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Combined Effect of Factor V Leiden and Prothrombin 20210A on the Risk of Venous Thromboembolism

Pooled Analysis of 8 Case-control Studies Including 2310 Cases and 3204 Controls

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Keywords

Factor V Leiden, factor II G20210A, deep vein thrombosis, oral contraceptive

Summary

Factor V Leiden and factor II G20210A mutations are two frequent genetic risk factors involved in venous thromboembolism (VTE) The goal of this pooled analysis of 8 case-control studies, comprising a total of 2310 cases and 3204 controls, was to precisely estimate the risk of VTE in patients bearing both mutations (double heterozygotes) Odds ratios for VTE were 4 9 (95% CI, 4 1-5 9) for the factor V Leiden and 3 8 (3 0-4 9) for the factor II G20210A mutation Fifty-one cases (2 2%) and none of the controls were double heterozygotes The odds ratio for venous thrombosis in double heterozygotes was 20 0 (11 1-36 1) Twelve percent of patients heterozygous for factor V Leiden were also heterozygous for factor II G20210A and conversely 23% of patients heterozygous for factor II G20210A were also heterozygous for factor V Leiden Furthermore, in this large population we analyzed the effect of oral contraceptive (OC) in women carrying one of these mutations Odds ratio for VTE associated with OC was 2 29 (1 72-3 04) In factor V Leiden carriers using OC, the odds ratio for VTE was 10 25 (5 69-18 45) The odds ratio of the association of factor II mutation and OC use was 7 14 (3 39-15 04) Finally, we also confirmed that the frequency of factor V Leiden was lower in patients with pulmonary embolism than in patients with deep vein thrombosis without PE (odds ratio 0 69) Conversely, factor II G20210A mutation was equally balanced in both patient groups

Introduction

Venous thromboembolism (VTE) is a common disorder with a yearly incidence of about one in 1000 subjects in developed countries (1) Genetic risk factors responsible for thrombophilia have been recognized to have an important role in Caucasian populations (review in 2) Furthermore, it has become apparent during the last few years that the development of venous thrombosis is multifactorial, and that acquired risk factors interacting with genes or gene-gene interactions are involved in a large proportion of patients (3-5) Factor V Leiden (FV G1691A) and prothrombin G20210A mutations are the most frequent genetic risk factors involved in deep vein thrombosis (DVT) and/or pulmonary embolism (PE) (6-8) Among Caucasians factor V Leiden frequency varies between 2 and 15%, and is found in 15 to 25% of unselected patients with DVT (9-11) Heterozygosity for factor V Leiden increases the thrombotic risk three- to eightfold, and the risk in homozygotes was estimated to be increased 80-fold (12) The prothrombin G20210A mutation is found in 1-3% of Caucasians (13) and in 6-16% of patients with unselected DVT (8, 14-21) These two mutations are common in Caucasian populations with venous thrombosis, thus representing a good model for the study of gene-gene interaction

Due to the relatively low prevalence of these polymorphisms in the general population (5% for the factor V Leiden mutation and 2% for the prothrombin G20210A), the expected prevalence of double heterozygotes for the factor V Leiden mutation and the prothrombin mutation is about 1 per 1000 Very large series are thus necessary to study gene-gene interaction in order to give reliable information concerning the thrombotic risk estimate In the present study, we pooled the individual data of 8 case-control studies to evaluate the risk associated with the combined genetic risk factors This also allowed us to compare the

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Center	n	cases	n	controls
Leiden (NL)	474	consecutive outpatients with a first DVT	474	population controls, age and sex-matched
Malmo (SW)	99	consecutive outpatients with DVT	281	healthy controls
Manchester (UK)	194	first or recurrent DVT or PE referred for thrombophilia screening	161	healthy plasma donors
Campinas (BR)	176	first or recurrent DVT or PE	130	staff of the hospital (student, lab workers and doctors)
Napoli and Palermo (I)	348	first or recurrent DVT or PE referred to a thrombosis center	850	healthy controls
Milano (I)	435	first or recurrent DVT	559	population controls, patients with unconfirmed DVT, staff
Roma (I)	314	first or recurrent DVT or PE	351	blood donors and hospital staff
Paris (F)	270	first or recurrent DVT or PE	398	healthy controls age- and sex- matched
Total	**2310**		**3204**	

Table 1 Number and type of recruitment for cases and controls in the eight centers

	% of FV G1691A		% of FII G20210A	
Center	**cases**	**controls**	**cases**	**controls**
Leiden (NL)	19 5	3 0	6 2	2 3
Malmo (SW)	11 4	2 8	7 0	1 8
Manchester (UK)	14 9	4 3	5 7	1 3
Campinas (BR)	8 5	6 1	5 1	1 5
Napoli and Palermo (I)	18 9	5 1	14 9	4 6
Milano (I)	19 7	2 9	11 7	2 5
Roma (I)	18 8	2 6	8 0	2 6
Paris (F)	20 4	3 8	11 9	2 8

Table 2 Prevalence (%) of the factor V Leiden (G1691A) and the prothrombin 20210A allele in each center

Table 3 Characteristics of cases and controls (VTE venous thromboembolism, DVT deep vein thrombosis, PE pulmonary embolism) * $p < 0.01$

	Cases (2310)	Controls (3204)
Female (n,%)	1316 (57%)	1680 (52%)*
Male (n,%)	994 (43%)	1524 (48%)*
Mean age of the population	43 0	42 2
Mean age at the first event	40 4	-
% with first episode of VTE	75 5	-
% with isolated DVT	74 1	-
% with isolated PE	12 4	
% with DVT+PE	13 5	-
% with idiopathic episode	52 9	-

effect of oral contraceptive use on the risk for VTE in women carrying one of the thrombophilic mutations with that observed in non-carriers (22) Finally we evaluated in this large population the risk for pulmonary embolism associated with the factor V Leiden and prothrombin G20210A mutations (23)

Methods

The individual data of eight case control studies from six different countries that have genotyped patients and controls for the factor V Leiden and prothrombin G20210A mutations were pooled The originating centre, the number of cases and controls, and the recruitment criteria for controls in each study are listed in Table 1 Seven out of the 8 centres were in Europe, from Scandinavia to Italy, and only one includes subjects from South America (Campinas, Brazil) Except for this last study all the other centres included

Table 4 Genotypes of the factor V G1691A (factor V Leiden) und factor II G20210A mutations foi combined cases and controls

	Factor V G1691A			Factor II G20210A		
	GG	GA	AA	GG	GA	AA
Cases [n(%)]	1869 (81 4%)	398 (17 3%)	30 (1 3%)	2088 (90 6%)	211 (9 2%)	5 (0 2%)
Controls [n(%)]	3044 (95 5%)	140 (4 4%)	4 (0 1%)	3106 (97 1%)	93 (2 9%)	0 (0 0%)

mainly Caucasian individuals Each of these studies has been previously published elsewhere (8, 14 20) Cases included patients who had had at least one objectively confirmed episode of VTE Foi controls, various healthy individuals were recruited and ranged fiom populations that were age and sex matched to blood donors oi hospital staff The prevalence of factoi V Leiden and factor II 20210A allele in cases and controls, in each centre, is given in Table 2

Characteristics of cases and controls foi the pooled population are given in Table 3 The cases and controls were well matched for age, but there was a significant difference in the sex-ratio, with 57% of women among cases and 52% among controls ($p < 0.01$) Information on recurrence of VTE was not available in 21% of cases Information concerning the type of venous thrombo sis (PE and/oi DVT) was not available for 22% of cases

All centres except two used a strategy for the detection of the factor V Leiden mutation and the prothrombin 20210 allele similar to the originally described methods (7, 8) Factor V Leiden was determined by SSCP (single strand conformation polymorphism) in Napoli (24), and in Paris factor V Leiden and prothrombin mutations were determined as previously described (20) Each centre reported that their molecular biology protocols were robust

Statistical Analysis

Patient characteristics were presented as means with standard deviations for continuous variables and counts and percentages for categorical variables Groups were defined according to the presence of factor V Leiden (G1691A) and prothrombin G20210A mutations, and use of oral contraception The event was defined as the occurrence of DVT and/or PE Comparisons between groups were carried out using the unpaired Student t test or Mann-Whitney U-test for continuous variables according to their distribution and a Chi square or Fisher's exact test for categorical variables Hardy Weinberg equilibrium was tested for by a Chi-square test with 1 D F , separately in cases and controls from the different recruitment centres Allele frequencies were calculated from the genotype frequencies, and differences between the cases and controls were identified by means of a Chi-square test (1 D F)

The results from each study were summarized in two by two tables for each potential risk factor for VTE Carriers for mutations of factor V G1691A and prothrombin G20210A were heterozygous or homozygous for the mutation As was previously done for the meta analysis of therapeutic trials (25) and as there was no reason to favour a particular effect model, we used various methods based on both fixed and random effect models – that is, the combined logarithm of the odds ratio, Mantel-Haenszel, Cochran, Peto, and percentage difference (both fixed and random effects models) Because the results obtained from the different methods were similar, only the odds ratios calculated with Peto's method were given with the corresponding 95% confidence intervals When no events were reported for a group a value of 0 25 was automatically attributed for the calculation of the odds ratio Association and heterogeneity tests were performed for each analysis (26) Heterogeneity was considered to be present when the p value from the homogeneity test was less than or equal to 0 05 Whenever applicable the causes of heterogeneity were sought A p value of 0 01 or less from an association test was taken to be significant

Statistics were computed with Statview 5 statistical software, SAS, Cary, NC, USA The meta-analysis was conducted with EasyMA (a program for the meta analysis of clinical trials available at http //www spc univ lyon1 fr/~mcu/ma/html/logiciels htm)

Results

Single Gene Defect

A total of 2310 patients and 3204 controls were analyzed Data concerning the factor V genotype were lacking for 29 subjects (13

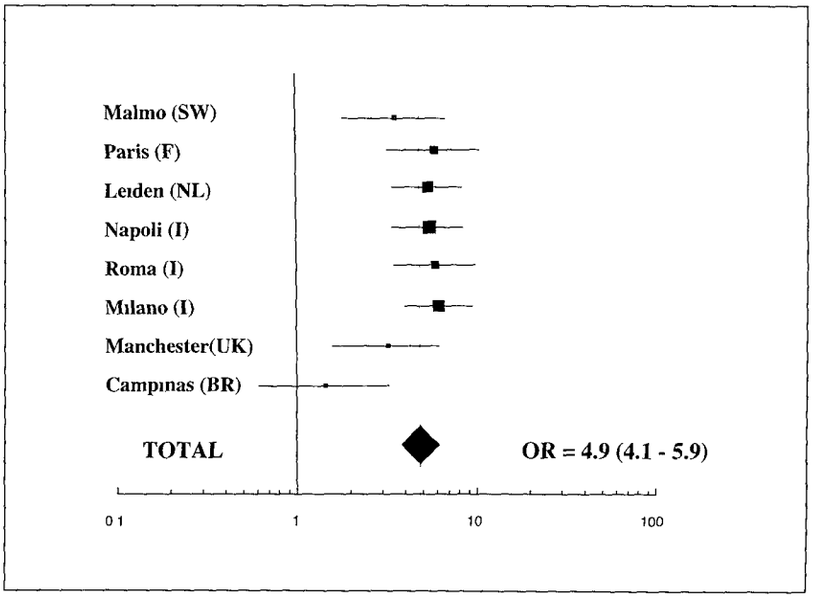

Fig 1 Odds ratios for venous thromboembolic dis ease associated with the presence of both factor II G20210A and factor V G169[illegible]A mutations Total odds ratio was computed with Peto's method (test of hetero geneity, p = 0 49, Q Cochran test)

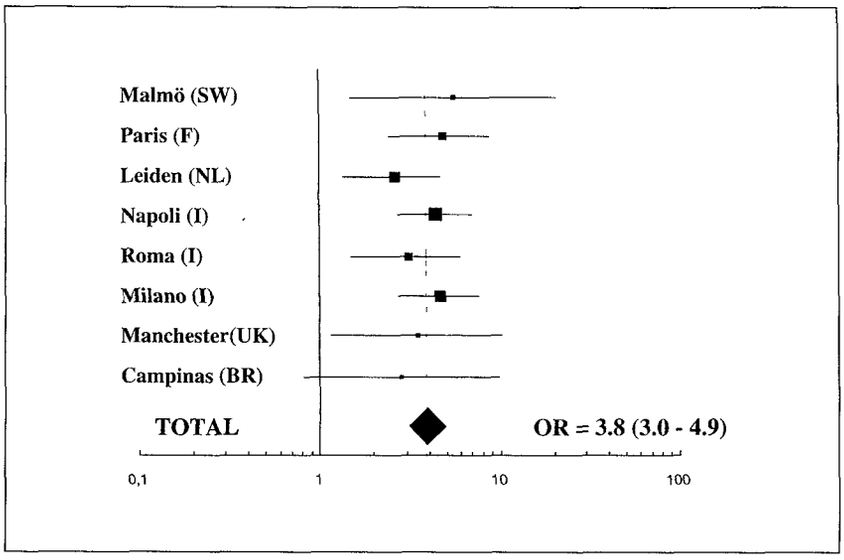

Fig. 2 Odds ratios for venous thromboembolic disease associated with factor II G20210A mutation. Total odds ratio was computed with Peto's method (test of heterogeneity, p = 0.83, Q Cochran test)

cases and 16 controls) and concerning prothrombin in 11 subjects (6 cases and 5 controls). Among the cases, 428 (18.6%) carriers of the factor V Leiden mutation were identified (including 30 homozygotes), and 216 (9.4%) carriers of the prothrombin G20210A mutation (including 5 homozygotes) were identified (Table 4). Among the controls, 144 (4.5%) subjects carried the factor V Leiden mutation (including 4 homozygotes), and 93 (2.9%) carried the prothrombin G20210A mutation. Odds ratios associated with venous thrombosis for the factor V Leiden and prothrombin G20210A mutations are indicated for each centre in Figs. 1 and 2 respectively. For the whole population, odds ratios for venous thrombosis associated with the factor V Leiden and the prothrombin mutations were 4.9 (95% CI; 4.1-5.9) and 3.8 (95% CI; 3.0-4.9), respectively.

Thirty cases were homozygous for the factor V Leiden mutation (1.3%) compared to 4 controls (0.13%). The odds ratio for venous thromboembolism associated with homozygosity for factor V Leiden was 9.85 (95% CI; 4.83-20.09). We were unable to estimate the risk of homozygous prothrombin G20210A mutation, as 5 cases (found in a single center) and no controls were too few to allow a meaningful estimation to be performed.

Combined Defect (Double Heterozygote)

Fifty-one cases (2.21%) and none of the controls were double heterozygotes. The odds ratio for venous thrombosis in carriers of both the factor V Leiden and the prothrombin mutations was 20.0 (CI 95%; 11.1-36.1) (Fig. 3). Among the 398 cases heterozygous for factor V Leiden, 48 (12%) were also heterozygous for prothrombin G20210A. Conversely, among the 211 cases heterozygous for prothrombin G20210A, 48 (23%) were also heterozygous for factor V Leiden. Two cases homozygous for factor V Leiden were also heterozygous for prothrombin G20210A (6.6% of the 30 FV Leiden homozygotes). One among 5 homozygotes for prothrombin G20210A was also heterozygous for factor V Leiden.

In double heterozygous cases, the age of the first episode of VTE was significantly younger than the remaining cases (34.7 vs 40.6 years; p <0.01). The odds ratio for occurrence of VTE below the median of age (44 years) was 2.21 (95% CI; 1.22-3.98) for carriers of both mutations but was not increased for each mutation alone (odds ratios respectively 1.02 and 1.06 for carriers of the factor V or prothrombin mutation).

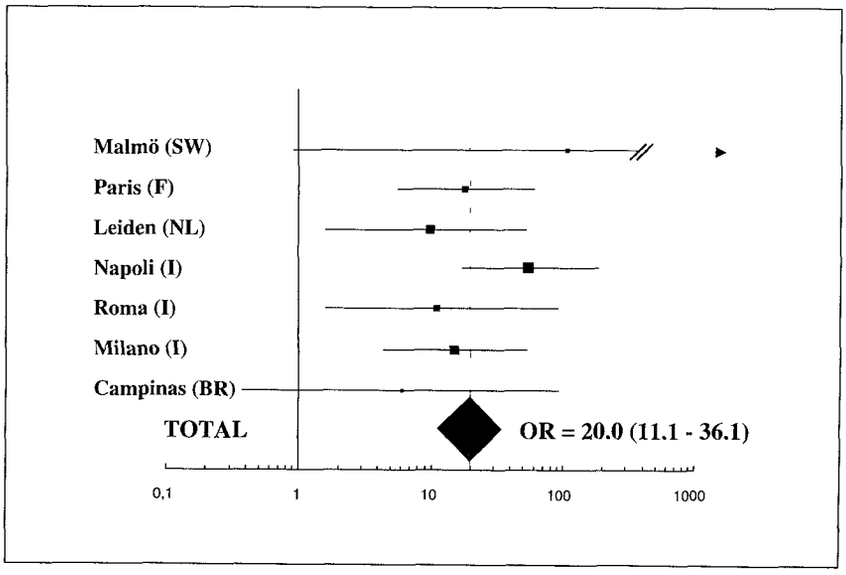

Fig. 3 Odds ratios for venous thromboembolic disease associated with factor V G1691A mutation. Total odds ratio was computed with Peto's method (test of heterogeneity, p = 0.07, Q Cochran test)

Table 5 Odds ratios for venous thromboembolism associated with oral contraceptive, factor V Leiden mutation and/or factor II G20210A in women aged 15 to 49 years in the 3 case control studies in which information was available (517 cases and 518 controls)

Oral contraceptive	Factor V Leiden	Factor II G20210A	OR (95% CI)	p heterogeneity
-			1 (Ref)	
+		-	2 29 (1 72 3 04)	0 59
	+		5 88 (3 52 9 82)	0 42
-		+	3 21 (1 44-7 15)	0 54
-	+	+	14 67 (3 47 62 03)	0 94
+	+	-	10 25 (5 69 18 45)	0 91
+	-	+	7 14 (3 39 15 04)	0 98
+	+	+	16 97 (3 95-72 80)	0 95

Oral Contraceptive Use

Information concerning the use of oral contraceptives in cases and controls was fully available in 3 centres (Leiden, Milano, Paris) The total number of women, aged 15 to 49 years, was 1035 (517 cases and 518 controls) Fifty two per cent (270/517) of the cases and 30% (153/518) of the controls used oral contraceptives For this subset of the whole female population, the odds ratio for VTE associated with oral contraceptive use was 2 29 (95% CI 1 72-3 04) (Table 5) In factor V Leiden carriers using oral contraceptive, the odds ratio for VTE was 10 25 (95% CI, 5 69-18 45) The odds ratio for the association of the prothrombin G20210A mutation and oral contraceptive use was slightly lower [7 14 (3 39-15 04)] Double heterozygous women who used oral contraceptives had an odds ratio of 16 97 (95% CI 3 95-72 80)

Pulmonary Embolism

In order to determine if the prevalence of the mutations were equally balanced between PE and DVT cases, we analyzed genotype prevalence in patients with each clinical entity The frequency of factor V Leiden was lower in patients with symptomatic PE (isolated or associated with DVT) than in patients who had DVT without symptomatic PE (14 7% and 20 0% respectively, OR 0 69, 95% CI 0 51-0 92) (Table 6) Conversely, the carriers of the prothrombin G20210A mutation or with both mutations were equally distributed among patients with either DVT or symptomatic PE

Discussion

The understanding of the causes of VTE, a multifactorial disease due to a combination of genetic and environmental factors, is a major goal in order to achieve better prevention and treatment of the disease The most efficient design for genetic studies of multifactorial diseases is the comparison of allele frequencies in unrelated cases and controls in studies of at least 1000 cases and controls or large family studies (27) To study gene-gene or gene-environment interactions, larger populations are required to achieve a sufficient number of cases and controls who combine 2 or more risk factors For factor V Leiden and prothrombin G20210A numerous studies have given concordant results concerning the risk associated with each mutation, but the odds ratio in double heterozygotes was unknown Several studies have pointed out an overrepresentation of carriers of both mutations in patients with VTE, but due to lack of power none of them was able to precisely estimate the risk (17-20, 28-30) Most of them have shown that factor V Leiden carriers who had a VTE episode were more frequently carriers of the prothrombin G20210A mutation, but in most cases the association was absent in controls

In this pooled analysis of 2310 cases and 3204 controls the odds ratio for VTE in carriers of FV Leiden was 4 9 (95% CI 4 1 5 9) The odds ratio in carriers of the prothrombin 20210A allele was 3 8 (95% CI 3 0-4 9) The estimated prevalence of carriers of both mutations in control Caucasian populations is 1 1000 Thus, we expected to find 3 double heterozygotes among the 3204 controls but none was observed In the present study 51 of the 2310 cases (2 2%) carried both mutations

Table 6 Prevalence and OR (95% CI) for symptomatic PE (with or without DVT) by reference to isolated DVT associated with factor V, factor II and association of the two mutations (N = 1791 patients)

	Factor V		Factor II		FV GG	FV GA or AA
	GG	GA or AA	GG	GA or AA	+ FII GG	+ FII GA or AA
PE or PE+DVT [n(%)]	396 (85 3)	68 (14 7)	416 (89 7)	48 (10 3)	453 (97 6)	11 (2 4)
Isolated DVT [n(%)]	1062 (80 0)	265 (20 0)	1190 (89 7)	137 (10 3)	1292 (97 4)	35 (2 6)
OR (95% CI) for PE associated with mutation	0 69 (0 51 0 92)		1 00 (0 71-1 42)		0 90 (0 45 1 78)	
p	0 011		1 00		0 75	

Double heterozygosity was associated with an odds ratio for venous thrombosis of 20 0 (95% CI, 11 1 36 1) The 2 2% prevalence of double heterozygotes in patients with venous thromboembolism refines the estimation of 1 to 5%, found in smaller populations (31-33) Compared to the odds ratios obtained for carriers of either the factor V Leiden mutation or the prothrombin G20210A mutation alone, a complete multiplicative effect of the association was observed (synergy index 1 07) (5) If double heterozygotes are relatively infrequent, it should be noted that 12% of patients with the factor V Leiden mutation also bear the prothrombin 20210A allele and conversely that 23% of patients heterozygous for the prothrombin G20210A mutation also carry the factor V Leiden mutation Furthermore, double heterozygous patients had thrombosis at a significantly younger age compared to other cases

We also took advantage of this large series to estimate the odds ratio associated with homozygosity for each mutation The odds ratio for venous thromboembolism associated with homozygosity for factor V Leiden was lower than the estimation of an increased risk of 80-fold found by Rosendaal et al (12) In this last study, 8 homozygotes were found among 471 patients (1 7%) and none in 474 age- and sex matched controls leading to an estimation of the odds ratio of 79 4 with a broad 95% confidence interval (22-289) The present results, based on a much larger population, estimate a 10-fold increased risk for factor V Leiden homozygotes (OR 9 85, 95% CI 4 83-20 09) However, due to the very low prevalence in the normal population, we cannot consider this estimation as fully reliable Nevertheless, it confirms that if homozygotes for factor V Leiden have an undoubtedly relatively high risk of thrombosis, they can also remain asymptomatic even after their sixties (34) We have no clear explanation for this discrepancy, but it may be due to the difficulty to precisely estimate a risk when none of the controls carries the risk factor (35)

The risk of oral contraceptive use alone or associated with the factor V Leiden and prothrombin G20210A mutations was analyzed by pooling the data from three studies (Leiden, Milano, Paris) The odds ratio for venous thromboembolism associated with oral contraceptive use was 2 29 (95% CI 1 72-3 04) This is in accordance with the less than 3 fold increase found in a recent review (36), but slightly lower than the 3 to 4-fold increased risk found in the Leiden Thrombophilia Study (22, 37) Since controls were very well matched in this last study our result is likely to be an underestimate Nevertheless, the range of the odds ratio that we found clearly overlaps the data found in previous studies, giving support to the coherence of our estimation involving a much larger population The odds ratios for the association of oral contraceptive use with the factor V Leiden or the prothrombin 20210A mutation were 10 25 (95% CI 5 69 18 45) and 7 14 (95% CI 3 39 to 15 04), respectively (Table 5) The joint relative risks are clearly higher than the effect of each risk factor alone, and exceed the sum of the relative risks indicating a supra-additive effect (5) In other words, the relative risk caused by the use of oral contraceptives is of similar magnitude in carriers and non-carriers for both of these frequent mutations, but the absolute risk is much higher Due to the small number of women bearing both mutations and using oral contraceptives the risk estimation is less precise (OR 16 97, 95% CI 3 95 72 80)

We confirmed that factor V Leiden is more strongly associated with the occurrence of deep vein thrombosis than with pulmonary embolism (23, 38-41), and conversely that the prothrombin G20210A mutation is evenly distributed in patients with deep vein thrombosis or pulmonary embolism These data are based on 78% of the whole study population and on the symptomatic events presented by each patient We cannot exclude that some patients with symptomatic DVT had asymptomatic PE and *vice versa* Due to the fact that some centres included only patients with DVT, the number of patients with either isolated PE or PE associated with DVT is only 25% of the whole population This could induce an important bias Nevertheless, in a large cohort of unselected patients with deep vein thrombosis or pulmonary embolism, we found a different prevalence of the factor V Leiden and prothrombin G20210A mutations This suggests that different patterns of venous thrombosis are not always associated with similar relative risks to specific risk factors (42) Furthermore, a lower frequency of proximal DVT (most likely associated with PE) in patients with the factor V Leiden mutation, compared to non-carriers, was recently demonstrated (43)

Reliable information on the role of genetic polymorphisms in venous thromboembolism will help to clarify disease mechanisms A problem with estimating the genetic risk for venous thromboembolism is either that the effect of any common polymorphism at a single locus is only moderate, or that the risk of the interrelation of 2 or more polymorphisms is high but difficult to assess reliably when too few cases and controls are involved Such relatively small case-control strategies are still not able to analyze actual synergistic effect, i e that two single polymorphisms are not individually risk factors for venous thromboembolism, but that their joint effect in the same individual increases the risk of the disease In the absence of studies of sufficient size, meta analysis can help to refine the estimation of the risk (44) However, because more extremely positive results are more likely to be published, the combination of published studies may tend to overestimate the strength of any association Also, meta analysis of case-control studies present particular challenges because of inherent bias such as differences in recruitment and study designs In the present study, one centre (Campinas, Brazil) had a much lower incidence of the factor V Leiden mutation compared to the other centres (Table 2) Nevertheless, the odds ratios for the pooled population given in Figs 1 to 3 were not significantly modified after exclusion of the Brazilian population (data not shown) The fact that we pooled individual data with very few missing genotypes (0 5% for the factor V Leiden mutation and 0 2% for the prothrombin G20210A mutation) strengthens the value of our results Despite the large number of subjects analyzed, we cannot consider the estimation of the thrombotic risk in double heterozygotes fully reliable due to the absence of such a genotype among the 3204 controls If one considers that it is relevant to study gene gene or gene environment interactions in venous thromboembolism, studies with more than 5000 cases and 5000 controls may well be needed and will be difficult to conduct in a single centre Large multicentre trials in well defined populations, including if possible a follow-up after a first event, would clarify the role and the interactions of the more numerous genetic or non genetic risk factors for VTE

Addendum

Joseph Emmerich coordinated the pooled analysis wrote the manuscript and is at the origin of the project Frits Rosendaal, Marco Cattaneo Maurizio Margaglione, Valerio De Stefano, Tony Cumming, Valder Hillarp were respectively in charge of the pooled analysis and correction of the manuscript for each center in Leiden Milano, San Giovanni Rotondo Roma, Manchester Campinas and Malmo Jean-Luc Reny performed the statistical analysis and contributed to the correction of the manuscript

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