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Prevalence of Molecular Risk Factors FV Leiden, FV HR2, FII 20210G>A and MTHFR 677C>T in Different Populations and Ethnic Groups of Germany, Costa Rica and India

F.H.Herrmann[1], Lisbeth Salazar-Sanchez[2], Winnie Schröder[1], Rita Grimm[1], Gudrun Schuster[1], G. Jimenez-Arce[2], M. Chavez[2] and J.R. Singh[3]

1 Institute of Human Genetics, Ernst –Moritz-Arndt-University, Greifswald,Germany
2 Unidad de Hemostasia y Thrombosis CIHATA, Universidad de Costa Rica, San José, Costa Rica
3 Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar, India

KEY WORDS FVL; FVHR2; MTHFR; FII20210G>A; epidemiology.

ABSTRACT The prevalences of the molecular risk factors FVLeiden, FVHis1299Arg (R2), FII 20210G>A and MTHFR 677C>T were studied in blood donors from NE Germany, India (Punjab), San José (Costa Rica), and from two tribes (Chorotegas, Bribri) of Indians and Blacks from Costa Rica. The prevalences of FVL heterozygotes in blood donors from Germany, Costa Rica and India are 6.5, 2 and 1.2% resp. Heterozygosity of R2 allele of FVHR2 was found in 15.5 % in Germany, 13.3% in India. None of the Indians and Blacks of Costa Rica carried FVL, but heterozygotes R1R2 were extremely frequent found in both Indian tribes (44,7% and 50,6%,resp.); homozygosity for R2R2 was 11%. In Blacks the rare R3 polymorphism was found. The FII 20210G>A polymorphism is missing in the Chorotegas Indians and Blacks of Costa Rica and in the population from India. Concerning MTHFR the prevalence of the homozygous mutant genotype is 7.7% in Germany, 5.3% among the Blacks of Costa Rica and 2.7% in India. In the Indian tribes of Costa Rica the prevalence of homozygotes are extremely high: 31.6 % in Chorotegas and 46.7 % in Bribri Indians. The prevalence of genetic risk factors in various populations and ethnic groups is discussed.

INTRODUCTION

Several genetic variants are currently identified as risk factors for venous and arterial thrombosis (deep venous thrombosis, myocard infarction and stroke). Activated protein C resistance due to the factor V Leiden mutation (FVL) and the 20210 G>A mutation in the factor II (FII, Prothrombin) gene are well established causes of thrombophilia. Concerning the risk of myocardial infarction and stroke the results are different. The 677C>T mutation in the methylenetetrahydrofolate reductase gene (677C>T MTHFR), which causes a mild hyperhomocysteinemia is considered to be a risk factor for coronary heart disease (Kluijtmans et al. 1996; Morita et al. 1997), venous thrombosis and stroke (Kluijtmans et al. 1998; Margaglione et al. 1998), but the results are controversial.

Recently new polymorphic markers of FV gene were described (Bernardi et al.1997; Lunghi et al.1996; Castoldi et al. 1997). A specific factor V gene haplotype (HR2) was defined by five restriction polymorphisms in exon 13 and a sequence variation located in exon 16. The exon 13 markers include the Rsa I polymorphic site, the rare allele of which (R2) has been previously found to be associated with partial FV deficiency in the Italian population (Lunghi et al. 1996). The nucleotide change 4070 G>A underlying the R2 allele gives rise to an amino acid change His to Arg at position 1299. Bernardi et al. (1997) demonstrated, that the FV gene marked by the HR2 haplotype, which was invariantly found to underlie the R2 marker, is both able to contribute by itself to determine a mild APC resistance phenotype and to interact synergically with the FV Leiden mutation Arg506Gln to produce a severe APC resistance phenotype. Carriers of the R2 allele are more frequent among patients of carotid endarterectomy (Marchetti et al. 1999) and the carriership of the R2 allele is associated with an increased risk for coronary artery disease (Hoekema et al. 1999) and venous thromboembolism (Faioni et al. 1999)

In order to estimate the role of these factors mentioned above as risk factors in a certain population it is necessary to know their prevalences

Correspondence to: Prof. Dr. Falko H. Herrmann, Institut für Humangenetik, Ernst- Moritz-Arndt Universität, Fleischmannstr.42-44, D-17487 Greifswald, *Tel:*+49-3834-86 5371, *Fax:* +49-3834-865393,
e-mail: herrmafh@mail.uni-greifswald.de

as far as they can vary widely in different populations world wide (Herrmann et al. 1997; Rosendaal et al. 1998; Sacchi et al. 1997; Kluijtmans et al. 1998; Song et al. 1999). Only few studies are known about their frequencies in different ethnic groups.

In this study we determined the prevalences of FV Leiden, FV His1299 Arg (R2), FII 20210G>A, as well as MTHFR 677C>T in different populations and ethnic groups of Costa Rica (two Indian tribes, Blacks and Costarician blood donors) and in blood donors from Germany and from India, region Punjab.

SUBJECTS AND METHODS

Blood samples were obtained from blood donors from Germany (n:170), North Eastern Germany; and from India (n: 150), Region Punjab (Amritsar). In Costa Rica blood samples were collected from 195 Blood donors (from San Jose) as well as from different ethnic groups: two tribes of Indians: Chorotegas (n: 75) from Western Costa Rica and Bribri (n: 77) from South-Eastern Costa Rica and from Blacks from the area of Limon (n: 95).

DNA Extraction

Genomic DNA was extracted from blood samples by standard methods (Miller et al. 1988). For some analyses blood samples soaked onto filter paper cards from the probands were used for PCR as described previously (Schröder et al.1996; Herrmann et al. 1997).

Determination of Mutations and Polymorphisms

For the DNA analysis of the variants of FV, FII and MTHFR DNA or blood samples soaked onto filter paper cards from the probands were used for PCR. The analysis of the three molecular markers has been performed by standard methods by PCR and restriction analysis as described elsewhere (Bertina et al. 1994; Poort et al. 1996; Frosst et al. 1995).

The FV HR2 haplotype was analysed by PCR amplification of the exon 13 RsaI polymorphic site and digestion with RsaI, as described by Bernardi et al. 1997b. Primers were directed against nt 3579-3600 and nt 4280-4261 of the factor V gene sequence (Lunghi et al. 1996).

Statistic Analysis

Allele frequencies were calculated by counting genes from the observed genotypes. The genotypes among different ethnic groups were determined and allele frequencies and genotypes were compared by the chi-square test.

RESULTS

The prevalence of various mutations and polymorphisms of cardiovascular risk factors was studied in blood donors from NE Germany, from India (Punjab) and from blood donors from San Jose, Costa Rica, as well as from two tribes (Chorotegas and Bribri) of Indians and Blacks from Costa Rica.The prevalences of the genotypes and the allele frequencies are summarized in table1.

FV-Leiden

In NE Germans the prevalence of FVLeiden heterozygotes is 6.5 %, the allele frequency 0.03. Only four out of 195 Costaricanian blood donors carried the FV Leiden mutation in heterozygous form, which gives a prevalence of 2 % (allele frequency of 0.01). In the population from India, region Punjab, the prevalence of heterozygotes is 1.3%, the allele frequency 0.007.

In comparison to the study group of NE-Germany the FV Leiden mutation is very rare in Costa Rica. It is significantly lower in Costa Rica blood donors than in German controls (χ^2 4.401, P = 0.0359 for allele frequency, χ^2 4.489, P = 0.0341 for heterozygous genotype) and much more lower in the population from Punjab (χ^2 5.27, P = 0.0216 for allele frequency, χ^2 5.379, P = 0.020 for heterozygous genotype). None of the 152 Indians and 95 Blacks studied in Costa Rica carried this mutation!

FV-His1299 Arg (HR2)

The R2 allele was found in 27 heterozygous subjects among 170 Germans (15.9%, allele frequency 0.079). In the studied population from India 20 heterozygotes were identified among 150 subjects (13.3%, allele frequency 0.067). In Costa Rica heterozygous carriers for the R2 allele are extremely frequent in both Indian tribes

Table 1: Prevalence of mutations/polymorphisms of FV Leiden, FV HR2, FII 20210 and MTHFR in populations and ethnic groups of Germany, Costa Rica and India

			Costa Rica								*India*	
	NE-Germany Blood donors		*Blood donors*		*Indians Chorotega*		*Indians Bribri*		*Blacks (Limon)*		*(Punjab) Blood donors*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
FV-Leiden												
Genotypes	170		195		75		77		95		150	
1691 G/G	159	93,5	191	97.9	75	100	77	100	95	100	148	98.7
1691 G/A	11	6,5	4	2.1	-	-	-	-	-	-	2	1.3
1691 A/A	-	-	-	-	-	-	-	-	-	-	-	-
Allele frequencies												
1691 G	0.967		0.990		1		1		1		0.993	
1691 A	0.033		0.010		0		0		0		0.007	
FV-HR 2												
His 1299 Arg												
Genotypes	170		188		76		77		95		150	
R1R1	143	84.1	143	76.0	34	44.7	29	37.7	88	92.6	130	86.7
R1R2	27	15.9	43	22.9	34	44.7	39	50.6	4	4.2	20	13.3
R2R2	-	-	2	1.1	8	10.6	9	11.7	-	-	-	-
R1R3	-	-	-	-	-	-	-	-	3	3.2	-	-
Allele frequencies												
R1	0.921		0.875		0.671		0.630		0.966		0.933	
R2	0.079		0.125		0.329		0.370		0.021		0.067	
R3	-		-		-		-		0.016		-	
FII-Prothrombin												
Genotypes	170		192		76		77		95		148	
20210 G/G	168	98.8	189	98.4	76	100	76	98.7	95	100	148	100
20210 G/A	2	1.2	3	1.6	-	-	1	1.3	-	-	-	-
20210 A/A	-	-	-	-	-	-	-	-	-	-		
Allele frequencies												
20210G	0.994		0.992		1		0.994		1		1	
20210A	0.006		0.008		0		0.006		0		0	
MTHFR												
Genotypes	170		194		76		77		95		150	
677 C/C	84	49.4	85	43.8	9	11.8	5	6.6	69	72.6	104	69.3
677 C/T	73	42.9	64	33.0	43	56.6	36	46.7	21	22.1	42	28.0
677 T/T	13	7.7	45	23.2	24	31.6	36	46.7	5	5.3	4	2.7
Allele frequencies												
677 C	0.709		0.603		0.401		0.299		0.837		0.834	
677 T	0.291		0.397		0.599		0.701		0.163		0.166	

(Chorotegas 44.7% and Bribri 50.6%). Additionally 17 homozygotes (R2R2) were detected in 153 Indians (prevalence 11%). The R2 allele frequencies in both tribes are 0.329 and 0.370 respectively; and significantly higher than in Germans (χ^2 49.446, P< 0.0001; χ^2 63.347, P< 0.0001, resp.). The R2 frequency in Blacks from Limon/ Costa Rica is significantly lower than in German controls (χ^2 7.524, P= 0.0061). In Blacks the very rare R3 allele could be detected heterozygously in 3 subject (R3 allele frequency 0.016). This R3 polymorphism His1254Arg mimics the R2 polymorphism His1299Arg in subjects of African origin (Lunghi et al.1998). In the Costarician blood donors from San Jose and in the Indians the R3 polymorphism is absent. The recent Costaricians represents an ancestral gene combination of 10-15 % African, 30% Amerindian and 50-60% Causasian origin. The prevalence of HR2 haplotype of the studied Costaricians is higher than in German controls (R2 allele frequency 0.125 versus 0.079, χ^2 3.999, P=0.045), but significant lower than in the Indians of Costa Rica. 2 homozygotes (R2R2) among 188 blood donors were detected (1,1%).

FII 20210 G>A

The prothrombin mutation 20210G>A was

found in 2 subjects among 170 Germans (prevalence of heterozygotes 1.2%) . In the population from Amritsar (Punjab, India) this mutation is missing. In Costa Rica this mutation is also absent in 76 Chorotegas Indians as well as in 95 Blacks. Only one heterozygote has been found among 76 Bribri Indians. Among the blood donors from Costa Rica we found three heterozygotes out of 192 subjects (prevalence 1.6 %).

MTHFR Mutation

The prevalence of the 677C>T MTHFR mutation in NE-Germany, India and various ethnic groups of Costa Rica is very different. Concerning the homozygous TT genotype the prevalence in NE Germany is 7.7%, 23.2% in the group of blood donors from Costa Rica and 5.3% among the Blacks, and 2,7% among the population from Punjab (India). In the two Indian tribes of Costa Rica the prevalence of homozygotes is extremely high ↑ : 31.6 % in Chorotegas and 46.7 % in Bribri Indians. The frequency of the mutant allele differs only slightly between both Indian tribes (0.599 in Chorotega Indians and 0.701 in the Bribri Indians). This allele frequency is significantly different from the NE-German population (χ^2 41.817, P< 0.0001; χ^2 73.084, P<0.0001, resp.) and from the Costa Rica control group (χ^2 17.907, P< 0.0001; χ^2 40.828, P<0.0001, resp.). In Blacks from Limon/Costa Rica the lowest frequency was detected (0.163 versus 0.397 in blood donors of Costa Rica, χ^2 31.967, P<0.0001).

DISCUSSION

Cardiovascular diseases and venous thrombosis are multifactorial diseases. Risk factors results from genetics, environment and behavior. The concept of the multicausal disease has received much attention in the last years. One of the reasons is that some of the genetic risk factors concern single point mutations, that are quite common in the general populations. The search for molecular risk factors remains intensive. There are known molecular factors, which increase the relatively risk for the disease and others, who have protective effects. The genetic background is given by the combination of all these molecular markers. The prevalences of these markers can vary widely in different populations world wide , as we have summarized for FVLeiden recently (Herrmann et al.1997). In order to estimate the role of these factors as risk factors in a certain population it is necessary to know their prevalences.

In Caucasians the FV Leiden mutation and the 20210A mutation of prothrombin are known as risk factors for venous thrombosis (thrombophilia) and discussed as risk factors for atherial thrombosis. The 677C>T mutation of MTHFR causes a mild hyperhomocysteinemia and seems to be a risk factor for coronary heart disease (Kluijtmans et al. 1996; Morita et al. 1997), venous thrombosis and stroke (Kluijtmans et al. 1998; Margaglione et al. 1998). Recently it was shown, that in Caucasiens the R2 allele of FVHR2 is associated with an increased risk for coronary artery disease (Hoekema et al. 1999) and venous thromboembolism (Faioni et al. 1999).

The results of our study have shown, that clear differences exist in the genetic background of these molecular risk factors in various populations/ subpopulations of Germany, India and Costa Rica. The role of FV Leiden mutation and the G20210A mutation of prothrombin as risk factors in Europeans are well known, and the prevalence of these factors is the basis for genetic diagnosis of thrombophilia. The recently described R2 allele (1299Arg) of the His1299Arg polymorphism of the FV gene haplotype HR2 is associated with lower APC resistance (Bernardi et al. 1997; Hoekema et al. 1999) and interacts with the FV Leiden mutation to produce a severe APC resistance phenotype (Hoekema et al. 1999; Faioni et al. 1999; de Visser et al.1999; Schröder et al. 2001).The R2 allele seems to be associated with venous and arterial thrombosis (Faioni et al. 1999; Mingozzi et al. 1999; Marchetti et al. 1999; Hoekema et al. 1999). In our study we have shown that the prevalence of heterozygotes is 15.9 % in Germans and double heterozygote carriers (FVL and FVHR2) comprise 1.76% of all subjects investigated, 11.11% of all R2 carriers or 27.27% of all FVL carriers. The genetic background for thrombosis is influenced by the pattern of these three molecular factors.

FV Leiden is absent in both Indian tribes

(Chorotegas and Bibri) and Blacks of Costa Rica. In contrast to the absence of this polymorphism the R2 haplotype of FVHR2 is very frequent: In both Indian tribes of Costa Rica about 50% of the individuals are heterozygous for this allele (Chorotegas 44.7% and Bribri 50.6%) and 17 homozygotes for the R2 allele were determined (11%). Hoekema et al. (1999) have shown in an APC resistance test, quantifing the ability of FV to act as cofactor in APC-catalyzed FVIII(a) inactivation, that the APC sensitivity ratios for homozygous carriers are significantly lower than in controls. These data indicate that R2-FV has reduced ability to act as a cofactor in the APC catalyzed FVIII (a) inactivation. Because FVL is absent in Indian and Black populations in Costa Rica no compound-heterozygotes could be detected. Whether the observed homozygosity of the R2 allele in Indians of Costa Rica is associated with a higher risk has to be estimated in further clinical studies.

In the studied population from India the prevalence of FVL is very low (heterozygosity 1.3%, allele frequency 0.007), but the prevalence of heterozygous R2 (genotype R1R2) is in the range of to the German population. The absence or reduced prevalence of FVL is not combined with a higher prevalence of FV-HR2 polymorphism.

Concerning the prothrombin mutation the prevalence in Europe is about 2% (Rosendaal et al. 1998). In Germans we have determined a prevalence of 1.2% for heterozygotes. The mutant allele as risk factor for thrombophilia is well known and the determination of this mutations belongs to the diagnostic pattern is of genetic risk factors in familiar thrombosis. This mutation is absent in the population of Amritsar (India), in Chorotegas Indians and Blacks and extremely rare in the Bribri Indians (1 heterozygot among 77 subjects). FVL and the G20210A mutation of prothrombin don't play an important role as genetic risk factors in this population.

In 3 of 95 Blacks of Costa Rica the very rare R3 allele was determined. This polymorphism was firstly described in subjects of Somali (6 out of 40 persons) and in one out of 146 Greek cypriots. This new R 3 polymorphism mimics the R2 polymorphism in subjects of African origin (Lunghi et al. 1998).

The 677C>T MTHFR polymorphism causes a mild hyperhomocysteinemia. Homozygosity of this mutation is in discussion as risk factor for coronary heart disease (Kluijtmans et al. 1996; Morita et al. 1997), venous thrombosis and stroke (Kluijtmans et al. 1998; Margaglione et al. 1998).The prevalence of homozygotes in the studied different populations of blood donors from NE-Germany, India and Blacks from Costa Rica is in the range from 2.7-7.7%. This mutation is very frequent in Indians of Costa Rica. The reported mutant allele frequency of 0.701 of the 677C>T polymorphism of the MTHFR gene in the Bribri Indians of Costa Rica and the prevalence of 46.7% for homozygous mutant subjects is the highest reported in the literature. In Yupka Indians from Western Venezuela we determined a allele frequency of 0.45 (homozygosity 15%) (unpublished). In five Brazilian Amazonian tribes of Indians Franco et al. (1998) found an allele frequency of 0.240 (homozygosity 7.8%). Arruda et al.(1998) described in an other tribe of the Amazonian Indians (Tupy) the frequency of 0.114 (homozygosity 1.2%). It seems that intertribal heterogeneity exists, which might be caused by isolation of small subpopulations and a high degree of consanguinity, as well as by genetic drift (Zago et al. 1996). The extremely high prevalence of homozygotes for the mutant allele in Costa Rica (Indians and blood donors) is remarkable, particularly under the point of view that homozygosity is discussed as a risk factor for cardiovascular diseases caused by mild homocysteinemia. Clinical studies are necessary to clear up the influence of this genotype on the prevalence of cardiovascular diseases in various ethnic groups and in relation to their different life style.

The prevalence data of the studied mutations/ polymorphisms of the blood donors from San Jose are different from Indians and Blacks of Costa Rica. These results can be explained by the fact, that the recent Costaricians represent an ancestral gene combination of 10-15% African, 30% Amerindian and 50.60% Caucasien (mostly from Spain) origin (Barrantes 1998).

The ethnic differences in the pattern of genetic risk factors of various populations or ethnic group indicate that the prevalence of these factors is the precondition of the characterization

of the role of such factors in an given population. These results indicate that we have to analyse not only one molecular risk factor for determination of the risk/predisposition for a given disease, but we have to study a panel of these molekular risk factors as genetic background for trying to answer the questions for genetical predisposition. The general risk is than the result of the interaction of this genetic background with the common environmental risk factors (e.g. surgery, use of contraceptiva, pregnancy etc. for thrombosis and dietary habit, mild hyper-homocysteinemia, age, sex, smoking etc. for cardiovascular diseases). Additional clinical studies are necessary to study the influence of the different genetic background in relation to the environmental factors for the prevalence of cardio- and cerebrovascular diseases in the given population. It is known that the dietary habits modify the relationship between homocysteine level and MTHFR mutation as well as triglyceride levels and FVII polymorphism for example. Under these circumstances the study of the life style of the subpopulations and ethnic groups should be very useful for understanding the differences in the epidemiology of diseases between these populations.

REFERENCES

Arruda VR, Siqueira LH, Goncalves MS, von Zuben PM, Soares MC, Menezes R, Annichino-Bizzacchi JM, Costa FF 1998. Prevalence of the mutation C677àT in the methylenetetrahydrofolate reductase gene among distinct ethnic groups in Brazil. *Am J Med Genet,* **78**: 332-335.

Barrantes R 1998. Desarrollo y perspectivas de la genética humana en Costa Rica. *Acta Pediátrica Costarricense,* **12**: 87-94.

Bernardi F, Faioni EM, Castoldi E, Lunghi B, Castaman G, Sacchi E, Mannucci PM 1997. A factor V gene component differing from factor V R506Q contributes to the activated protein C resistance phenotype. *Blood,* **90**: 1552-1557.

Bertina RM, Koeleman BC, Koster T, Rosendaal RF, Dirven RJ, Ronde de H, Velden van der PA, Reitsma PH 1994. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature,* **369**: 64-7.

Castoldi E, Lunghi B, Mingozzi F, Ioannou I, Marchetti G. Bernardi F 1997. New coagulation factor V gene polymorphisms define a single and infrequent haplotype underlying the factor V Leiden mutation in mediterranean populations and indians. *Thromb Haemostas,* **78**:1037-1041.

de Visser MCH, Guasch JF, Kamphuisen PW, Vos HL, Rosendaal FR, Bertina RM 1999. The HR2 haplotype of factor V: effects on factor V levels, normalized APR sensitivity ratios and the risk of venous thrombosis. *Thromb Haemost,* **82 (Suppl)**: 203-4.

Faioni EM, Franchi F, Bucciarelli P, Margaglione M, de Stefano V, Castaman G, Finazzi G, Casorelli I, Mannucci PM 1999. The HR2 haplotype in the factor V gene confers an increased risk of venous thromboembolism to carriers of factor V R506Q. *Thromb Haemost,* **82**: 418.

Faioni EM, Franchi F, Bucciarelli P, Margaglione M, de Stefano V, Castaman G, Finazzi G, Casorelli I, Mannucci PM 1999. Conheritance of the HR2 haplotype in the factor V ene confers an increased risk of venous thromboembolism to carriers of factor V R506Q (factor V Leiden) *Blood,* **94**: 3062-6.

Franco RF, Araujo AG, Guerreiro JF, Elion J, Zago MA 1998. Analysis of the 677 CàT mutation of the methylenetetrahydrofolate reductase gene in different ethnic groups. *Thromb Haemost,* **79**: 119-121.

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijmans LAJ, van den Heuvel LP, Rozen R 1995. A candidate genetic risk factor for vascular diseases: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet,* **10**: 111-3.

Herrmann FH, Koesling M, Schröder W, Altman R, Jimenez Bonilla R, Lopaciuk S, Perez-Requejo JL, Singh JR 1997. Prevalence of factor V Leiden mutation in various populations. *Genet Epidemiol,* **14**: 403-411.

Hoekema L, Castoldi E, Tans G, Manzato F, Bernardi F, Rosing J 1999. Characterization of blood coagulation factor V (a) encoded by the R2-gene. *Thromb Haemost,* **82 (Suppl)**: 68.

Kluijtmans LAJ, den Heijer M, Reitsma PH, Heil SG, Blom HJ, Rosendaal FR 1998. Thermolabile Methylenetetrahydrofolate reductase and factor V Leiden in the risk of deep-vein Thrombosis. *Thromb Haemost,* **79**: 254-8.

Kluijtmans LAJ, van den Heuvel LPWJ, Boers GHJ, Frosst P, Stevens EMB, van Oost BA, den Heijer M, Trijbels FJM, Rozen R, Blom, HJ 1996. Molecular genetic analysis in mild hyperhomocys-teinämia: A common mutation in the methylenetetra-hydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet,* **58**: 35-41.

Lunghi B, Castoldi E, Mingozzi F, Bernardi F 1998. A new factor V gene polymorphism (His1254Arg) present in subjects of african origin mimics the R2 polymorphism (His1299Arg). *Blood,* **91**: 364-5.

Lunghi B, Iacoviello L, Gemmati D, DiIasio MG, Castoldi E, Pinotti M, Castaman G, Redaelli R, Mariani G, Marchetti G, Bernardi F 1996. Detection of new polymorphic markers in the factor V gene: Association with factor V levels in plasma. *Thromb Haemost,* **75**: 45-48.

Marchetti G, Ferraresi P, Quaglio S, Taddia C, Chiozzi A, Cataldi A, Bernardi F, Mascoli F 1999. Study of FV genetic markers in carotid artery disease. *Thromb Haemost,* **82 (Suppl)**: 450.

Margaglione M, D'Andrea G, d´Addedda M, Giuliani N, Cappucci G, Iannaccone L, Vecchione G, Grandone E, Brancaccio V, D Minno G 1998. The methylenetetrahydrofolate reductase TT677 genotype is associated with venous thrombosis independently of the coexistence of the FV Leiden and the prothrombin A^{20210} mutation. *Thromb Haemost,* **79**: 907-911.

Mingozzi F, Lunghi B, Ferraresi P, Gastoldi E, Legnani C, Gemmati D, Pancani C, Palareti G, Marchetti G, Bernardi F 1999. Factor V markers for the detection of genetic components of APC-resistance in venous thrombosis. *Thromb Haemost*, **82 (Suppl)**: 404.

Morita H, Taguchi H, Kurihara H, Kitaoka M, Kaneda H, Kurihara Y, Maemura K, Shindo T, Minamino T, Ohno M, Yamaoki K, Ogasawara K, Aizawa T, Suzuki S, Yazaki Y 1997. Genetic polymorphism of 5,10-methylenetetrahydrofolate reduc-tase (MTHFR) as a risk factor for coronary artery disease. *Circulation*, **95**: 2032-6.

Poort SR, Rosendaal FR, Reitsma PH, Bertina RM 1996. 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.

Rosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Sisovick DS, Hillarp A, Watzke HH, Bernardi F, Cumming AM, Preston FE, Reitsma PH 1998. Geographic distribution os the 20210 G to A prothrombin variant. *Thromb Haemost*, **79**: 706-708.

Sacchi E, Tagliabue L, Duca F, Manucci PM 1997. High freqency of the C677 mutation in the methylenetetrahydrofolate reductase (MTHFR) gene in Northern in Italy. *Thromb Haemost*, **78**: 963-964.

Schröder W, Koesling M, Wulff K, Wehnert M, Herrmann FH 1996. Large-scale screening for factor V Leiden mutation in a North-Eastern German population. *Haemostasis*, **26**: 233-236.

Schröder W, Konrad R, Grimm R, Schuster G, Herrmann FH 2001. Factor V variants – FV Leiden, FV R2 polymorphism (ex 13), FV Ddel polymorphism (int 16) – Risk factors for venous thrombosis? Results of a pilot study. In: I Scharrer, W Schramm (Eds.): *30th Hemophilia Symposium Hamburg 1999*, Springer Verlag Berlin Heidelberg 2001 pp 261-265.

Song KS, Park YS, Kim HK, Choi IR 1999. Absence of the prothrombin gene variant in Koreans. *Thromb Haemost*, **81**: 990.