz SM, et al. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. *Blood* 1997;89:2817-21.
24. Bertolaccini ML, Atsumi T, Hunt BJ, Amengual O, Khamashta MA, Hughes GR. Prothrombin mutation is not associated with thrombosis in patients with antiphospholipid syndrome. *Thromb Haemost* 1998;80:202-3.
25. Girolami A, Zanon E, Zanardi S, Saracino MA, Simioni P. Thromboembolic disease developing during oral contraceptive therapy in young females with antiphospholipid antibodies. *Blood Coagul Fibrinolysis* 1996;7:497-501.
26. Julkunen HA. Oral contraceptives in systemic lupus erythematosus: side-effects and influence on the activity of SLE. *Scand J Rheumatol* 1991;20:427-33.
27. Krnic-Barrie S, O'Connor CR, Looney SW, Pierangeli SS, Harris EN. A retrospective review of 61 patients with antiphospholipid syndrome. Analysis of factors influencing recurrent thrombosis. *Arch Intern Med* 1997;157:2101-8.
28. Horbach DA, van Oort E, Donders RC, Derksen RH, de Groot PG. Lupus anticoagulant is the strongest risk factor for both venous and arterial thrombosis in patients with systemic lupus erythematosus. Comparison between different assays for the detection of antiphospholipid antibodies. *Thromb Haemost* 1996;76:916-24.
29. Nojima J, Suehisa E, Akita N, et al. Risk of arterial thrombosis in patients with anticardiolipin antibodies and lupus anticoagulant. *Br J Haematol* 1997;96:447-50.
30. Christopher R, Nagaraja D, Dixit NS, Narayanan CP. Anticardiolipin antibodies: a study in cerebral venous thrombosis. *Acta Neurol Scand* 1999;99:121-4.
31. de Godoy JM, de Godoy MF, Braile DM, Torres CA. Prevalence of anticardiolipin antibodies in peripheral arterial thrombosis. *Angiology* 2000;51:473-7.