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Prevalence of activated protein C resistance and analysis of clinical profile in thromboembolic patients. A Belgian prospective study

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Abstract. Hainaut P, Azerad M-A, Lehmann F, Schlit A-F, Zech F, Heusterspreute M, Philippe M, Col C, Lavenne E, Moriau M. (Cliniques Universitaires St Luc, Bruxelles, Belgium). Prevalence of activated protein C resistance and analysis of clinical profile in thromboembolic patients. A Belgian prospective study. *J Intern Med* 1997; **214:** 427–33.

Objectives. To assess the prevalence of activated protein C resistance (APC-R) among healthy subjects and thromboembolic patients and to determine the clinical characteristics associated with APC-R.
Design. A prospective study.
Setting. One academic medical centre.
Subjects. 91 health controls and 126 thromboembolic patients.
Measurements. Patients and control were genotyped for the factor V Leiden (VaQ506) mutation. The anticoagulant response of the patient's plasma to activated protein C was also determined.
Results. The frequency of APC-R was 3.3% among healthy control subjects and 22% among thrombotic patients of whom 18% were heterozygous and 4% were homozygous. The mean age at the first thrombotic event and the severity of thrombotic disease including the proportion of proximal deep vein thrombosis and the frequency of lung embolism were identical among APC-R positive and negative patients. A family history of thromboembolic disease was elicited more frequently in APC-R positive than in APC-R negative patients (57% vs. 22%, $P < 0.001$). The recurrence rate was higher for APCR-R positive patients (57% vs. 34%, $P < 0.05$). The percentage of cases with a factor predisposing to thrombosis was very similar in APC-R positive (57%) and negative (68%) patients.
Conclusions. A familial history of thromboembolic disease and recurrences are significantly more frequent among APC-R positive than APC-R negative patients.

Keywords: activated protein C resistance, APC-R, coagulation, deep venous thrombosis, thromboembolism, thrombophilia.

Introduction

Thromboembolic disease is a major cause of mortality and morbidity in the Western world [1]. The frequent finding of a positive familial history underlies the role of genetic factors in the genesis of thromboembolism. Until recently, inherited deficiency of physiological inhibitors (antithrombin III, protein C, protein S) accounted for most cases of hereditary thrombophilia; however, the combined incidence of these deficits accounts for less than 15% of patients suffering from thromboembolic disease [2]. In 1993, Dahlbäck and colleagues described a family with a high incidence of thrombosis whose members displayed a weak plasmatic response to the anticoagulant action of activated protein C (APC). This inherited abnormality was termed activated protein C resistance (APC-R) [3]. The underlying genetic defect was characterized as a factor V point mutation inducing a substitution of an Arg by a Gln at position 506 [4–7]. The mutated factor Va molecule was referred to factor VaQ506 or factor V Leiden. This finding contributed to the elucidation of the APC-R mechanism: APC plays its anticoagulant role, through serine protease action, by inactivating factors Va and factor VIIIa. The modification of one

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of the three activated protein-C cleavage sites located on the heavy chain renders factor V Leiden less sensitive to inactivation by APC. As factor V procoagulant activity is left unchanged, the mutation unbalances the procoagulant and the anticoagulant properties leading to a hypercoagulable state [3, 5].

APC-R is transmitted in an autosomal dominant fashion. It was found in 1.6% to 14.7% of healthy subjects with a higher incidence being reported in the Northern countries and lower figures in African and Asian populations [3, 8, 9–13]. Among thromboembolic patients, the reported prevalence ranged from 8% to 64% [8, 9, 14–17]. Although preliminary reports also advocated an increased incidence of arterial thrombosis linked to mutated factor V [18–19], large-scale studies ultimately discarded this association [20–21].

Aside from the epidemiological interest, the detection of thrombophilia in an individual patient influences the prognosis and possibly the therapy. These findings prompted us to study the prevalence of APC-R, along with other thrombophilic factors and precipitating circumstances, in our thromboembolic population. The aim was to fix the incidence and the clinical characteristics of APC-R in a Belgian population. Additionally, we correlated the APC ratio, determined by coagulation assay, with DNA analysis of factor V Leiden mutation, in order to evaluate whether the APC ratio would discriminate between negative, homozygous and heterozygous positive APC-R patients.

Patients and methods

Patients with thrombosis and healthy controls

The study population included prospectively 126 patients and 91 healthy volunteers recruited in a single academic hospital between June 1993 and September 1995. Twenty-eight patients were hospitalized while 98 were outpatients. Ninety-one normal Caucasian individuals (54 women and 37 men) without either personal or familial history of thrombosis were included as control subjects.

Assays

Blood sampling. Blood was collected through a clean venipuncture into tubes containing 0.106 trisodium citrate for coagulation assay and kalium-EDTA (1.6 mg EDTA/ml blood) for DNA processing. At blood sampling none of the patients were receiving any medication that would interfere with the coagulation study.

Coagulation assays. Platelet-free plasma was obtained by centrifugation at 5000 *g* for 20 min at 4°C and was stored at −20°C until required. Protein C and antithrombin III levels were determined through a functional test with chromogenic substrates kits Chromogenix® on a centrifugal autoanalyser ACL200 (Instrumentation laboratory, Milan, Italy). Protein S was analysed by enzyme-linked immunosorbent assay testing (free protein S) [22]. Lupus anticoagulant was evaluated by three screening tests (activated recalcification time, activated partial thromboplastin time (APTT), tissue thromboplastin inhibition test (TTI; using Innovin Ortho thromboplastin) and two confirming methods involving plasmatic mixture and platelet neutralization. The anticoagulant response of the patient's plasma to APC was determined using the Coatest APC Resistance kit (Chromogenix, Sweden) on an ACL 200. Results are expressed at the ratio of the APTT clotting time of the plasma in the presence of exogenous APC to the APTT clotting time without exogenous APC.

DNA amplification and sequencing. DNA was extracted from peripheral blood leukocytes using a QI Aamp Blood kit (Qiagen, Germany). The Arg 506-Gln mutation of the factor V gene resulting from G-A 1691 substitution was detected using digestion by the restriction enzyme Mnl of the products amplified by polymerase chain reaction, as described by Bertina [5], and sequencing was done using a Taq Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems, CA, USA).

Diagnosis of thromboembolism

Deep venous thrombosis was confirmed by duplex Doppler ultrasonography or venography. Lung embolism was diagnosed by high-probability lung scintigraphy according to Pioped criteria or angiography.

Statistical analysis

Dichotomic values were compared using the χ^2 test with Yate's continuity correction. When appropriate, results were expressed as odds ratios. Multivariate

analysis was performed using Mantel-Haenszel's test after stratification.

Results

Correlation of coagulation APC ratio assay and DNA testing

Both APC coagulation assay and gene testing were performed in 91 controls and 108 consecutive patients with thrombotic disease. Patients were classified as APC-R negative, heterozygous and homozygous according to factor-V mutation gene testing. Considering the controls and the patients who benefitted from both tests, the median value ($\pm$SD) of the APC ratios were 3.5 ($\pm$0.55), 2.25 ($\pm$0.5) and 1.5 ($\pm$0.27) for APC-R negative, heterozygous and homozygous positive subjects, respectively. Data are shown in Fig. 1. When using a unique cut-off of 2.6 for all cases, the sensitivity is 79% and the specificity, 96%. If separate cut-offs are used according to sex and current hormonal therapy as proposed by others [23; 2.8 for men, 3.0 for women without oral contraception and 2.5 for women with oral contraception], the sensitivity is 84% and the specificity 95%.

Prevalence of APC resistance in healthy controls

Among 91 controls with a mean age of 28 years (range, 20 to 64), the prevalence of APC resistance was 3.3% including only heterozygous factor V mutation (Table 1).

Prevalence and clinical profile of thrombotic patients regarding APC resistance

A total of 126 patients with thromboembolic disease were analysed: their mean age was 41 years (range, 16 to 80) at the time of inclusion. Twenty-two per cent exhibited an APC resistance: 4% were homozygous and 18% were heterozygous for factor V mutation (Table 1). The clinical profile of the patients is summarized in Table 1. The mean age at the time of first thrombotic episode did not differ between APC-R positive and negative patients: they were 41 years (range, 21 to 69) and 40 years (range, 16 to 78), respectively. The APC-R homozygous patients suffered a first thrombotic event at a younger age but because of the small number of patients the difference was not statistically significant. The type of presentation of the thromboembolic manifestation was similar in both groups with a predominance of proximal deep vein thrombosis. Moreover, the presence of either symptomatic or asymptomatic lung embolism was assessed in 101 patients: the overall prevalence of lung embolism was 38% (38 out of 101 patients) with no significant difference between APC-R positive (24%, 5 out of 21 patients) and negative patients (41%, 33 out of 80). A striking preponderance of females was found in both the overall population and the APC-R-negative group while the sex ratio completely inverted in the APC-R-positive group which displayed a slight preponderance of males ($P < 0.02$). A familial history was highly predictive of APC resistance as it was found in

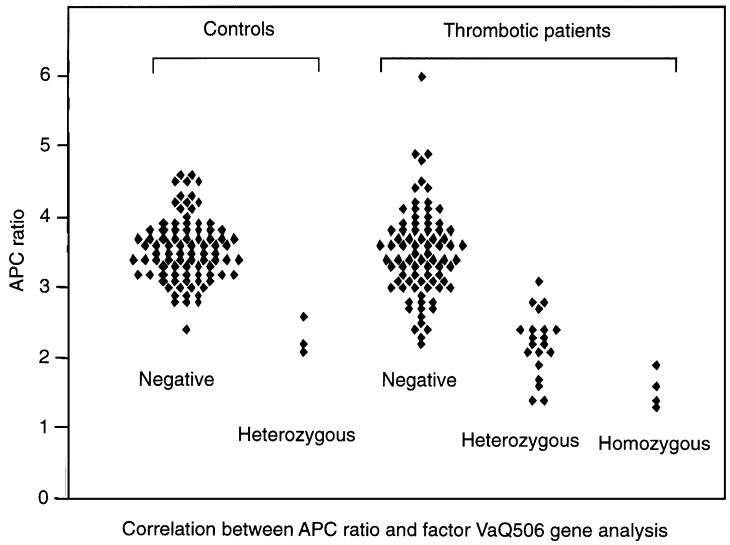

Fig. 1 Controls and patients were classified as activated protein C resistance (APC-R) negative, heterozygous and homozygous according to factor Va mutation (VaQ506) gene testing. Individual values of activated protein C (APC) ratio are plotted for each group.

Table 1 Clinical profile of patients with thromboembolic disease

	Overall	APC-R positive			APC-R negative	*P*
		Total	Homozygous	Heterozygous		
Healthy controls						
Number	91	3	0	3	88	—
Sex (male/female)	37/54	1/2	0	1/2	36/52	—
Thromboembolic patients						
Number	126	28	5	23	98	—
Sex (male/female)	41/85	15/13	3/2	12/11	26/72	< 0.02
Mean age at 1st thrombotic event (years)	40	41	33	43	40	NS
Presenting thrombotic event						
Proximal DVT	63	16	4	12	47	—
Distal DVT	53	11	1	10	42	—
Lung embolism	5	0	0	0	5	—
Varia	5	1	0	1	4	—
No. of patients with positive family history of thromboembolic disease (%)	38 (30%)	16 (57%)	4 (80%)	12 (52%)	22 (22%)	< 0.001
No. of patients with recurrence of thromboembolic disease (%)	49 (39%)	16 (57%)	2 (40%)	14 (61%)	33 (34%)	< 0.05
No. of patients with predisposing risk factors	83 (66%)	16 (57%)	4 (80%)	12 (52%)	67 (68%)	NS

APC-R, activated protein C resistance; DVT, deep vein thrombosis; NS, not significant.

Table 2 Prevalence of predisposing factors in patients with thromboembolic disease

Thromboembolic patients	Overall	APC-R positive			APC-R negative
		Total	Homozygous	Heterozygous	
Number	126	28	5	23	98
No. of patients with predisposing risk factors					
Total	83 (66%)	16 (57%)	4 (80%)	12 (52%)	67 (68%)
Hormonotherapy	15	3	1	2	12
Other haemostatic abnormalities	13	1	0	1	12
Traumatism	12	2	0	2	10
Post-partum	13	3	1	2	10
Immobilization	9	2	2	0	7
Varices	9	1	0	1	8
Postoperative state	6	3	0	3	3
Inflammatory disease	3	0	0	0	3
Neoplastic disease	3	1	0	1	2

APC-R, activated protein C resistance.

57% of APC-R-positive patients but only 22% of APC-R-negative patients ($P < 0.001$). The recurrence rate was higher among APC-R-positive patients (odds ratio: 2.6; $P < 0.05$). The percentage of patients for which another predisposing factor was identified was quite similar in APC-R-positive (57%) and negative (68%) groups. Likewise, the prevalence of risk factors analysed individually was grossly equivalent for the APC-R positive and negative thrombotic populations (Table 2). The multivariate analysis demonstrates that familial history (odds ratio: 4.5; $P < 0.002$) and male sex (odds ratio: 3.1; $P < 0.02$) were two independent predictors of APC resistance. On the other side, APC-R was significantly associated with recurrences in our population.

Analysis of in- and outpatients

As the study population included both outpatients attending a phlebology clinics and hospitalized patients, we combined both subgroups in a Mantel-Haenszel's test regarding the clinical characteristics

Table 3 Comparison of in- and outpatients with thromboembolic disease

	Inpatients			Outpatients		
	Total	APC-R positive	APC-R negative	Total	APC-R positive	APC-R negative
Number of patients	28	7	21	98	21	77
Sex (male/female)	15/13	6/1	9/12	26/72	9/12	17/60
Mean age (years) at 1st thrombotic event	53	47	55	36	39	35
Presenting thrombotic event						
Proximal DVT	23	5	18	40	11	29
Distal DVT	3	1	2	50	10	40
Lung embolism	1	0	1	4	0	4
Varia	1	1	0	4	0	4
No. of patients with positive family history of thromboembolic disease (%)	7 (25%)	5 (71%)	2 (10%)	31 (32%)	11 (52%)	20 (26%)
No. of patients with recurrence of thromboembolic disease (%)	13 (46%)	4 (57%)	9 (43%)	36 (37%)	12 (57%)	24 (31%)

APC-R, activated protein C resistance.

associated with APC-R. The relationship between APC-R and the sex ratio ($P < 0.02$), the familial history ($P < 0.001$) and the rate of recurrence ($P < 0.05$) was confirmed independently from the stratification (Table 3).

Discussion

We conducted a prospective study of APC resistance (APC-R) in thromboembolic patients and healthy control subjects attending one Belgian academic hospital. Patients and controls were tested for APC-R by both APC ratio coagulation assay and gene analysis of factor V mutation (F Va Q506). The APC ratio discriminates accurately between APC-R negative, heterozygous and homozygous subjects although results of the APC ratio assay shows some overlap between the three groups. When using a cut-off of 2.6, the APC ratio provided, in our series, a sensitivity of 79% and a specificity of 96%. The APC ratio determination can be further refined using a modified test that confers increased sensitivity and specificity and allows APC ratio assessment in individuals currently receiving coumadine derivatives or bearing a lupus anticoagulant [24–26]. This method was not yet available at the beginning of the study.

The frequency of APC-R found in our thromboembolic patients (22%) and controls (3.3%) is in concordance with the results reported in the literature. As previously recalled, the values suffer large variations depending on ethnic groups, APC-R assay, patients' selection and associated risk factors [27–28]. The figure of 22% is close to the results found in both the large Leiden study (21%) [8] and French (17%) [29–30] and Australian (27%) [31] reports. Figures as high as 50 to 60% were obtained in small series generally selected with restrictive criteria such as young age and absence of classical risk factors [15].

Regarding APC resistance, the clinical profiles of thrombotic patients raise pertinent issues. In an individual patient, the most powerful clinical predictors of APC-R are a family history of thromboembolism, and male sex. As APC-R has been firmly established as an autosomal dominant trait [32], the first point is quite plausible even if it has not been found in some other studies [29]. The second finding is rather more intriguing. In fact, the female preponderance found in the whole cohort is inverted in the APC-R positive subgroups. The high proportion of women in our thromboembolic population reflected the preponderance of female patients attending phlebology clinics whereas the in-patient subgroup involved males and females in similar proportions. A potential bias in the analysis was discarded by performing a Mantel-Haenszel test after stratification for both subgroups (in- and outpatients). This test confirmed the over-representation of males among APC-R positive patients. Thus, the relationship between APC-R and male sex was found to be independent of the subgroups. The result is hardly compared with other studies as either they included a limited number of male patients or they

did not address the link between the sex ratio and APC-R. One can speculate that APC-R weakens the influence of hormonotherapy as a causative agent of thromboembolism. Whatever, it must be stressed that APC-R is only one risk factor for thromboembolism alongside other precipitating circumstances. Associated predisposing factors were found in APC-R positive as frequently as in APC-R negative patients underlying the fact that APC-R is probably only a weak thrombogenic factor acting together with other stimuli. In accordance with previous reports [29], the age at the time of the first thrombotic episode was not significantly different between APC-R positive and negative. One exception is the homozygous patients who develop the first thrombotic event at an earlier age but the small number of homozygous patients in our series precludes firm conclusions. Interestingly APC resistance did not influence the location or the severity of thrombotic disorder. The proportion of either symptomatic or asymptomatic lung embolism was similar in APC-R positive and APC-R negative patients. Finally, the rate of recurrence was also found to be higher among APC-R positive patients. It must be recalled, however, that the recurrences were not assessed prospectively but were established according to the medical chart obtained at enrollment. However, as the age at the first thrombotic event was identical for APC-R positive and negative patients, the analysis probably remains valid in spite of the retrospective design. This issue was not addressed in other large studies since relapsing patients were generally excluded [8, 29]. Multivariate analysis confirm that both factors, i.e. male sex and family history, were independent predictors of APC resistance. Reciprocally, APC-R is significantly associated with recurrence.

In conclusion, we confirmed in a prospective study using APC-R ratio and gene testing of factor V mutation that APC resistance is a frequent risk factor of thromboembolism. The clinical profile of patients highlights that APC-R positive thrombotic patients more frequently exhibit a positive family history and recurrences of thrombosis. Additionally, APC-R-related thromboembolism affects male patients as frequently as females. APC-R should be looked for since the findings may be helpful for genetic counselling and appropriate therapy management, at least in relapsing patients.

References

1 Schafer AI. Low-molecular-weight heparin: an opportunity for home treatment of venous thrombosis. *N Engl J Med* 1996; **334:** 724–5.

2 Hirsh J, Prins MH, Samama M. Hemostasis and thrombosis. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. *Basic Principles and Clinical Practice*. Philadelphia: JB Lippincott, 1994; 1543–61.

3 Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognised mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci* 1993; **90:** 1004–8.

4 Voorberg J, Roelse J, Koopman R, Buller H, Berends F, ten-Cate JW *et al*. Association of idiopathic venous thromboembolism with single point mutation at Arg506 of factor V. *Lancet* 1994; **343:** 1535–6.

5 Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de-Ronde H *et al*. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994; **369:** 64–7.

6 Dahlbäck B, Hildebrand B. Inherited resistance to activated protein C is corrected by anticoagulant cofactor activity found to be a property of factor V. *Proc Natl Acad Sci* 1994; **91:** 1396–1400.

7 Zoller B, Dahlbäck B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. *Lancet* 1994; **343:** 1536–8.

8 Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet* 1993; **342:** 1503–6.

9 Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med* 1994; **330:** 517–22.

10 Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. *Lancet* 1995; **346:** 1133–4.

11 Halbmayer WM, Haushofer A, Schön R, Fischer M. The prevalence of poor anticoagulant response to activated protein C (APC resistance) among patients suffering from stroke or venous thrombosis and among healthy subjects. *Blood Coagul Fibrinolysis* 1994; **5:** 51–7.

12 Cadroy Y, Sie P, Boneu P. Frequency of a defective response to activated protein C in patients with a history of venous thrombosis. *Blood* 1994; **83:** 2008–9.

13 Zöller B, Nordlund L, Leksell H, Nilsson JE, von Schenk H, Rosén U, Jeppson JO *et al*. High prevalence of the FVR506Q mutation causing APC resistance in a region of southern Sweden with a high incidence of venous thrombosis. *Thromb Res* 1996; **83:** 475–7.

14 Cumming AM, Fildes S, Pylypczuk CC, El-Metaal M, Burn AM, Wensley RT, Tait RC. Low incidence of resistance to activated protein C in patients with venous thrombosis. *Br J Haematol* 1994; **86** (Suppl. 1): 34.

15 Griffin JH, Evatt B, Wideman C, Fernandez JA. Anticoagulant protein C pathway defective in majority of thrombophilic patients. *Blood* 1993; **82:** 1989–93.

16 Cadroy Y, Sié P, Alhenc-Gelas M, Aiach M. Evaluation of APC resistance in the plasma of patients with Q506 mutation of factor V (factor V Leiden) and treated by oral anticoagulants. *Thromb Haemost* 1995; **73:** 734–5.

17 Legnani C, Palareti G, Biagi R, Coccheri S. Activated protein C resistance in deep-vein thrombosis. *Lancet* 1994; **343:** 541–2.
18 Lindblad B, Svensson P, Dahlbäck B. Arterial and venous thromboembolism with fatal outcome and resistance to activated Protein C. *Lancet* 1994; **343:** 917.
19 Samani NJ, Lodwick D, Martin D, Kimber P. Resistance to activated protein C and risk of premature myocardial infarction. *Lancet* 1994; **344:** 1709–10.
20 Ridker P, Hennekens C, Lindpainter K, Stampfer M, Eisenberg P, Miletich J. 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.
21 Kontula K, Ylikorkala A, Miettinen H, Vuorio A, Kauppinen-Makelin R, Hamalainen L *et al*. Arg506Gln Factor V Mutation (Factor V Leiden) in patients with ischaemic cerebrovascular disease and survivors of myocardial infarction. *Thromb Haemost* 1995; **73:** 558–60.
22 Bertina RM. Protein S antigen. In: Jespersen J., Bertina RM, Haverkate F, eds. *Ecat Assay Procedures*. Lancaster: Kluwer Academic Publishers, 1992; 99–108.
23 Henkens CM, Bom VJ, Seinen AJ, van der Meer J. Sensitivity to activated protein C: influence of oral contraceptives and sex. *Thromb Haemost* 1995; **73:** 402–4.
24 Trossaert M, Conard J, Horellou MH, Samama MM, Ireland H, Bayston TA, Lane DA. Modified APC resistance assay for patients on oral anticoagulants. *Lancet* 1994; **344:** 1709.
25 Tosetto A, Rodeghiero F. Diagnosis of APC resistance in patients on oral anticoagulant therapy. *Thromb Haemost* 1995; **73:** 732–3.
26 Le DT, Griffin JH, Greengard JS, Mujumdar V, Rapaport SI. Use of a generally applicable tissue factor-dependent factor V assay to detect activated protein C-resistant factor Va in patients receiving warfarin and in patients with a lupus anticoagulant. *Blood* 1995; **85:** 1704–11.
27 Vandenbroucke JP, Koster T, Briet E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. *Lancet* 1994; **344:** 1453–7.
28 Dahlbäck B. Inherited thrombophilia: resistance to activated protein C as a pathogenic factor of venous thromboembolism. *Blood* 1995; **85:** 607–14.
29 Trossaërt M, Conard J, Horellou MH, Samaha M, Elalamy I., Samama MM. Résistance à la protéine C activée dans les accidents thrombo-emboliques veineux. Fréquence et manifestations cliniques. *Presse Med* 1995; **24:** 209–12.
30 Samaha M, Trossart M, Conard J, Horellou MH, Elalamy I, Samama MM. Prevalence and patient profile in activated protein C resistance. *Am J Clin Pathol* 1995; **104:** 450–4.
31 Ma DDF, Aboud MR, Williams GB, Isbister JP. Activated protein C resistance (APC) and inherited factor V (FV) missense mutation in patients with venous and arterial thrombosis in a haematology clinic. *Aust NZ J Med* 1995; **25:** 151–4.
32 Zöller B, Svensson PJ, He X, Dahlbäck B. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. *J Clin Invest* 1994; **94:** 2521–4.

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