in vivo *26*: 165-172 (2012)

Potential Prevention of Thromboembolism by Genetic Counseling and Testing for Two Common Thrombophilia Mutations

CHRISTOS YAPIJAKIS[1,2], THALIA ANTONIADI[3], KATERINA SALAVOURA[4], COSTAS VOUMVOURAKIS[5] and ELEFTHERIOS VAIRAKTARIS[1]

[1]*Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Attikon Hospital, Athens, Greece;*
[2]*Laboratory of Molecular Genetics, Cephalogenetics Research Center, Athens, Greece;*
[3]*Bristol Genetics Laboratory, Southmead Hospital, Bristol, United Kingdom;*
[4]*First Department of Pediatrics, University of Athens Medical School, Aghia Sofia Childrens' Hospital, Athens, Greece;*
[5]*Second Department of Neurology, University of Athens Medical School, Attikon Hospital, Athens, Greece*

Abstract. *Background/Aim: Thrombophilia is a multifactorial predisposition for thromboembolism affecting about a tenth of any population. We investigated whether genetic counseling combined with molecular testing for two common dominant mutations (coagulation factor V Leiden and prothrombin G20210A) may increase prevention of venous thromboembolic incidents in individuals with a positive family history compared to the general population. Patients and Methods: Mutation detection was carried out by Restriction Fragment Length Polymorphism analysis in DNA samples of 96 unrelated healthy Greeks (group A) who asked for genetic counseling for various reasons and had at least two relatives with thromboembolic incidents and 100 unrelated healthy Greeks (group B). Results: In group A, both mutations were detected at five-fold higher frequencies (33.33% for Leiden and 19.79% for G20210A) compared to group B, which had frequencies typically found in the Greek population (6% and 4%, respectively). Conclusion: In populations with a high prevalence for these two common mutations, genetic counseling and molecular testing of at-risk individuals may significantly increase prevention of thromboembolic disease.*

Correspondence to: Christos Yapijakis, D.M.D., M.S., Ph.D., Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Attikon Hospital, Rimini 1, Athens 12461, Greece. Tel: +30 2103230000, Fax: +30 2106813995, e-mail: cyapijakis_ua_gr@yahoo.com

Key Words: Thromboembolism, thrombosis, coagulation factor V, prothrombin, genetics, genetic counseling.

Thrombophilia (OMIM 188050) is a multifactorial predisposition for thrombosis, caused by inborn hypercoagulation of the blood and affecting 10-15% of individuals in any given population (1-3). Thromboembolic incidents include brain stroke, myocardial infarction, deep vein thrombosis and obstetrical complications, including about 60% of spontaneous abortions (3-10). The disorder may occur on a familial basis as a result of one or more mutant genes encoding any one of a considerable number of clotting, anticoagulant or thrombolytic factors functioning alone, or in association with other genetic and environmental factors (2-9). Genetic causes are present in approximately one third of unselected thrombosis cases and up to two thirds of familial cases (3).

One of the most common inherited defects causing thrombophilia in Europeans is the coagulation factor V Leiden mutation (G1691A) (11-13). The factor V gene encodes a plasma glycoprotein which is activated by thrombin/factor Xa and converted into factor Va. The mutation destroys a cleavage site of the anticoagulant-activated protein C in factor Va. Several studies have reported a high frequency of factor V Leiden mutation in Caucasians (1.5-8.8%), while in other populations, the mutation is rare (3, 11, 14-18). This mutation is responsible for 20-25% of isolated thrombotic events and for 40-45% of cases of familial thrombophilia and fetal loss (1, 4-6, 9, 10, 17, 19-23).

Another common defect, with an allelic frequency of 1.3-4.5% in Caucasian populations, is a guanine to adenine transition in the 3′ untranslated region of the prothrombin gene (G20210A) (3, 16, 24). Also known as coagulation factor II, prothrombin is a plasma glycoprotein which is activated to thrombin by factors Xa and Va. The G20210A mutation is related to elevated plasma prothrombin levels, and increased risk of venous thrombosis (20, 24, 25).

Accumulating evidence suggests that these two genetic alterations display a dominant predisposition effect (1, 3, 16). Heterozygotes with the factor V Leiden mutation have a 5 to 10-fold risk, and heterozygotes with the prothrombin G20210A mutation have a 2 to 4-fold risk of venous thrombotic incidents compared with individuals without these mutations (1, 3, 16). Homozygotes for one mutation or double heterozygotes have a 50 to 100-fold risk compared to normal population (1, 3, 16).

Genetic counseling may play an important role in the prevention of thrombophilia (25, 26). Evaluation of family data may reveal parameters helpful for risk calculation and the ability to routinely detect the inherited gene defects may significantly contribute to early diagnosis. Thromboembolic events are associated with significant risk of morbidity and mortality, therefore, when a person's genetic predisposition is known, preventive anticoagulant therapy may safeguard that individual from life-threatening incidents and improve their health status (3, 27).

Leiden and G20210A mutations are important susceptibility factors for thromboembolic incidents in certain populations, such as in Greeks, in whom high frequencies of 5% and 4.5%, respectively, have been detected (15, 17, 18, 28). Here, we report the molecular testing results for these two common thrombophilia mutations in two cohorts of healthy Greeks: a) index cases with a family history of idiopathic thrombosis, and b) individuals of the general population. Analysis of the presented data illustrates the significant impact of genetic counseling on prevention of thromboembolic incidents.

Patients and Methods

Participants. Blood samples were collected from two different groups of unrelated and apparently healthy individuals of Greek origin (N=196). All studied individuals were fully informed about the potential meaning of test results and willingly participated in the study.

Group A consisted of 96 unrelated healthy individuals (26-55 years old), who were referred for genetic counseling for various reasons (Table I), and their family pedigree analysis revealed that, besides their reason of reference, they had at least one first-degree and one second-degree relative with a thromboembolic incident (in brain, heart, lungs, deep veins *etc*.). Thrombophilia-related details of family history of these individuals are presented in Table I, while three characteristic pedigrees are presented in Figure 1. Regarding the etiology of reference for genetic counseling, there were seven defined categories: a) neurogenetic disorders (N=19), including cases of neurofibromatosis I, Charcot-Marie-Tooth I, Becker muscular dystrophy, Duchenne muscular dystrophy, myotonic dystrophy, and X-linked spastic paraplegia; b) syndromes (N=11), including cases of Alagille, CADASIL (cerebellar autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), cerebro-oculo-facio-skeletal, Cowden, cri-du-chat, de Lange, Dubowitz, frontonasal dysplasia, Opitz-G, Saethre-Chotzen and Waardenburg syndromes; c) familial cancer (N=6); d) endocrinological problems (N=6); e) fertility problems (N=8); f) recurrent spontaneous abortion (N=8); g) familial idiopathic thrombosis (N=38).

Group B consisted of 100 unrelated healthy individuals of comparative sex and age (23-61 years old), who were tested in the frame-work of a routine biochemical, hematological and molecular check-up, without obtaining any information on their family history. There were no exclusion criteria for these individuals.This group served as a control group representing the general Greek population.

Molecular testing. DNA was extracted from blood with the use of NucleoSpin™ kit (Macherey-Nagel GmbH & Co, Dfiren, Germany). Molecular analysis was performed as previously described, with a combination of PCR and endonuclease *Taq* I digestion analysis for thrombophilia-causing mutations Leiden (G1691A) and G20210A in the genes of coagulation factors V and II (prothrombin), respectively (13, 18, 24). In both cases, a *Taq* I recognition site found in normal alleles is lost in PCR products containing a mutant allele. All molecular analyses were blindly performed twice.

Statistical analysis. Carrier frequencies for both mutations found in group A were compared to the respective frequencies of the control group B. Statistical analysis for comparisons between categorical variables was performed using the chi-square test and Fisher's exact test. All statistical differences were two-sided, and the significance level was set at $p<0.05$.

Results

In group A, mutant alleles were detected in 47 out of 96 individuals (48.96%), while they were found in 10 out of 100 individuals of group B (10%). More specifically, factor V Leiden was detected in 32 out of 96 individuals of group A (33.33% carrier frequency), while it was detected in 6 out of 100 individuals of the group B (6% carrier frequency). The prothrombin G20210A mutation was detected in 19 out of 96 individuals in group A (19.79% carrier frequency) and in 4 out of 100 individuals in group B (4% carrier frequency). Both mutations were detected in only four individuals of group A (Table I). A significant difference between the two groups was found for both mutations ($\chi^2=9.36$, $p<0.005$ for Leiden and $\chi^2=6.03$, $p<0.025$ for G20210A).

Further analysis was performed in two subgroups of group A, examining the frequency of both mutations compared to the general population. The first subgroup included 46 individuals whose referral for genetic counseling was possibly related to thrombophilia: 38 with a family history of thromboembolic episodes and 8 with recurrent spontaneous abortions. As one would expect, the prevalence of both mutations in this subgroup was much higher in comparison to those of the control group: 41.3% for factor V Leiden ($\chi^2=30.17$, $p<0.001$) and 32.6% for the prothrombin mutation ($\chi^2=25.09$, $p<0.001$).

In the remaining 50 cases of group A, with a referral reason irrelevant to thrombophilia, only the observed frequency for Leiden mutation was also statistically different

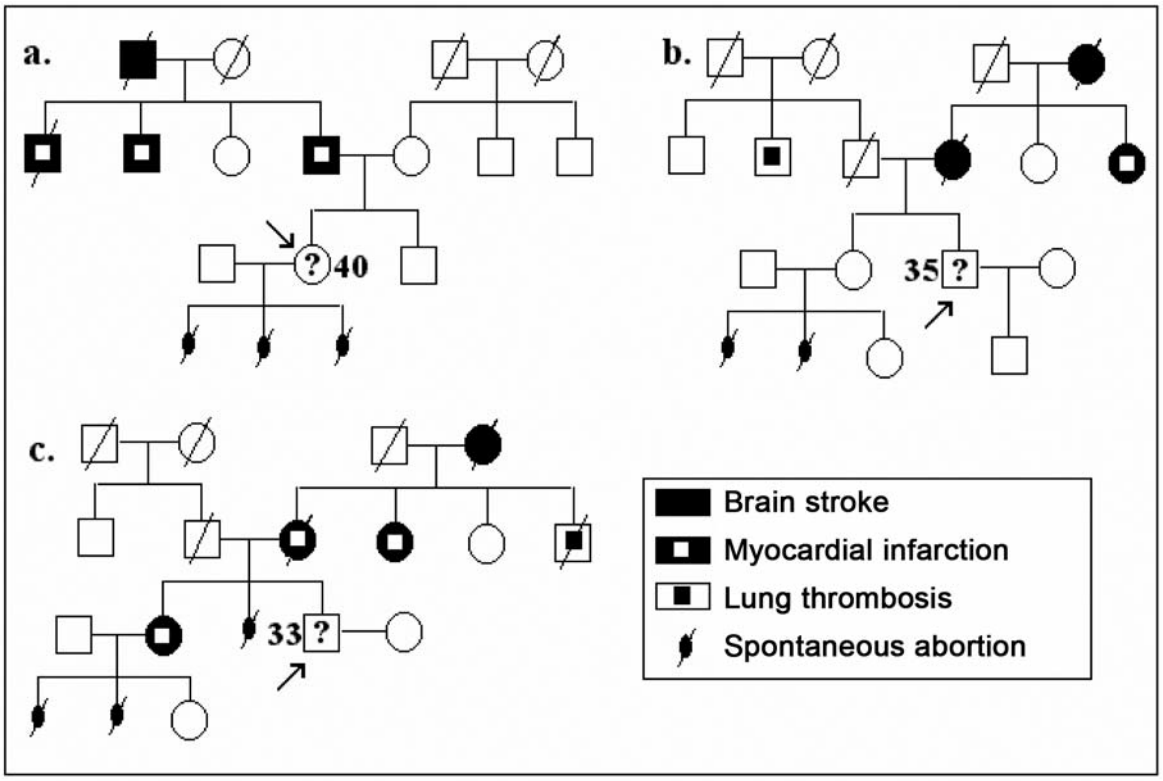

Figure 1. *Characteristic pedigrees of index cases of group A. An arrow marks studied index cases with their age besides them. An unknown predisposition for thrombosis at the time of family history taking is depicted by a question mark. a: Forty-year-old woman who was referred for genetic counseling because of three spontaneous abortions at first trimester (family 51); b: 35-year-old father of a child with a syndrome which was diagnosed as cri-du-chat (family 23); c: 33-year-old man with oligospermia (family 49). The latter two index cases were found to have the factor V Leiden mutation.*

compared to the control group: 26% (χ^2=15.39, p<0.001). On the contrary, in this subgroup the detected frequency for G20210A (8%) was not significantly different from that observed for the control group (0.25<p<0.10).

Discussion

Thrombophilia is a common multifactorial predisposition for thromboembolism, that may cause life-threatening cardiovascular disease, as well as serious complications after surgery (3, 8, 29-31). Several susceptibility genes for thrombophilia are known but very few of them have been shown to be singularly significant for thrombosis, such as the common mutated ones coding for coagulation factor V and prothrombin. It is clear that a combination of genetic variations along with other factors increases the risk for thromboembolic events (1-3, 8, 21). A characteristic and rather common example of such an effect of interaction is homocysteinemia due to homozygosity for 677C→T polymorphism in the methylenetrahydrofolate reductase gene in combination with limited dietary uptake of folic acid (3, 32, 33).

The fact remains that the factor V Leiden and the prothrombin G20210A dominant mutations are strong contributors to thrombosis since their combined frequencies are about 3.5-11% in Caucasian populations (16). This contribution seems to be most important in populations of South and East European decent such as Greeks, Italians, Argentineans and Polish, in which the distribution of the two mutations shows a nearly two-fold higher prevalence in comparison to Northern Europeans (15-18, 34). In such a population, in which the mutant allele frequencies of strong contributors to thrombophilia are high, genetic counseling may be very important for prevention of life-threatening thromboembolic incidents in individuals with an inherited predisposition for thrombosis (25-27). Molecular findings of thrombophilia susceptibility in individuals at risk may offer the possibility of early preventive anticoagulant therapy and prevention of serious thromboembolic complications.

In the present study, we investigated whether genetic counseling combined with molecular testing for two common thrombophilia-causing mutations could potentially contribute to increase prevention of disease in healthy individuals with

Table I. *Analytical presentation of individuals tested for thrombophilia-causing mutations (Group A): family history regarding thromboembolic events and molecular screening results. 1st, 2nd, 3rd, 4th: First, second, third and fourth degree of kinship relation. The numbers in these columns indicate the number of family members, of the respective relative degree, that presented with the observed problem. F: Female, M: male; FV: factor V Leiden mutation, PT: prothrombin G20210A mutation; m: presence of the mutation, –: absence of the mutation; THR-E: thromboembolic event; NF1: neurofibromatosis type 1; MD: muscular dystrophy; BS: brain stroke, MI: myocardial infarction, SA: spontaneous abortion, OTE: other thromboembolic events (deep venous thrombosis, pulmonary embolism); +: indicates death of that person due to the thromboembolic event.*

					Group A	Family history															
						1st				2nd				3rd				4th			
Family	Gender	Age (years)	FV	PT	Reason for referral	BS	MI	SA	OTE	BS	MI	SA	OTE	BS	MI	SA	OTE	BS	MI	SA	OTE
1	F	44	–	–	NF1		1+						1		1+						
2	M	30	–	–	NF1		1			2+					1+						
3	F	55	–	–	NF1	1+	1+				2(1+)				1						
4	M	37	m	–	NF1		1			1		2		1+		3					
5	M	32	–	–	NF1		1+				1				1+						
6	M	40	–	–	NF1		1				1+				1+						
7	F	35	–	–	NF1	1+					1+		1								
8	F	51	m	–	NF1		1			2		1		2				1+			
9	F	31	m	–	Charcot-Marie-Tooth I	1+			1			1		1+	1+		1+	1+	1+		
10	F	37	m	–	Charcot-Marie-Tooth I	1	1				1		1		2+		1+	2+			
11	M	41	–	–	Duchenne MD		1				1+		1+	1+	1						
12	M	34	–	–	Duchenne MD	1					1+			2+				1			
13	M	42	–	–	Becker MD		1				1+			1+	3 (1+)		1+		1		
14	M	32	–	–	Becker MD	1					1+		1	1+	1+	1		1	1+		
15	F	33	–	–	Myotonic dystrophy	1+					1			1	1+		1				
16	F	37	–	–	Myotonic dystrophy		1+			1	1				1+	1					
17	M	39	–	–	Spastic paraplegia		1+				1+			1	1+		1				
18	F	40	–	m	Spastic paraplegia		1		1		1+			1+	2			1+			
19	M	43	–	–	Spastic paraplegia	1+				1		1			1+	1					
20	F	36	–	m	Spastic paraplegia		1		1		1+			1+	1+		1		1+		
21	F	31	m	–	Frontonasal dysplasia	1						1			1+						
22	M	36	–	–	CADASIL	1+				1+				2+			2		2		2
23	M	35	m	–	Cri-du-chat	1+				1+					1	2	1				
24	F	34	m	–	Opitz-G		1	1					1+	2+							
25	F	29	–	m	Cowden				1	1+				1+							
26	F	26	m	–	Saethre-Chotzen		1					1				1+					
27	F	27	–	–	Dubowicz		1+				1+			1+	1+		2	1	1	2	
28	M	35	–	–	Waardenburg I		1+				1+				1			1			
29	M	38	–	–	Alagille	1		1				1	1+		1+						
30	F	32	–	m	De Lange		1			1+					1+			1			
31	F	43	–	–	Hereditary cancer	1		1				1	1				1				
32	M	41	–	–	Hereditary cancer		1		1	1+	1+		1	1	1+	1					
33	M	30	–	–	Hereditary cancer		1				1+			1+							
34	F	37	–	–	Hereditary cancer	1				1+		1		1	1+	2					
35	F	43	–	–	Hereditary cancer	1			1+	2 (1+)			1	2+	1						
36	F	33	–	–	Hereditary cancer		1				1+			1+							
37	M	31	–	–	Hormonal problems		1+			1+				1+	2						
38	F	52	–	–	Hormonal problems		1				1				1+		1				
39	F	26	m	–	Hormonal problems				1	1+					1+						
40	F	52	–	–	Hormonal problems		1				1				1		1				
41	F	29	m	–	Hormonal problems				1	1+				1+							
42	F	32	m	–	Fertility problems		1				1+				1+			1			
43	M	50	–	–	Fertility problems	1			1		1	2				1+					
44	M	37	–	–	Fertility problems		1			1+		1				8	1				
45	M	33	m	–	Fertility problems		1						1+		2						
46	F	30	–	–	Fertility problems				1		1+				1						
47	M	42	–	–	Fertility problems		1			1				1							
48	M	39	–	–	Fertility problems	1				1+		3				3		1+			

Table I. *Continued*

Table I. *Continued*

Family	Gender	Age (years)	FV	PT	Group A Reason for referral	Family history 1st BS	1st MI	1st SA	1st OTE	2nd BS	2nd MI	2nd SA	2nd OTE	3rd BS	3rd MI	3rd SA	3rd OTE	4th BS	4th MI	4th SA	4th OTE
49	M	33	m	–	Fertility problems		1+			1+	1	1			1	2	1+				
50	F	32	–	–	Fertility problems		1					1+					1				
51	F	40	–	–	3 SA		1	3		1+					2 (1+)						
52	F	36	–	–	3 SA	1+		3			1+					1	1				
53	F	34	–	m	2 SA		1	2			1+				1		2				
54	F	31	–	m	2 SA			2	1	1+						1	2				
55	F	40	–	–	2 SA		1	2			1+				3 (1+)						
56	F	31	m	–	2 SA			8	1		1+				1+	4	3				
57	F	31	m	–	2 SA		1	3			1+		1	1+		3		1	1+		
58	F	37	m	–	2 SA	1		4		1					1+	2			1+		1+
59	M	45	–	m	THR-E		1+				1+			1				1			
60	M	30	m	–	THR-E		1					1	1+		1	2					
61	M	40	–	–	THR-E	1				1+											
62	M	52	–	m	THR-E		1+				1+										
63	F	30	m	–	THR-E		1				1+			1							
64	F	55	m	–	THR-E				1+		1+				1+						
65	F	37	–	–	THR-E		2+			1+	1+				1+						
66	F	39	–	–	THR-E				1+	1+				1+	1						
67	M	39	–	m	THR-E		2+			2+	1+						1				
68	M	47	–	m	THR-E		1+				1+										
69	M	55	m	–	THR-E				1+	1+				1+	1+					4	1
70	M	54	–	–	THR-E	1+					1+				1+						
71	M	51	–	–	THR-E				1	2+					2						
72	M	48	m	–	THR-E		1+				2 (1+)				1+				2+		
73	M	45	m	m	THR-E				1				1+		2+				1+		1+
74	M	55	–	–	THR-E		1		1+				1	1+							
75	M	42	m	m	THR-E	1	1+				1+	3	1		1+		1				
76	M	30	m	–	THR-E		1					1+		1	1	1+					
77	M	40	m	–	THR-E		1+			1+					1+						
78	M	52	–	m	THR-E	1+		1			1+	2									
79	F	30	–	–	THR-E		1+				1			1	1						
80	F	55	m	–	THR-E		1+				1+				1+						
81	F	37	–	m	THR-E		1+		1		1+		1+		1+						
82	F	39	–	–	THR-E	1+							1+	1	1+				1+		
83	M	39	–	m	THR-E	1	1+			1+	2 (1+)		1+		1+	1					
84	M	47	–	–	THR-E		1+			1					1+						
85	M	55	m	–	THR-E		1+			1+				1+	1+	3			1+		1
86	M	54	m	–	THR-E		1+				1+			1+					2+		
87	M	51	–	–	THR-E	1				1+	1+			1	1+						
88	M	48	–	–	THR-E		1+			1+	1+	2		1+					1+		1+
89	M	31	m	m	THR-E		1+				1+		1+	1+	1+		1		1+		
90	M	55	–	–	THR-E	1	1+				1+				1+			1+			
91	M	34	m	m	THR-E	1	1+			1	1+	2			1+	2		1+			
92	M	54	m	–	THR-E	1				1		3	1+		1+	6			1+		
93	F	32	–	m	THR-E		2 (1+)				1+				2 (1+)						
94	F	46	–	m	THR-E		2+		1	1+	2		1				1+		2 (1+)		
95	F	35	m	–	THR-E	1					1+		1		1	3					
96	M	42	–	–	THR-E		1			1+											

a family history of idiopathic thrombosis, compared to random check-up of the general population. Molecular testing revealed that one third of the studied at risk individuals of group A had the factor V Leiden mutation and one fifth of them the G20210A mutation. The overall observed frequency of both mutations was 48.96%, corresponding surprisingly well to the expected respective risk of individuals who have a parent with an autosomal

dominant disorder (50%). This overall frequency is five-fold greater than that of group B, which was found to be 10% (6% for factor V Leiden, and 4% for G20210A), corresponding roughly to the combined frequencies of both mutations (9.5-11%) previously observed in the Greek population (15, 17, 18, 23, 28).

All individuals from both groups who were found to be carriers of either mutation were referred to hematologists who advised some of them to receive preventive anticoagulant therapy. They were also advised to inform certain at-risk relatives about testing. Women in particular were counseled to a) avoid taking contraceptive pills without the advice of a hematologist, and b) consult a hematologist in addition to an obstetrician in case of pregnancy.

The optimal management of asymptomatic mutation carriers remains unclear, but it is generally agreed that thromboprophylaxis, at least during risk periods, should be provided (35-46). Asymptomatic close relatives of thrombotic patients who also carry a mutated gene may benefit from prophylactic treatment with anticoagulants, especially when their risk of thrombosis is increased by temporal factors, such as pregnancy or surgery (5, 39, 40, 43, 45, 46).

Prevention of idiopathic thrombosis is imperative, since it is very common and life-threatening. Genetic counseling is very important for people with a family history of thrombophilia (26). Geneticists may play a very significant role in prevention of this complex disorder if, during their routine collection of family history data in a counseling session, they recognize individuals at risk for thrombosis and inform them about preventive measures, including the available molecular tests (47). Obviously, during genetic counseling, it is important to address psychological issues in regard to the impact of predictive testing on the wellbeing of testees and their family, as in other late-onset hereditary diseases (48, 49). A number of studies have indicated that pre-test genetic counselling would be helpful in reducing confusion about thrombophilia facts and anxiety (26, 50, 51).

It seems imperative that both clinicians and patients obtain accurate information concerning appropriate use of genetic testing in order to achieve an acceptable cost/benefit ratio safeguarding the life and health status of individuals at risk. As genetic testing becomes a routine approach, it is expected that it will be extensively used both in hospital and community preventive medicine.

Acknowledgements

The Authors wish to thank everyone who participated in this study by donating blood, as well as referring clinicians Dr. George Antonopoulos and Dr. Helena Skouteli. This article is dedicated to the memory of friend and colleague Dr. Nikos Koufaliotis. This work was supported in part by a Bioerevna Research grant to C.Y.

References

1 Seligsohn U and Lubetsky A: Genetic susceptibility to venous thrombosis. N Engl J Med *344*: 1222-1231, 2001.
2 Kearon C: Epidemiology of venous thromboembolism. Semin Vasc Med *1*: 7-26, 2001.
3 Gathof BS, Picker SM and Rojo J: Epidemiology, etiology and diagnosis of venous thrombosis. Eur J Med Res *9*: 95-103, 2004.
4 Blumenfeld Z and Brenner B: Thrombophilia-associated pregnancy wastage. Fertil Steril *72*: 765-774, 1999.
5 Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z and Lanir N: Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Haemost *82*: 6-9, 1999.
6 Dilley A, Austin H, El-Jamil M Hooper WC, Barnhart E, Evatt BL, Sullivan PS, Ellingsen D, Patterson-Barnett A, Eller D, Randall H and Philipp C: Genetic factors associated with thrombosis in pregnancy in a United States population. Am J Obstet Gynecol *183*: 1271-1277, 2000.
7 Sanson BJ, Lijmer JG, Mac Gillavry MR, Turkstra F, Prins MH, and Büller HR: Comparison of a clinical probability estimate and two clinical models in patients with suspected pulmonary embolism. Thromb Haemost *83*: 199-203, 2000.
8 Masuda J, Nabika T and Notsu Y: Silent stroke: pathogenesis, genetic factors and clinical implications as a risk factor. Curr Opin Neurol *14*: 77-82, 2001.
9 Masuhr F, Mehraein S and Einhaupl K: Cerebral venous and sinus thrombosis. J Neurol *251*: 11-23, 2004.
10 Coppage KH, Hinton AC, Moldenhauer J, Kovilam O, Barton JR and Sibai BM: Maternal and perinatal outcome in women with a history of stroke. Am J Obstet Gynecol *190*: 1331-1334, 2004.
11 Beauchamp NJ, Daly ME, Hampton KK, Cooper PC, Preston FE and Peake IR: High prevalence of a mutation in the factor V gene within the U.K. population: relationship to activated protein C resistance and familial thrombosis. Br J Haematol *88*: 219-222, 1994.
12 Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA and Reitsma PH: Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature *369*: 64-67, 1994.
13 Bertina RM: Factor V Leiden and other coagulation factor mutations affecting thrombotic risk. Clin Chem *43*: 1678-1684, 1997.
14 Rees DC, Cox M and Clegg JB: World distribution of factor V Leiden. Lancet *346*: 1133-1134, 1995.
15 Antoniadi T, Hatzis T, Kroupis C, Economou-Petersen E and Petersen MB: Prevalence of factor V Leiden, prothrombin G20210A, and *MTHFR* C677T mutations in a Greek population of blood donors. Am J Hematol *61*: 265-267, 1999.
16 Hessner MJ, Luhm RA, Pearson SL, Endean DJ, Friedman KD and Montgomery RR: Prevalence of prothrombin G20210A, factor V G1691A (Leiden) and methylenetetrahydrofolate reductase (*MTHFR*) C677T in seven different populations determined by multiplex allele-specific PCR. Thromb Haemost *81*: 733-738, 1999.
17 Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, Zournatzi V, Makris PE, Bontis J and Kotsis A: Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. Hum Reprod *15*: 458-462, 2000.

18 Vairaktaris E, Yapijakis C, Wiltfang J, Ries J, Vylliotis A, Derka S, Vassiliou S and Neukam FW: Are factor V and prothrombin mutations associated with increased risk for oral cancer? Anticancer Res *25*: 2561-2566, 2005.
19 Ridker PM, Hennekens CH, Lindpainter K, Stampfer MJ, Eisenberg PR and 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 *332*: 912-917, 1995.
20 De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, Rossi E and Leone G: The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med *341*: 801-806, 1999.
21 Caprini JA, Glase CJ, Anderson CB and Hathaway K: Laboratory markers in the diagnosis of venous thromboembolism. Circulation *109*: 4-8, 2004.
22 Hatzis T, Cardamakis E, Drivalas E, Makatsoris K, Bevan D, Pantos C, Malliopoulou V, Tsagaris N, Kreatsa O, Antoniadi T, Petersen MB, Karageorgiou H and Mantouvalos H: Increased resistance to activated protein C and factor V Leiden in recurrent abortions. Review of other hypercoagulability factors. Eur J Contracept Reprod Health Care *4*: 135-144, 1999.
23 Ioannou HV, Mitsis M, Eleftheriou A, Matsagas M, Nousias V, Rigopoulos C, Vartholomatos G and Kappas AM: The prevalence of factor V Leiden as a risk factor for venous thromboembolism in the population of North-Western Greece. Int Angiol *19*: 314-318, 2000.
24 Poort SR, Rosendaal FR, Reitsma PH and 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 *88*: 3698-3703, 1996.
25 Turchetti D and Romeo G: Problems related to counseling in genetic thrombophilias. Pathophysiol Haemost Thromb 32: 254-257, 2002.
26 Reich LM, Bower M and Keys NS: Role of the geneticist in testing and counseling for inherited thrombophilia. Genet Med *5*: 133-143, 2003.
27 Cosmi B and Palareti G: Extended treatment for venous thromboembolism: How long is long enough? Curr Hematol Rep *3*: 375-381, 2004.
28 Gialeraki A, Politou M, Rallidis L, Merkouri E, Markatos C, Kremastinos D and Travlou A: Prevalence of prothrombotic polymorphisms in Greece. Genet Test *12*: 541-547, 2008.
29 Ganotakis E, Vrentzos GE, Gazi IF, Papadakis JA, Jagroop IA, Paraskevas KI, Nair DR and Mikhailidis DP: Fibrinogen, lipoprotein (a), albumin and bilirubin (F-L-A-B) levels and cardiovascular risk calculated using the Framingham equation. In Vivo *21*: 685-694, 2007.
30 Gluba A, Banach M, Mikhailidis D and Rysz J: Genetic determinants of cardiovascular disease: The rennin/angiotensin/aldosterone system, paraoxonases, endothelin-1, nitric oxide synthase and adrenergic receptors. In Vivo *23*: 797-812, 2009.
31 Handschel J, Willamowski C, Smeets R, Ommerborn MA, Naujoks C, Kübler NR and Depprich R: Complications after oral surgery in patients with congenital or drug-induced bleeding disorders. In Vivo *25*: 283-286, 2011.
32 Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J and Rozen R: Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation *93*: 7-9, 1996.
33 Aroni K, Ioannidis E, Voudouris S and Yapijakis C: Homocysteinemia-associated anetoderma in a young woman with anorexia nervosa history. Int J Dermatol *50*: 343-345, 2011.
34 Herrmann FH, Koesling M, Schroder W, Altman R, Jimenez Bonilla R, Lopaciuk S, Perez-Requejo JL and Singh JR: Prevalence of factor V Leiden mutation in various populations. Genet Epidemiol *14*: 403-411, 1997.
35 Middeldorp S, Meinardi JR, Koopman MM, van Pampus EC, Hamulyák K, van Der Meer J, Prins MH and Büller HR: A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. Ann Intern Med *135*: 322-327, 2001.
36 Simioni P, Tormene D, Prandoni P, Zerbinati P, Gavasso S, Cefalo P and Girolami A: Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Blood *99*: 1938-1942, 2002.
37 Langlois NJ and Wells PS: Risk of venous thromboembolism in relatives of symptomatic probands with thrombophilia: a systematic review. Thromb Haemost *90*: 17-26, 2003.
38 Coppens M, van de Poel MH, Bank I, Hamulyak K, van der Meer J, Veeger NJ, Prins MH, Buller HR and Middeldorp S: A prospective cohort study on the absolute incidence of venous thromboembolism and arterial cardiovascular disease in asymptomatic carriers of the prothrombin 20210A mutation. Blood *108*: 2604-2607, 2006.
39 Horne MK 3rd and McCloskey DJ: Factor V Leiden as a common genetic risk factor for venous thromboembolism. J Nurs Scholarsh *38*: 19-25, 2006.
40 Taniguchi S, Fukuda I, Daitoku K, Minakawa M, Odagiri S, Suzuki Y, Fukui K, Asano K and Ohkuma H: Prevalence of venous thromboembolism in neurosurgical patients. Heart Vessels *24*: 425-428, 2009.
41 King A and Markus HS: Doppler embolic signals in cerebrovascular disease and prediction of stroke risk: a systematic review and meta-analysis. Stroke 40: 3711-3717, 2009.
42 Zubkov AY and Wijdicks EF: Deep venous thrombosis prophylaxis in cerebral hemorrhage. Rev Neurol Dis *6*: 21-25, 2009.
43 de Maistre E, Terriat B, Lesne-Padieu AS, Abello N, Bouchot O and Steinmetz EF: High incidence of venous thrombosis after surgery for abdominal aortic aneurysm. J Vasc Surg *49*: 596-601, 2009.
44 Kakkos SK, Sabetai M, Tegos T, Stevens J, Thomas D, Griffin M, Geroulakos G and Nicolaides AN: Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) Study Group: Silent embolic infarcts on computed tomography brain scans and risk of ipsilateral hemispheric events in patients with asymptomatic internal carotid artery stenosis. J Vasc Surg *49*: 902-909, 2009.
45 Selby R, Geerts W, Ofosu FA, Craven S, Dewar L, Phillips A and Szalai JP: Hypercoagulability after trauma: hemostatic changes and relationship to venous thromboembolism. Thromb Res *124*: 281-287, 2009.
46 Said JM, Higgins JR, Moses EK, Walker SP, Borg AJ, Monagle PT and Brennecke SP: Inherited thrombophilia polymorphisms and pregnancy outcomes in nulliparous women. Obstet Gynecol *115*: 5-13, 2010.
47 Donnai D and Elles R: Integrated regional genetic services: current and future provision. Br Med J *322*: 1048-1052, 2001.

48 Evers-Kiebooms G, Welkenhuysen M, Claes E, Decruyenaere M and Denayer L: The psychological complexity of predictive testing for late-onset neurogenetic diseases and hereditary cancers. Soc Sci Med *51*: 831-841, 2000.

49 Evers-Kiebooms G, Nys K, Harper P, Zoeteweij M, Durr A, Jacopini G, Yapijakis C and Simpson S: Predictive DNA-testing for Huntington's disease and reproductive decision making: a European collaborative study. Eur J Hum Genet *10*: 167-176, 2002.

50 Saukko PM, Richards SH, Shepherd MH and Campbell JL: Are genetic tests exceptional? Lessons from a qualitative study on thrombophilia. Soc Sci Med *63*: 1947-1959, 2006.

51 Federici C, Gianetti J and Andreassi MG: Genomic medicine and thrombotic risk: Who, when, how and why? Int J Cardiol *106*: 3-9, 2006.

Received July 18, 2011
Revised October 3, 2011
Accepted October 5, 2011