Haematologica 2000; 85:1271-1276
orginal paper

Hemostasis & Thrombosis

Risk of venous thrombosis in carriers of the prothrombin G20210A variant and factor V Leiden and their interaction with oral contraceptives

JUSTO AZNAR,* AMPARO VAYÁ,* AMPARO ESTELLÉS,° YOLANDA MIRA, * RAFAEL SEGUÍ,° PIEDAD VILLA,* FERNANDO FERRANDO,* CRISTINA FALCÓ,° DOLORES CORELLA,# FRANCISCO ESPAÑA°

**Department of Clinical Pathology and °Research Centre, "La Fe" University Hospital and #Department of Preventive Medicine and Public Health, School of Medicine, Valencia, Spain*

ABSTRACT

Background and Objectives. **The prothrombin G20210A mutation and factor V Leiden have been found to be associated with an increased risk of venous thrombosis, but the reported prevalences of the prothrombin gene variant both in the normal population and in patients with deep venous thrombosis (DVT) vary greatly in the literature. Moreover, the influence of oral contraceptives (OC) on thrombotic events in patients with the prothrombin G20210A variant has not been well established. In this study we evaluate both circumstances.**

Design and Methods. **A case-control study was run on 229 patients with DVT and 246 healthy controls. The patients' history of thrombosis and acquired thrombotic risk factors, especially OC, were recorded. Prothrombin G20210A mutation, factor V Leiden, antithrombin, heparin II cofactor, plasminogen and proteins C and S were evaluated.**

Results. **Seven and a half percent of the patients and 2.9% of the controls were carriers of the prothrombin mutation, while 12.2% of the patients and 1.6% of the controls had factor V Leiden. Among the 229 DVT patients there were 130 patients with clinically suspected thrombophilia (first thrombotic event occurring before the age of 45 years or positive family history of thrombosis or recurrent venous thrombosis). Ten percent of these 130 patients were carriers of the prothrombin G20210A mutation and 18.5% had the factor V Leiden mutation. The odds ratios (OR) for DVT risk were: 2.4 (95% CI, 1.0-6.3) for the total DVT patients and 5.2 (95% CI, 1.4-19.5) for the patients with clinically suspected thrombophilia with the prothrombin mutation. The risk of thrombosis was 6.9 (95% CI, 2.3-20.6) for the DVT patients and 14.3 (95% CI, 3.3-64.6) for the patients with clinically suspected thrombophilia with factor V Leiden. Fifty-five percent of the patients with combined congenital defects (prothrombin mutation G20210A plus another congenital defect) had recurrent thrombosis. In women receiving OC the risk of DVT was 3.5 (95% CI, 1.5-8.2) that of the patients not receiving OC. When women with combined defects were also taking OC, the risk of thrombosis increased significantly.**

Interpretation and Conclusions. **The prevalence of the prothrombin G20210A mutation in the healthy population in our study is similar to that observed in other southern European countries. The prothrombin G20210A mutation does not by itself seem to be a high thrombotic risk factor. However, when it is present together with other thrombotic risk factors, the predicted risk of thrombotic events increases. The use of OC by women with the prothrombin G20210A variant or FV Leiden, either alone or combined with other thrombotic risk factors, was associated with a significant increase in the risk of venous thrombosis.**

Key words: prothrombin G20210A; acquired thrombophilic risk factors; oral contraceptives; factor V Leiden; thrombosis

Correspondence: Justo Aznar MD, Ph.D., Department of Clinical Pathology, La Fe University Hospital, Avda. Campanar 21, 46009 Valencia, Spain. Fax: international +34-96-3868789 – E-mail: aznar_jus@gva.es

Several genetic defects have been identified as venous thrombosis risk factors.[1] A single G to A nucleotide transition at position 20210 in the 3'-untranslated region of the prothrombin gene was found to be associated with an increased prothrombin level and an increased risk of venous thrombosis.[2] This mutated allele has been identified in 0.7-6.5% of normal subjects,[2-18] in 4.3-11.3% of unselected patients with a first episode of deep venous thrombosis (DVT)[2-7,13,16,18] and in 7.9-24.1% of patients with a personal or family history of venous thrombosis or thrombophilia.[2,8-10,12,18] The increased risk of DVT in patients with the prothrombin G20210A mutation as compared to in controls varies from an odds ratio (OR) of 2.0 to 6.6.[2-6,13,16,18] As can be observed, these data indicate wide variations both in the prevalence and risk of venous thrombosis in patients with the prothrombin G20210A variant. In fact, in a broad study performed in 11 different centers and involving 5,527 patients[19] the prevalence estimates varied from 0.7% to 4.0 %. In that study, the prevalence in southern Europe was higher than in northern Europe (2.3-3.7% vs 1.3-2.2%). These data agree with those of other studies that give values of 2.2% to 7% in northern European populations[2-4,6,10,20] and 1.6% to 2.5% in the USA.[1,15,21] Howev-

er, the data from southern Europe are more heterogeneous, because some authors reported values similar to those of northern Europe (1.0-2.5%),[7,12-14,16] while other reports gave higher prevalences (3.7%-6.5%).[8,11,17,18] This variability may be due to the heterogeneity of the groups studied and/or to racial or geographic variations. At any rate, it is evident that further studies are needed. In relation to the risk of DVT in patients with prothrombin G20210A, recent reports have described it to be enhanced in these patients when associated with factor V Leiden.[22,23] In the present study we examine the prevalence of the prothrombin mutation G20210A and factor V Leiden in 229 patients with DVT and in 246 healthy blood donors from the Mediterranean area. We also analyze other congenital and acquired thrombotic risk factors, especially oral contraceptives (OC), in order to evaluate their influence on thrombotic events in these patients.

Design and Methods

Clinical group

The total group included 229 patients [age at first DVT: 44±18 years (mean ± SD), 42 (median)] with a confirmed diagnosis of DVT of the lower limbs who were consecutively referred to our Thrombosis Unit. Forty-two of the 229 patients with DVT had pulmonary embolism. One hundred and thirty [age at first DVT: 33±12 years (mean ± SD), 32 (median)] of the 229 DVT patients were selected according to the presence of clinically suspected thrombophilia (first thrombotic event occurring before the age of 45 years or positive family history of thrombosis or recurrent venous thrombosis). Nineteen patients had recurrences, seventy-six patients had thrombotic event before 45 years old, six patients had a family history of thrombosis, and the rest of the patients (n=29) had two clinical signs of thrombophilia.

In all the patients the thrombosis location and recognized predisposing risk factors (prolonged immobilization, trauma or surgery within the past three months), and prothrombotic medical situations (varicose veins, congestive heart failure, obesity, chronic obstructive pulmonary disease, pregnancy and oral contraceptives) were recorded. Malignancy was excluded. The episode was considered spontaneous in the absence of environmental risk factors. Objective procedures for diagnosis of thrombosis were performed in all patients. DVT of the lower limbs was established by ultrasonography or venography, and pulmonary embolism was diagnosed by ventilation-perfusion lung scanning or pulmonary angiography. The thrombophilic study done on all the patients evaluated the following parameters: antithrombin, protein C, protein S, plasminogen, heparin cofactor II (HCII), activated protein C (APC) resistance, factor V Leiden and the prothrombin G20210A mutation.

While patients were being recruited, a control group of 246 healthy blood donors (40±13 years) from the same geographic area as the DVT patients was studied. The control group was similar in sex and age to the patients. In all, the control group comprised 156 subjects younger than 45 years (32±8 years) who were compared with the 130 selected patients with clinically suspected thrombophilia. The absence of thrombotic events or a family history of thrombosis was verified by means of a validated questionnaire.[24] All the women in the patient and control groups were asked about contraceptive use at the time of the study. The thrombophilic study was also done in the control group subjects. Informed consent to participation in the study was obtained from all the subjects examined.

Blood collection

Blood was collected at least 6 months after the thrombotic event, and at least 15 days after anticoagulant treatment had ended. Upon suspending anticoagulation, treatment with low molecular weight heparin was applied when necessary.

Blood samples were collected into vacuum tubes with 0.129 M trisodium citrate as anticoagulant. Samples were centrifuged at 1,500 g for 15 min to obtain platelet-poor plasma, which was stored at –70º C until tested. For DNA studies, venous blood was collected in EDTA.

Laboratory methods

Antithrombin antigen was measured by radial immunodiffusion (Behringwerke AG, Marburg, Germany). Anti factor Xa activity was measured in the presence of heparin using the Coamatic Antithrombin Kit (Chromogenix AB, Mölndal, Sweden), and the assay was performed on an ACL 7000 autoanalyzer (Instrumentation Laboratory, Milan, Italy).

Protein C activity was analyzed by Coamate PC (Chromogenix, Mölndal, Sweden). Total and free protein S were evaluated by enzyme immunoassay (Asserachrom Diagnostica, Stago, Asniéres, France). Protein S activity was measured with the IL test (Instrumentation Laboratory, Milan, Italy). The modified APC-resistance assay was performed after diluting the patient's plasma with factor V-depleted plasma, as previously reported.[25] DNA was extracted from whole blood samples using the Genomic Purification System (Promega, Madison, USA) following the manufacturer's protocol. The factor V Leiden mutation was studied following the method

described by Gandrille *et al*.[26] The prothrombin gene G20210A variant was detected using a previously described polymerase chain reaction technique.[2]

HCII activity was determined quantitatively by a chromogenic substrate method,[27] using the Stachrom HCII assay provided by Diagnostica Stago (Asniéres, France). HCII antigen was measured by an ELISA (Enzyme Research Lab Ltd., Swansea, UK); a well established protocol was used.[28] Plasminogen activity was analyzed by Coamate Plasminogen (Chromogenix, Mölndal, Sweden).

Statistical analyses

All the assays were performed using the statistical package SPSS Release 6.0 for Windows (SPSS Inc, Chicago, USA). Pearson's χ^2 test was used to compare percentages. Differences between the ages of the groups were compared by the Mann-Whitney test. When the expected frequencies were <5, Fisher's exact test was applied. Values of $p<0.05$ were considered to be statistically significant.

Results

Among the 229 DVT patients the prevalence of thrombophilic defects was 22.3% (n=51), and among the 130 patients with clinically suspected thrombophilia, it was 32.3% (n=42) (Table 1). Seventeen patients (7.5 %) from the total group of DVT patients were carriers of prothrombin G20210A, while 28 patients (12.2 %) had factor V Leiden. Eight patients (3.5 %) had other single defects (4 had protein S deficiency, 2 protein C deficiency, 1 antithrombin deficiency and 1 heparin cofactor II deficiency). Nine patients (3.9%) showed combined defects (3 were carriers of prothrombin G20210A and FV Leiden, 4 FV Leiden and another thrombophilic deficiency, 1 protein C deficiency and prothrombin G20210A, and 1 antithrombin and heparin cofactor II deficiencies). In the group of 246 healthy subjects (control group) the prevalence of the prothrombin G20210A mutation was 2.9% (n=7) and that of factor V Leiden was 1.6% (n=4). The percentage of patients with single or combined thrombophilic defects among the patients with clinically suspected thrombophilia was always higher than among the total DVT patients. The risk of DVT when the prothrombin G20210A mutation was present gave an OR of 2.4 (95% CI, 1.0-6.3) and for factor V Leiden an OR of 6.9 (95% CI 2.3-20.6) among the total DVT patients (Table 1). With the prothrombin mutation the OR was 5.2 (95% CI, 1.4-19.5) and with factor V Leiden it was 14.3 (95% CI, 3.3-64.6) among the patients with clinically suspected thrombophilia (Table 2).

Among the 178 patients with no recognized thrombophilic defect who formed part of the DVT group of 229 patients, 20.2% had two (n=33) or three (n=3) thrombotic events. The percentage of thrombotic recurrence was 19.0% for patients with a single genetic risk factor and 55.5% for patients with combined genetic defects. The time elapsed from the first event to the referral of the patient to our Thrombosis Center was quite similar in patients with a single genetic risk factor (mean=3.8 years) and in patients wih combined defects (mean=3.6 years). The median age at the first DVT and at the time of referral was 35 years and 40 years, respectively, for the patients with a single genetic risk factor and 26 years and 34 years, respectively, for patients with combined defects.

When we compared the age at which DVT appeared in the group of 229 patients, we found (Table 3) that this disorder appeared at an earlier age in women than in men ($p<0.05$). When all the DVT patients were divided into two groups according to whether or not they had thrombophilic defects, similar differences between males and females were found in both groups (Table 3), but these differences were not statistically significant, probably because of the limited number of cases.

When the risk of DVT was evaluated with

Table 1. Prevalence of thrombophilic defects and risk of deep vein thrombosis.

Total DVT patients	*Patients (n=229)*	*Controls (n=246)*	*Odds ratio (95% CI)*	*p value*
No recognized thrombophilic defect	178 (77.7 %)	235 (95.5 %)	1 (ref.)	
With thrombophilic defect	51 (22.3%)	11 (2.4%)	6.1 (3.1-12.1)	< 0.0001
Prothrombin G20210A alone or combined	17 (7.5%)	7 (2.9%)		
alone	13 (5.7%)	7 (2.9%)	2.4 (1.0-6.3)	0.06
FV Leiden alone or combined	28 (12.2%)	4 (1.6%)		
alone	21 (9.2%)	4 (1.6%)	6.9 (2.3-20.6)	< 0.0001
Other single defects	8 (3.5 %)	0 (0%)		< 0.002
Combined defects	9 (3.9%)	0 (0%)		< 0.002
prothrombin G20210A and FV Leiden	3 (1.3%)	0 (0%)		NS
other combined defects	6 (2.6%)	0 (0%)		< 0.01

Table 2. Prevalence of thrombophilic defects and risk of deep vein thrombosis in DVT patients with and without clinically suspected thrombophilia.

DVT patients with clinically suspected thrombophilia

	Patients (n=130)	*Controls (n=156)*	*Odds ratio (95% CI)*	*p value*
No recognized thrombophilic defect	88 (67.7 %)	151 (96.8 %)	1 (ref)	
With thrombophilic defect	42 (32.3 %)	5 (3.2%)	14.4 (5.5-37.8)	< 0.0001
Prothrombin G20210A alone or combined	13 (10.0%)	3 (1.9%)		
alone	9 (6.9%)	3 (1.9%)	5.2 (1.4-19.5)	< 0.005
FV Leiden alone or combined	24 (18.5%)	2 (1.3%)		
alone	17 (13.1%)	2 (1.3%)	14.3 (3.3-64.6)	< 0.0001
Other single defects	7 (5.4 %)	0 (0%)		< 0.002
Combined defects	9 (6.9%)	0 (0%)		< 0.002
prothrombin G20210A and FV Leiden	3 (2.3%)	0 (0%)		0.05
other combined defects	6 (4.6%)	0 (0%)		< 0.01

VT patients without clinically suspected thrombophilia

	Patients (n=99)	*Controls (n=90)*	*Odds ratio (95% CI)*	*p value*
No recognized thrombophilic defect	90 (90.9%)	84 (93.3%)	1 (ref)	
With thrombophilic defect	9 (9.1%)	6 (6.7%)	1.4 (0.48-4.10)	NS
Prothrombin G20210A alone or combined	4 (4.0%)	4 (4.4%)		
alone	4 (4.0%)	4 (4.4%)	0.9 (0.23-3.85)	NS
FV Leiden alone or combined	4 (4.0%)	2 (2.2%)		
alone	4 (4.0%)	2 (2.2%)	1.87 (0.33-10.46)	NS
Other single defects	1 (1.0%)	0 (0%)		NS
Combined defects	0 (0%)	0 (0%)		NS
prothrombin G20210A and FV Leiden	0 (0%)	0 (0%)		NS
other combined defects	0 (0%)	0 (0%)		NS

respect to the use of OC and the presence or absence of the prothrombin G20210A mutation and/or factor V Leiden in 89 healthy women from the control group and 84 women with DVT from the DVT patient group, all of whom were ≤ 45 years of age, it was observed that in the 84 women with DVT, 19 (22.6%) used OC, while among the 89 healthy women, 10 (11.2%) were OC users ($p<0.01$). Therefore, the risk of DVT in women using oral contraceptives had an OR of 3.5 (95% CI, 1.5-8.2) as compared with the risk in non-users. As none of the controls was a carrier of both prothrombin mutation G20210A and factor V Leiden and also a user of OC, the risk of DVT in women who were double carriers and users of OC could not be calculated. However, in the group of 84 patients there were nine women (10.7%) who used OC and had factor V Leiden, as compared with none in the control group ($p<0.005$). These differences were not significant for the prothrombin G20210A.

Discussion

Our data yield a prevalence of the prothrombin G20210A variant of 2.9% in the group of healthy subjects, which is similar to the values reported for the south of Europe by other authors, and could suggest, as do Rosendaal *et al.*,[19] that the prevalence in southern Europe is a little higher than in northern Europe and the USA. In other geographic areas, such as Asia, Africa and South America, the prevalence of the prothrombin variant appears to be lower.[5,19,29]

The discrepancies in the prevalence of the prothrombin G20210A variant in patients with DVT may also be due to the heterogeneity of the

Table 3. Deep venous thrombosis: patients' age (in years) at the time of the first thrombotic episode.

	All patients	*Men*	*Women*	*Mann-Whitney test men vs women*
Total patients				
Mean±SD	44 ±18	46±16	41±19	<0.05
Median	42	46	36	
Range	(15-90)	(16-87)	(15-90)	
Nº patients	(n=229)	(n=128)	(n=101)	
With no thrombophilic defect				
Mean±SD	46 ±18	47±16	44±20	=0.058
Median	45	48	42	
Range	(16-90)	(16-87)	(16-90)	
Nº patients	(n=178)	(n=99)	(n=79)	
With thrombophilic defect				
Mean±SD	38±17	41±16	33±17	NS
Median	34	39	27	
Range	(15-74)	(16-72)	(15-74)	
Nº patients	(n=51)	(n=29)	(n=22)	

groups studied. In consecutive patients with verified DVT, the prevalence varies from 5% to 11%.[2,7,13,18] In our group of 229 DVT patients the prevalence of the prothrombin variant was 7.5%. In our patients with clinically suspected thrombophilia the prevalence was 10.0%. The results given in the literature for patients with clinically suspected thrombophilia vary from 7.9% to 24%.[2,8-10,18,21]

The prevalence of factor V Leiden in the group of healthy subjects was 1.6%, while in the European population this value is about 5%,[30,31] with a range of 0.6% to 13.0%.[31] The prevalence of factor V Leiden in the group of 229 DVT patients was 12.2%, and 18.5% in the group of patients with clinically suspected thrombophilia. These data agree with most of the previous results in the literature. According to a recent review,[31] the prevalence of factor V Leiden carriers among Caucasian patients with DVT oscillates between 11.2% and 37.0%.

There are few reports in the literature on whether the presence of these risk factors increases the probability of recurrent thrombosis.[8,32,33] In our group of 229 patients a similar recurrence rate was observed in subjects with no recognized thrombophilic defect (20.2%) and in the patients with a single genetic risk factor (19.0%). However, in the patients with more than one congenital defect the recurrence rate was 55.5 %. These differences between the group of patients with one thrombophilic defect and that with more than one cannot be attributed to the time elapsed from the first event to the referral to the our Thrombosis Center, for it was very similar in the two groups. Two studies recently published[32,33] yielded results similar to the ones we have obtained, and show that the risk of recurrent DVT is similar among carriers of factor V Leiden or prothrombin G20210A mutation and patients without these mutations, but that carriers of both factor V Leiden and the prothrombin G20210A variant have an increased risk of recurrent DVT.

Our review of the relevant medical literature turned up no reports on whether sex can influence the age at which a first thrombotic event occurs. However, our results show (Table 3) that DVT occurred earlier in women (41±19 years) than in men (46±16 years) (p<0.05) in the DVT group of 229 patients. This difference was also seen in the group of 178 patients without thrombophilic defects (44±20 vs 47±16 years) and in the group of 51 patients with thrombophilic defects (33±17 vs 41±16 years). These differences in the age at which the first thrombotic event occurs may be attributable to acquired risk factors that are found only in women, such as oral contraceptives and pregnancy. In our group, the OC users had an increased risk of thrombosis, and this increase was substantially larger when the women taking OC also had combined genetic thrombophilic defects.

All these data suggest that oral contraceptives can be a circumstantial thrombotic risk factor when combined with another congenital risk factor, especially factor V Leiden. Martinelli *et al.*[34] also showed a high thrombotic risk factor in heterozygous patients with the prothrombotic mutation G20210A and/or factor V Leiden who took OC.

Contributions and Acknowledgments

JA co-ordinated and designed this study and was involved in writing the text. AV and YM were responsible for monitoring the patients. AE was involved in the molecular biological tests and co-wrote the text. RS and CF did the molecular biology tests. PV was responsible for the coagulation tests. FF was responsible for the coagulation tests and co-wrote the text. DC was involved in epidemiological aspects of the study.

Funding

Grant 96/1256 from the Fondo de Investigación Sanitaria (FIS), Spain; and grant PM97-0024 from the Dirección General de Enseñanza Superior, Ministerio de Educación y Cultura, Spain.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received February 25, 2000; accepted October 20, 2000.

Potential implications for clinical practice

- The prothrombin G20210A mutation does not by itself seem to be a strong risk factor for thrombosis. However, in the presence of additional thrombotic risk factors the predicted risk of thrombotic events increases.

References

1. Bertina RM. Factor V Leiden and other coagulation factor mutations affecting thrombotic risk. Clin Chem 1997; 43:1678-83.
2. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variant in the 3´- untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and increase in venous thrombosis. Blood 1996; 88:3698-703.
3. Hillarp A, Zöller B, Svensson JP, Dahlbäck B. The 20210 A allele of the prothrombin gene is a common risk factor among Swedish outpatients with verified deep venous thrombosis. Thromb Haemost 1997; 78:990-2.
4. Cumming AM, Keeney S, Salden A, Bhavnani M, Shwe KH, Hay CR. The prothrombin gene G20210A variant:

prevalence in a U.K. anticoagulant clinic population. Br J Haematol 1997; 98:353-5.
5. Arruda VR, Annichino-Bizzacchi JM, Goncalves MS, Costa FF. Prevalence of the prothrombin gene variant (nt20210A) in venous thrombosis and arterial disease. Thromb Haemost 1997; 78:1430-3.
6. Brown K, Luddington R, Williamson D, Baker P, Baglin T. Risk of venous thromboembolism associated with a G to A transition at position 20210 in the 3´-untranslated region of the prothrombin gene. Br J Haematol 1997; 98:907-9.
7. Corral J, Gonzalez-Conejero R, Lozano ML, Rivera J, Heras I, Vicente V. The venous thrombosis risk factor 20210 A allele of the prothrombin gene is not a major risk factor for arterial thrombotic disease. Br J Haematol 1997; 99:304-7.
8. Ferraresi P, Marchetti G, Legnani C, et al. The heterozygous 20210 G/A prothrombin genotype is associated with early venous thrombosis in inherited thrombophilias and is not increased in frequency in artery disease. Arterioscler Thromb Vasc Biol 1997; 17:2418-22.
9. Kapur RK, Mills LA, Spitzer SG, Hultin MB. A prothrombin gene mutation is significantly associated with venous thrombosis. Arterioscler Thromb Vasc Biol 1997; 17:2875-9.
10. Makris M, Preston FE, Beauchamp NJ et al. Co-inheritance of the 20210A allele of the prothrombin gene increases the risk of thrombosis in subjects with familial thrombophilia. Thromb Haemost 1997; 78:1426-9.
11. Bentolila S, Ripoll L, Drouet L, Crassard I, Tournier-Lasserve E, Piette JC. Lack of association between thrombosis in primary antiphospholipid syndrome and the recently described thrombophilic 3'-untranslated prothrombin gene polymorphism. Thromb Haemost 1997; 78:1415-21.
12. Alhenc-Gelas M, Le Cam-Duchez V, Emmerich J, et al. The A20210 allele of the prothrombin gene is not frequently associated with the factor V Arg 506 to Gln mutation in thrombophilic families. Blood 1997; 90: 1711.
13. Leroyer C, Mercier B, Oger E, et al. Prevalence of 20210 A allele of the prothrombin gene in venous thromboembolism patients. Thromb Haemost 1998; 80:49-51.
14. De Stefano V, Chiusolo P, Paciaroni K, et al. Prothrombin G20210A mutant genotype is a risk factor for cerebrovascular ischemic disease in young patients. Blood 1998; 91:3562-5.
15. Longstreth WT Jr, Rosendaal FR, Siscovick DS, et al. Risk of stroke in young women and two prothrombotic mutations: factor V Leiden and prothrombin gene variant. Stroke 1998; 29:577-80.
16. Margaglione M, Brancaccio V, Giuliani N, et al. Increased risk for venous thrombosis in carriers of the prothrombin G→A20210 gene variant. Ann Intern Med 1998; 129:89-93.
17. Zabalegui N, Montes R, Orbe J, et al. Prevalence of FVR506Q and prothrombin 20210A mutations in the Navarrese population. Thromb Haemost 1998; 80: 522-3.
18. Souto JC, Coll I, Llobet D, et al. The prothrombin 20210A allele is the most prevalent genetic risk factor for venous thromboembolism in the spanish population. Thromb Haemost 1998; 80:366-9.
19. Rosendaal FR, Doggen CJ, Zivelin A, et al. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998; 79:706-8.
20. Ehrenforth S, Ludwig G, Klinke S, Krause M, Scharrer I, Nowak-Gottl U. The prothrombin 20210A allele is frequently coinherited in young carriers of the factor V Arg 506 to Gln mutation with venous thrombophilia. Blood 1998; 91:2209-10.
21. Howard TE, Marusa M, Boisza J, et al. The prothrombin gene 3'-untranslated region mutation is frequently associated with factor V Leiden in thrombophilic patients and shows ethnic-specific variation in allele frequency. Blood 1998; 91:1092.
22. Salomon O, Steinberg DM, Zivelin A, et al. Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism: prevalence and risk assessment. Arterioscler Thromb Vasc Biol 1999; 19: 511-8.
23. Margaglione M, D'Andrea G, Colaizzo D, et al. Coexistence of factor V Leiden and Factor II A20210 mutations and recurrent venous thromboembolism. Thromb Haemost 1999; 82:1583-7.
24. Frezzato M, Tosetto A, Rodeghiero F. Validated questionnaire for the identification of previous personal or familial venous thromboembolism. Am J Epidemiol 1996; 143:1257-65.
25. Jorquera JI, Montoro JM, Fernández MA, Aznar JA, Aznar J. Modified test for activate protein C resistance. Lancet 1994; 344:1162-3.
26. Gandrille S, Alhenc-Gelas M, Aiach M. A rapid screening method for the factor V Arg 506 Gln mutation. Blood Coagul Fibrinol 1995; 6:245-8.
27. Tollefsen DM, Pestka CA, Monafo WJ. Activation of heparin cofactor II by dermatan sulphate. J Biol Chem 1983; 258:6713-6.
28. España F, Griffin JH. Determination of functional and antigen protein C inhibitor and its complexes with activated protein C in plasma by ELISA's. Thromb Res 1989; 55:671-82.
29. Rahimy MC, Krishnamoorthy R, Ahouignan G, Laffan M, Vulliamy T. The 20210A allele of prothrombin is not found among sickle cell disease patients from west Africa. Thromb Haemost 1998; 79:444.
30. Zoller B, García de Frutos P, Hillarp A, Dahlbäck B. Thrombophilia as a multigenic disease. Haematologica 1999; 84:59-70.
31. De Stefano V, Chiusolo P, Paciaroni K, Leone G. Epidemiology of factor V Leiden: clinical implications. Semin Thromb Hemost 1998; 24:367-79.
32. De Stefano V, Martinelli I, Mannucci PM, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 1999; 341:801-6.
33. Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost 1999; 81:684-9.
34. Martinelli I, Taioli E, Bucciarelli P, Akhavan S, Mannucci PM. Interaction between the G20210A mutation of the prothrombin gene and oral contraceptive use in deep vein thrombosis. Arterioscl Thromb Vasc Biol 1999; 19:700-3.