The risk of recurrent venous thromboembolism among heterozygous carriers of factor V Leiden or prothrombin G20210A mutation. A systematic review of prospective studies

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ABSTRACT

Factor V Leiden (FVL) and prothrombin G20210A mutation (PTM) are the two most common genetic polymorphisms known to predispose to a first episode of venous thromboembolism (VTE). However, whether these thrombophilic abnormalities are also risk factors for recurrent VTE is unclear. We conducted a systematic review of prospective studies to assess the risk of recurrent VTE associated with heterozygous carriage of each of these mutations. All randomized controlled trials and prospective cohort studies that reported the incidence of recurrent VTE in patients with and without FVL and PTM after discontinuation of anticoagulant treatment were collected and analyzed. The risk ratios (RR) and their 95% confidence intervals (CI) for recurrent VTE were calculated in heterozygous carriers of FVL or PTM and compared to those of non-carriers. Eleven studies fulfilled the inclusion criteria. Recurrent VTE occurred in 114 out of 557 heterozygous carriers of FVL (20.5%) as compared to 382 out of 2,646 non-carriers (14.4%); and in 38 out of 212 heterozygous carriers of PTM (17.9%) compared to 428 of 2,996 non-carriers (14.3%). The RR of VTE recurrence conferred by the heterozygous carriage of FVL and PTM was 1.39 (95% CI, range 1.15 to 1.67) and 1.20 (range 0.89 to 1.61), respectively, using the Mantel-Haenszel fixed-effects model; 1.45 (1.13 to 1.85) and 1.36 (1.02 to 1.82), respectively, using the Der Simonian and Laird random effects method. In symptomatic patients with VTE, heterozygous carriage of FVL is clearly associated with a definitely increased risk of recurrent thromboembolism. The risk is lower with PTM and is difficult to interpret since it varies according to the assessment method used.

Key words: venous thromboembolism, thrombophilia, anticoagulation, factor V Leiden, prothrombin G20210A.

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Following the discovery of factor V Leiden (FVL) and prothrombin G20210A mutation (PTM) in the first half of the 1990s, numerous case-control, cross-sectional, and family studies have provided the convincing evidence that heterozygosity for either mutation is associated with a significantly increased risk of venous thromboembolism (VTE).[1-3] This risk is higher in homozygous carriers and in carriers of double heterozygosity. While it is widely acknowledged that these mutations confer an increased risk of thrombosis, it is controversial whether patients who are heterozygous carriers of either mutation and develop a thrombotic episode have an increased risk of recurrent events in comparison with non-carriers.[1-3] As a consequence, it is still not known whether detection of these abnormalities, which are highly prevalent in western countries, has the potential to identify a subgroup of patients who might benefit from the adoption of individually adjusted prevention strategies following their first thrombotic episode.[4,5] Available literature dealing with this issue includes both prospective and retrospective investigations. By definition, prospective studies are methodologically stronger, as they imply the formation of an appropriate inception cohort, and provide adequate documentation of outcome events and independent assessment of objective tests and clinical outcomes.[6] Thus, their conclusions are likely to be more reliable. In recent years, a number of prospective cohort studies and randomized clinical trials have appeared which report on the long-term out-

come of VTE patients with or without FVL or PTM after discontinuing anticoagulation. We undertook the first systematic review and meta-analysis of available prospective investigations.

Search strategy and data collection

All randomized controlled trials and prospective cohort studies that reported VTE recurrence in relation to the presence of FVL and/or PTM were independently sought by two reviewers (AM and LM) through electronic searches of MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials (from January 1, 1993 up to July 31, 2006) using combinations of the following search terms: randomized controlled trial, controlled clinical trial, clinical trial, cohort studies, follow-up studies, pulmonary embolism, thrombophlebitis, vein thrombosis, venous thrombosis, deep vein thrombosis, recurrence, recurrent, factor V Leiden, factor V, prothrombin G20210A, prothrombin, factor II, risk factor(s), thrombophilia. The search strategy had no language restriction. Titles and abstracts of the reports were checked to eliminate those obviously irrelevant.

The full text of the remaining articles was obtained and independently examined by the same reviewers to determine their potential eligibility for the meta-analysis based on the following predetermined inclusion criteria: (i) prospective study design; (ii) inclusion of consecutive patients with the first episode of objectively confirmed (i.e., confirmed by widely accepted diagnostic methods) deep vein thrombosis (DVT) of the lower extremities and/or pulmonary embolism (PE); (iii) genetic assessment of FVL or PTM carriage (performed in at least 70% of patients); (iv) institution of proper anticoagulant therapy (i.e., unfractionated or low-molecular-weight heparin followed by at least six weeks of anti-vitamin K drugs); (v) adoption of prospective follow-up for a period of at least 6 months without anticoagulant treatment to detect recurrent thromboembolism that was confirmed by means of objective diagnostic tests using strict predefined criteria. Studies were excluded if they only considered patients with venous thrombosis in unusual sites (e.g., cerebral sinuses, upper limbs, mesenteric, renal) or also included patients with recurrent VTE (as an index event) without providing separate information on VTE recurrences among patients enrolled after their first thrombotic episode. Additional relevant publications, identified by reviewing the reference lists of articles retrieved, and by hand searches on last issues of relevant journals and conference proceedings in the field of VTE, were subjected to the same selection process. Data extraction and quality assessment of the potentially eligible studies were undertaken by one reviewer (AM), and verified by the other (LM). Data were extracted and summarized using extraction sheets that detailed study design (prospective cohort study, randomized clinical trial), year of commencement, kind of study centre, characteristics of patients considered (eligibility, number, type of VTE at baseline: proximal/distal DVT, PE, number of episodes with predisposing factors such as surgery, trauma, prolonged immobilization, oral contraceptives, pregnancy, puerperium (secondary VTE) and number of episodes with no apparent provoking factor (spontaneous or idiopathic VTE), number of non-carriers and carriers of heterozygosity, homozygosity and double heterozygosity of FVL and/or PTM, mean duration of oral anticoagulant treatment and mean length of follow-up in carriers and non-carriers of each polymorphism, number of VTE recurrences among carriers and non-carriers during the follow-up, inclusion or not of patients with antiphospholipid antibody syndrome and deficiencies of natural anticoagulants (i.e., antithrombin, protein C and protein S). If data were missing from the original reports, then attempts were made to contact the authors to obtain the missing information. The quality of each study was appraised using a checklist appropriate to the design to assess the validity of the methods used with regard to the purpose of the meta-analysis. Cohort studies were labelled as having a high quality if they met all the following predefined criteria (i) patients systematically genotyped for FVL and PTM; (ii) anticoagulant treatment of patients with FVL or PTM comparable to that of non-carriers; (iii) follow-up period completed by at least 90% of study patients. Randomized clinical trials were considered as having a high quality if they: (i) systematically genotyped patients enrolled for FVL and PTM; (ii) used a correct randomization system not influenced by the knowledge of the presence of FVL or PTM, with consequent comparable treatment and duration of follow-up among carriers and non-carriers of either abnormality; (iii) had an objective and independent assessment of recurrent VTE blinded to the presence of FVL or PTM; (iv) had a follow-up period completed by at least 90% of the patients. Among studies considering the same cohort of patients, only the one providing the most complete and updated information was included.

Statistical analysis

The fixed effects model by Mantel–Haenszel and, due to differences in basic design and patient populations included, the random effects method by Der Simonian and Laird were used to pool the data of the included studies.[7,8] Risk ratios (RR) and their 95% confidence intervals (CI) for recurrent VTE in heterozygous carriers of FVL or PTM in comparison with non-carriers were calculated using the fixed effects model, and compared with the results obtained with the random effects method. The Cochran's Q was calculated to formally identify statistical heterogeneity among studies. A p value of less than 0.05 was considered to denote statistically significant heterogeneity. The I^2 method was used to quantify inconsistency across studies and to assess its impact on the meta-analysis effect estimate (an I^2 greater than 50% was considered substantial).[9] One study at a time was removed from the model to assess the source of heterogeneity, if present. Sensitivity analyses were performed considering: (i) study design (randomised controlled studies and cohort studies); (ii) type of index

event considered (only DVT versus DVT plus PE, only unprovoked VTE versus unprovoked and secondary VTE); (iii) year of commencement (studies started enroling patients before and after the median year in which all studies began); (iv) mean duration of initial anticoagulant treatment (3 months versus 6 months or longer); (v) mean duration of follow-up (shorter or longer than 2 years, shorter or longer than 4 years); (vi) inclusion or not of patients with antiphospholipid antibody syndrome and deficiencies of natural anticoagulants; and (vii) study quality. In addition, to further investigate possible interference in the risk estimate by the concomitant presence of multiple thrombophilic conditions, the risk ratios for recurrent VTE of patients with only heterozygous carriage of FVL or PTM and no other recognized thrombophilic conditions (i.e., antithrombin, protein C and S defects, and antiphospholipid antibody syndrome) compared to patients with no thrombophilia was calculated by the Mantel–Haenszel and the Der Simonian and Laird models, based on the studies that provided the necessary information. The potential for publication bias was explored using funnel plots of effect size versus standard error. In addition, on the basis of the pooled prevalence of FVL and PTM derived from included studies that enrolled patients with spontaneous and secondary first VTE, and the pooled relative ratios for VTE recurrence conferred by either mutation, the population attributable risk percentages of both polymorphisms were calculated. Rev Man version 4.2 (The Cochrane Collaboration, Oxford, England, 2002) software was used to perform the statistical analyses.

Selection of studies

Our search strategy retrieved 266 reports, of which 244 were excluded on the basis of titles and abstracts as clearly irrelevant. After carefully examining the full text of the remaining 22 articles, 11 were excluded because of the following reasons: (i) lack of prospective design;[10-16] (ii) inclusion of patients with recurrent VTE as index event without providing separate information for those with a first event;[17] (iii) availability of updated and more complete reports.[18-20] The remaining 11 articles fulfilled the inclusion criteria, and were, therefore, included in the meta-analysis.

Heterozygous carriage of FVL and risk of VTE recurrence

Ten studies (seven prospective cohort studies and three randomised trials) examining the risk of recurrent VTE in heterozygous carriers of FVL fulfilled the inclusion criteria (Table 1).[21-30] They included a total of 3,203 patients with a first episode of VTE, of whom 557 (17.4%, 95% CI, 16.1 to 18.7) were heterozygous carriers of FVL. The prevalence of FVL in patients with first unprovoked VTE was 18.0% (95% CI, 16.9 to 22.9), while it was 16.9% (95% CI, 15.5 to 18.3) among patients with unprovoked and secondary VTE. The duration of follow up ranged from 0.75 to 8.3 years. Recurrent thromboembolism was experienced by 114 of the 557 heterozygous carriers of FVL (20.5%), and by 382 of the 2,646 non-carriers (14.4%). Pooling the results of the included studies using the Mantel-Haenszel fixed effects model, the RR of VTE recurrence conferred by the heterozygous carriage of FVL after a first VTE event was 1.39 (95% CI, 1.15 to 1.67) with non statistically significant heterogeneity (p=0.11, I^2=37.2%) (Figure 1A). The Der Simonian and Laird random effects model yielded a similar estimate of increased risk (RR, 1.45; 95% CI, 1.13 to 1.85)(Figure 1B). Of the 4 studies that fulfilled the high quality criteria,[23,28-30] only that by Palareti achieved a statistically significant increase of RR for recurrent VTE in heterozygous carriers of FVL (RR 2.69; 95% CI, 1.58 to 4.58).[23] A funnel plot of effect size versus standard error was broadly symmetrical (Figure 2), suggesting no major publication bias. Sensitivity analyses taking into account some relevant features of the included studies are reported in Table 2. All the analyses led to an association between FVL and VTE recurrence (RR >1). Higher RRs were obtained: (i) in cohort studies compared with randomized clinical trials; (ii) in studies considering only patients with DVT compared with those enrolling also patients with PE; (iii) in studies including all VTE compared with those including only idiopathic episodes; (iv) in studies with a shorter duration of initial anticoagulant treatment compared with those with a longer duration; and (v) in studies that enrolled patients with antiphospholipid antibody syndrome or deficiencies of natural anticoagulants compared with those which excluded these patients. The risk ratio for recurrent VTE of patients with only heterozygous carriage of FVL and no other thrombophilic conditions (i.e., PTM, antithrombin, protein C or S defects, antiphospholipid antibody syndrome) compared to patients with no thrombophilia was 2.15 (95% CI, range 1.59-2.89) by the Mantel–Haenszel fixed effects method and 2.19 (95% CI, range 1.63-2.95) by the Der Simonian and Laird random effects model, based on 1,067 patients of the only four studies included which provided the necessary information.[21,23,25,30] The population attributable risk percentage of VTE recurrence conferred by the heterozygous carriage of FVL, calculated on the basis of the pooled prevalence of the FVL polymorphism and the pooled RR for recurrence (and its 95% CI), resulting from the studies that enrolled patients with unprovoked and secondary first VTE episode was 6.2% (95% CI, range 2.6-10.1%).

Heterozygous carriage of PTM and risk of VTE recurrence

Ten studies (seven prospective cohort studies and three randomised trials) examining the risk of recurrent VTE in heterozygous carriers of PTM fulfilled the inclusion criteria (Table 3).[21-27,29-31] They included a total of 3,208 patients with a first episode of VTE, of whom 212 (6.6%, 95% range CI, 5.8-7.5) were heterozygous carriers of PTM. The prevalence of PTM in patients with first unprovoked VTE was 7.1% (95% CI, range 5.3-9.3), and 6.9% (95% CI, range 6.0-8.0) among patients with unprovoked and secondary VTE. The duration of follow-up ranged from 0.75 to 8.3 years. Recurrent thromboembolism was experienced by 38

Table 1. Studies examining heterozygous carriage of Factor V Leiden (FV 1691A gene) and risk of recurrent venous thromboembolism.

Study Author Publication year	*Study Design*	*Starting year*	*Patients / Center(s)*	*Index VTE*	*Only Idiopathic Index VTE ?*	*Prevalence heterozygous carriage FVL*	*OAT duration (months)*	*FVL n/N*	*Non-FVL n/N*	*Follow-up FVL/non-FVL (years)*
Lindmarker[26] 1999	RCT	1998	Age ≤70 years without deficiencies of NA, 87% genotyped for FVL/multiple centers in Sweden	DVT(pd), PE	No ≤	25.9%	1.5 or 6	19/118	42/338	4/4
Kearon[27] 1999	RCT	1994	Consecutive patients without known deficiencies of NA, 88% genotyped for FVL/multiple centers in Canada and USA	DVT(p), PE	Yes	23.2%	3	[2 /19]	[14/ 63]	0.75/0.75
Simioni[21] 2000	PC	1986	Consecutive patients without APS or deficiencies of NA, 74% genotyped for FVL/a specialist center in Padua (Italy)	DVT (pd)	No	16.3% [16.1%]	3	18/38° [8/18]°	49/210 [31/104]	8.3/8.3
Miles[22] 2001	PC	1982	Male physicians aged 40-84 years, all genotyped for FVL/multiple centers in USA	DVT, PE	No	13.3%°	3?	5/26°	21/189	4.1/6.7
Eichinger[28] 2002	PC	1992	Consecutive patients age >18 years without APS or deficiencies of NA, all genotyped for FVL/4 centers in Vienna (Austria)	DVT(p), PE	Yes	28.9%	10	[17/83]	[44/204]	3.2/2.9
Baglin[24] 2003	PC	1997	Consecutive patients, 85% genotyped for FVL/hematology department in Cambridge (UK)	DVT(pd), PE, arm	No	15.9%	6--> 6.75	9/77*	36/408*	Up to 2/Up to 2
Palareti[23] 2003	PC	1995	Consecutive patients, all genotyped for FVL/1anticoagulant clinic in Bologna (Italy)	DVT(pd), PE	No	12.1%	?3	15/68°	43/525	1.45/1.45
Christiansen[29] 2005	PC	1988	Consecutive patients aged 18 to 70 years, all genotyped for FVL/3 anticoagulant clinics in the Netherlands	DVT, arm	No	18.0%	3 to 12	19/84	70/382	7.3/7.3
Santamaria[25] 2005	RCT	1995	Consecutive patients age >18 years, 73% genotyped for FVL/ multiple centers in Italy	DVT (p)	Yes	[7.7%]	3 or 12	[5/15]	[44/180]	3.9/3.9
Gonzales-Porras[30] 2006	PC	1997	Consecutive patients without APS or deficiencies of NA with OAT <=6 months, a specialist center in Salamanca (Spain)	DVT(pd) ±PE	No	17.6%	3 or 6	5/29°	19/147	4.1/4.9
All studies						**17.4%**		**114/557 (20.5%)**	**382/2646 (14.4%)**	

*FVL: heterozygous carriers of factor V Leiden gene mutation (FV 1691A); Non-FVL: non carriers of factor V Leiden mutation (homozygous carriers of the FV 1691G "wild type" gene); n: the number with VTE recurrence; N: the number of patients with a first VTE; OAT: oral anticoagulant treatment; NA: natural anticoagulants (protein C, protein S and antithrombin); APS: antiphospholipid antibody syndrome; DVT: deep vein thrombosis; p: proximal; d: distal; PE: pulmonary embolism; []: idiopathic patients' data; *data kindly provided by the authors; °FVL/prothrombin G20210A compound heterozygosity excluded*

out of the 212 heterozygous carriers of PTM (17.9%), and by 428 of the 2,996 non-carriers (14.3%). The RR of VTE recurrence conferred by the heterozygous carriage of PTM was 1.20 (95% CI, range 0.89-1.61) using the Mantel-Haenszel fixed effects model (Figure 3A), and 1.36 (95% CI, range 1.02-1.82) by means of the Der Simonian and Laird random effects method (Figure 3B). No statistically significant heterogeneity was detected among the studies (p=0.53, I^2=0%). None of the three studies that fulfilled the high quality criteria showed an increased risk for recurrent VTE.[23,29,30] A funnel plot of effect size versus standard error was broadly symmetrical (Figure 4) suggesting no major publication bias. Sensitivity analyses taking into account some relevant features of the included studies are reported in Table 2. With the exception of the analyses considering the three studies that fulfilled the high quality criteria, and those enrolling patients with antiphospholipid antibody syndrome or deficiencies of natural anticoagulants, all the other analyses led to an association between PTM and VTE recurrence (RR >1). Higher RRs were obtained in (i) cohort studies compared with randomized clinical trials; (ii) in studies enrolling only patients with DVT; (iii) in those that excluded patients with antiphospholipid antibody syndrome and deficiencies of natural anticoagulants; (iv) in older studies compared with more recent studies; (v) in studies with shorter duration of initial anticoagulant treatment compared with those with a longer duration; and (vi) in studies with a longer duration of follow-up compared with those with a shorter duration. The risk ratio for recurrent VTE of patients with only heterozygous carriage of PTM and no other well recognized thrombophilic conditions (i.e., FVL, antithrombin, protein C or S defects, and

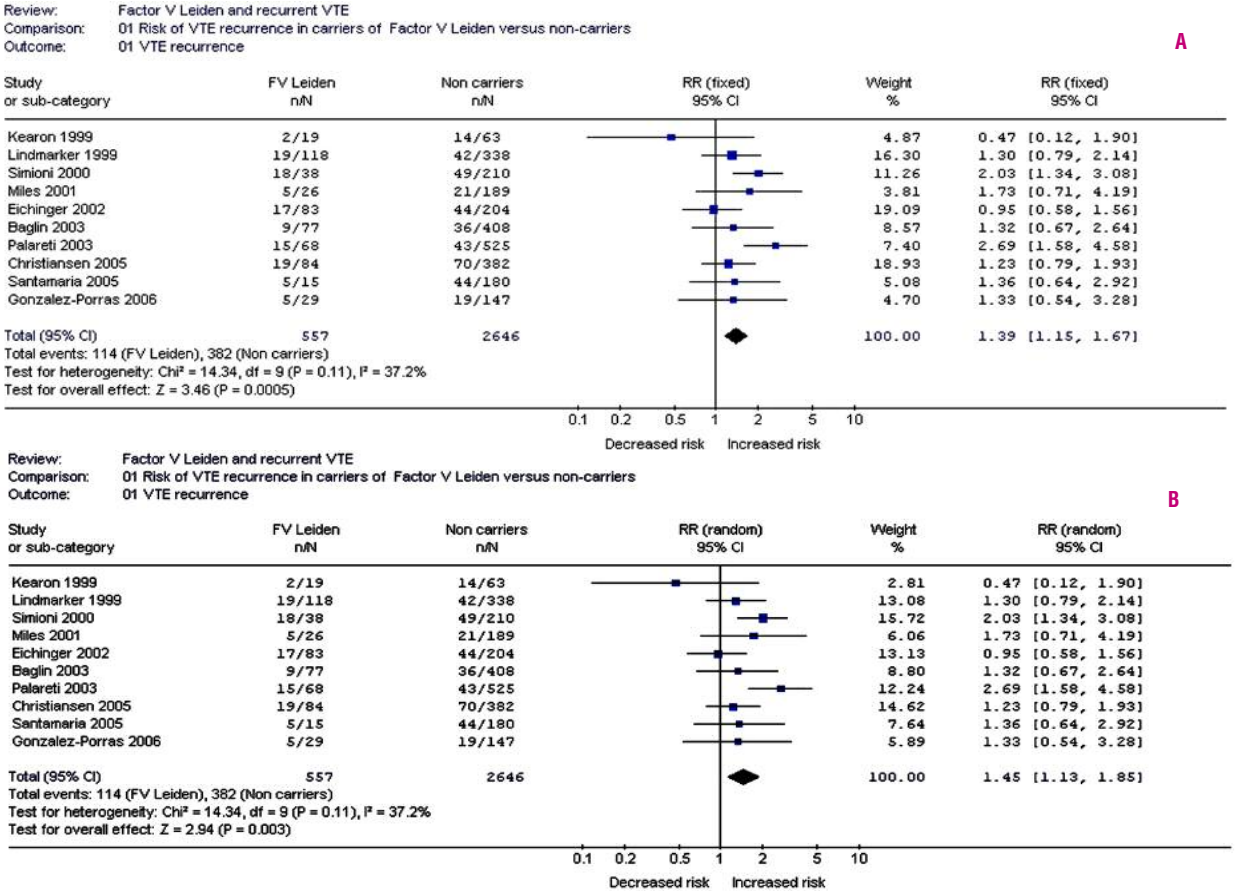

Figure 1 (above). A. Risk of VTE recurrence in heterozygous carriers of factor V Leiden (fixed-effects model).. B. Risk of VTE recurrence in heterozygous carriers of factor V Leiden (random-effects model).

Table 2. Sensitivity analyses.

Heterozygous carriage of Factor V Leiden and recurrent VTE			
Study feature	*RR (95% CI)*	*Study feature*	*RR (95% CI)*
Prospective Cohort	1.43 (1.16-1.77)	RCT	1.16 (0.77-1.73)
DVT only	1.51 (1.13-2.01)	DVT and/or PE	1.29 (1.01-1.64)
Idiopathic VTE	1.05 (0.75-1.47)	Idiopathic and secondary VTE	1.53 (1.24-1.89)
Started before 1995	1.26 (0.99-1.62)	Started in1995 or after	1.49 (1.13-1.98)
Follow-up ≤2 years	1.48 (1.00-2.20)	Follow-up> 2 years	1.33 (1.08-1.65)
Follow-up ≤4 years	1.26 (0.98-1.62)	Follow-up > 4 years	1.53 (1.16-202)
OAT 3 months	1.59 (1.10-2.30)	OAT ≥6 months	1.07 (0.71-1.59)
APS and NA deficiencies included	1.49 (1.14-1.96)	APS and NA deficiencies excluded	1.26 (0.97-1.63)
High quality	1.30 (1.00-1.70)	Non-high quality	1.43 (1.10-1.85)
Heterozygous carriage of Prothrombin 20210A polymorphism and recurrent VTE			
Study feature	*RR (95% CI)*	*Study feature*	*RR (95% CI)*
Prospective Cohort	1.26 (0.90-1.76)	RCT	1.02 (0.53-1.93)
DVT only	1.25 (0.84-1.87)	DVT and/or PE	1.15 (0.75-1.78)
Idiopathic VTE	1.44 (0.90-2.30)	Idiopathic and secondary VTE	1.24 (0.89-1.73)
Started before 1995	1.37 (0.94-2.00)	Started in 1995 or after	1.03 (0.64-1.64)
Follow-up≤2 years	1.16 (0.57-2.36)	Follow-up > 2 years	1.21 (0.87-1.68)
Follow-up≤4 years	1.07 (0.68-1.68)	Follow-up > 4 years	1.34 (0.90-1.97)
OAT 3 months	1.92 (1.25-2.95)	OAT ≥6 months	1.40 (0.64-3.04)
APS and NA deficiencies included	0.94 (0.58-1.53)	APS and NA deficiencies excluded	1.47 (1.01-2.13)
High quality	0.82 (0.45-1.49)	Non-high quality	1.44 (1.03-2.02)

RCT: randomised controlled trial; DVT: deep venous thrombosis; PE: pulmonary embolism; VTE: venous thromboembolism; OAT: oral anticoagulant treatment; APS: antiphospholipid antibody syndrome; NA: natural anticoagulants (protein C, protein S, antithrombin).

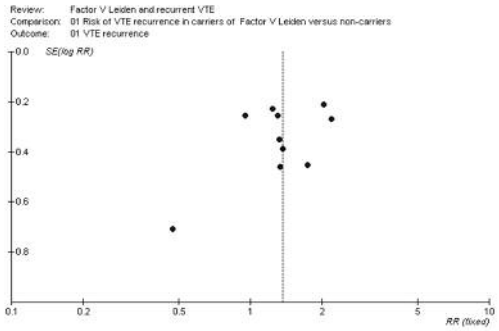

Figure 2. Funnel plot of published studies on heterozygous carriers of factor V Leiden and risk of recurrent VTE

antiphospholipid antibody syndrome) compared to patients with no thrombophilia was 1.74 (95% CI, range 1.02-2.98) by the Mantel–Haenszel fixed effects method and 1.62 (95% CI, range 0.74-3.57) by the Der Simonian and Laird random effects model, based on 1016 patients of the only four studies included which provided the necessary information. [21,23,25,30] The population attributable risk percentage of VTE recurrence conferred by the heterozy-

Table 3. Studies examining heterozygous carriage of prothrombin mutation (PT 20210A gene) and risk of recurrent venous thromboembolism.

Study Author Publication year	*Study Design*	*Starting year*	*Patients/Centers*	*Index VTE*	*Only Idiopathic Index VTE ?*	*Prevalence heterozygous carriage PTM*	*OAT duration (months)*	*PTM n/N*	*Non-PTM n/N*	*Follow-up PTM/non-PTM (years)*
Eichinger[31] 1999	PC	1992	Consecutive patients age >18 years without APS or deficiencies of NA, 82% genotyped for PTM/4 centers in Vienna (Austria)	DVT(pd), PE, arm	Yes #	[8.2%]	12 PTM 8 non-PTM	[3/24]	[29/268]	-2.2/-2.1
Lindmarker[26] 1999	RCT	1998	Age ≥70 years without deficiencies of NA, 85% genotyped for PTM/ multiple centers in Sweden	DVT(pd), PE	No	6.1%	1.5 or 6	4/28	57/428	4/4
Kearon[27] 1999	RCT	1994	Consecutive patients without known deficiencies of NA, 87% genotyped for PTM/multiple centers in Canada and USA	DVT(p), PE	Yes	3.6%	3	[1 /3]	[16/ 80]	0.75/0.75
Simioni[21] 2000	PC	1986	Consecutive patients without APS or deficiencies of NA, 74% genotyped for PTM/a specialist center in Padua (Italy)	DVT(pd)	No	10.7% [8.1%]	3	12/24° [6/8]°	55/224 [33/114]	8.3/8.3
Miles[22] 2001	PC	1982	Male physicians aged 40-84 years, all genotyped for PTM/multiple centers in USA	DVT, PE	No	6.9%	3?	2/11°	24/204	4.1/6.7
Baglin[24] 2003	PC	1997	Consecutive patients, 83% genotyped for PTM/hematology department in Cambridge (UK)	DVT(pd), PE, arm	No	4.2%	6--> 6.75	3/20*	39/454*	Up to 2/ Up to 2
Palareti[23] 2003	PC	1995	Consecutive patients, all genotyped for PTM/ 1anticoagulant clinic in Bologna (Italy)	DVT(pd), PE	No	7.0%	≥3	3/38	55/557	1.45/1.45
Christiansen[29] 2005	PC	1988	Consecutive patients aged 18 to 70 years, all genotyped for PTM/3 anticoagulant clinics in Netherlands	DVT, arm	No	6.1%	3 to 12	4/29	86/445	7.3/7.3
Santamaria[25] 2005	RCT	1995	Consecutive patients age >18 years, 73% genotyped for PTM/multiple centers in Italy	DVT(p)	Yes	[7.2%]	3 or 12	[3/14]°	[46/181]	3.9/3.9
Gonzales-Porras[30] 2006	PC	1997	Consecutive patients without APS or deficiencies of NA with OAT ≤6 months, a specialist center in Salamanca (Spain)	DVT(pd) ±PE	No	13.2%	3 or 6	3/21°	21/155	4.1/4.9
All studies						6.6 %		38/212 (17.9%)	428/2996 (14.3%)	

*PTM: heterozygous carriers of prothrombin mutation (PT 20210A, the gain-of-function gene); Non-PTM: non carriers of prothrombin mutation (homozygous carriers of PT20210G the "wild type" gene); n the number with VTE recurrence; N the number of patients with a first VTE; OAT: oral anticoagulant treatment; NA: natural anticoagulants (protein C, protein S and antithrombin); APS antiphospholipid antibody syndrome; DVT: deep vein thrombosis; p: proximal; d: distal; PE: pulmonary embolism; []: idiopathic patients data; #patients enrolled with secondary VTE were not considered because the data of patients with first VTE index events were reported pooled with those of patients enrolled at recurrent VTE; * data kindly provided by the authors; ° FVL /prothrombin G20210A compound heterozygosity excluded.*

gous carriage of PTM, calculated on the basis of the pooled prevalence of the polymorphism FVL and the pooled RR for recurrence (and its 95% CI), resulting from the studies that enrolled patients with unprovoked and secondary first VTE episode was 1.4% (95% CI, range 0-4.0%).

According to the results of our meta-analysis, the heterozygous carriage of FVL clearly confers an increased risk of VTE recurrence (by about 40%). The risk is lower with heterozygous carriage of PTM and is difficult to interpret since this varies according to the method used for its estimation, ranging between 20% (using the fixed effects model) and 36% (using the random effects model, which takes into greater account the inter-study variability). Interestingly, when the analysis was confined to subjects free from other thrombophilias, both the risk of VTE recurrence related to FVL and that related to PTM became stronger, although once again in the latter group the two methods used for its estimation offered different results. Our findings are consistent with those from earlier meta-analyses.[32-34] In 2000, for heterozygous carriers of FVL, Marchetti *et al.* reported an odds ratio for VTE recurrence of 1.36 (95% CI, range 1.05-1.78).[32] In 2003, Vink *et al.* reported an odds ratio for VTE recurrence of 1.3 (95% CI, range 1.0-1.7) for heterozygous carriers of FVL, and 1.4 (95% CI, range 0.9-2.0) for heterozygous carriers of PTM.[33] More recently, Ho *et al.* reported a higher odds ratio for VTE recurrence in heterozygous carriers of PTM (1.72; 95% CI, range 1.27-2.31) than in heterozygous carriers of FVL (1.41; 95% CI, range 1.14-1.75).[34] They included a substantial proportion of retrospective studies.[13,14,16] These have a higher potential for biases including patient selection, differences in treatment exposures, and inadequate identification of recurrent thrombotic disease.[6] Although their findings were confirmed even when the analysis was confined to prospective studies, they could not include two studies

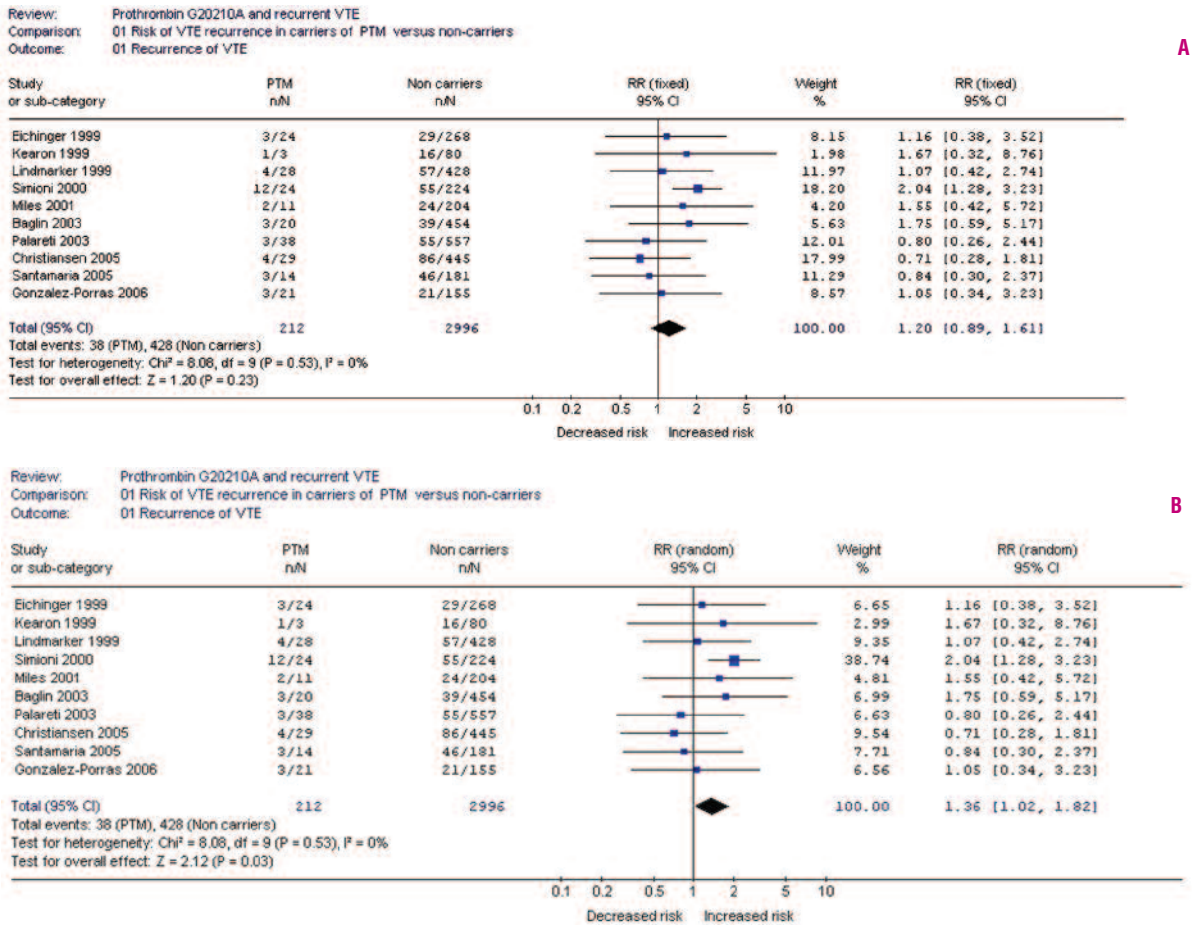

Figure 3. A. Risk of VTE recurrence in heterozygous carriers of prothrombin 20210A mutation (fixed-effects model) **B.** Risk of VTE recurrence in heterozygous carriers of prothrombin 20210A mutation (random-effects model).

that were published later on.[25,30] The strength of our study is that we systematically searched the literature to identify all investigations that examined the risk of VTE recurrence among heterozygous carriers of FVL and PTM compared with non-carriers, and confined our evaluation to the only prospective studies. Potentially confounding factors were suppressed by excluding from the analysis patients with previous thromboembolism. To evaluate potential under-reporting of negative studies we examined publication bias by means of funnel plots suggesting that major publication bias is unlikely to have occurred. Although the single studies showed considerable heterogeneity in terms of study quality and patients' features (modality of clinical presentation, location of thrombi, presence or absence of detectable risk factors, inclusion or exclusion of other thrombophilic conditions, duration of initial anticoagulant treatment which has evolved over the years in patients with idiopathic VTE according to the recommendations of international guidelines, and duration of follow-up), the sensitivity analyses that were performed demonstrated broadly similar and consistent results. In addition, they suggested a possible influence of some of these features (i.e., duration of initial anticoagulant treatment, and concomitant presence of other thrombophilic conditions) on the risk of recurrence conferred by heterozygous carriage of FVL and PTM. Most of the studies considered for our systematic review did not include the information necessary to separate the clinical outcome of patients with spontaneous VTE from that of patients with secondary VTE, and the outcome of patients presenting with primary pulmonary embolism from that of patients presenting with primary deep vein thrombosis. We failed to obtain this addition-

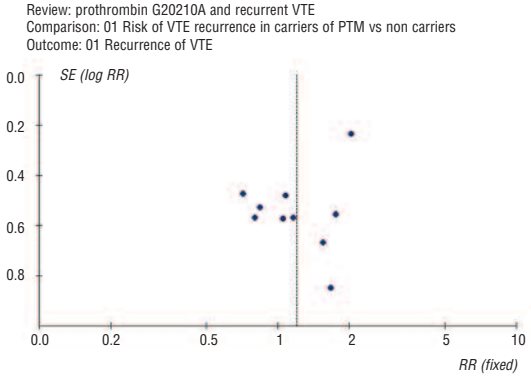

Figure 4. Funnel plot of published studies on heterozygous carriage of prothrombin 20210A mutation and risk of recurrent VTE

al information despite contacting the authors. For the same reasons, we could not adequately evaluate the influence of other thrombophilic abnormalities, such as hyperhomocysteinemia or increased factor VIII:C, which have been reported to have the potential to increase the risk associated with FVL or PTM carriage.[14]

In conclusion, heterozygous carriers of FVL who experience an episode of VTE have on average a 40% increased risk of recurrent VTE over non-carriers, and this increase is statistically significant. The risk is lower with heterozygosity of PTM, and is difficult to interpret since it varies according to the method used. More studies are needed to clarify this potentially important issue. The clinical relevance of these findings still remains to be assessed. For the time being, whether to prolong the duration of anticoagulant therapy beyond the recommended time period (3 to 12 months) in carriers of either polymorphism remains an individual decision to be made for each patient and depends on a variety of elements, including comorbidities and patient preference.

Authors' Contributions

AM and LM conducted the literature search and reviewed the studies. AM and MHP performed the statistical analysis. PP and AM wrote the first manuscript draft. All authors participated in the study conception and design, revised the manuscript critically for important intellectual content and approved the final version for publication.

Conflict of Interest

The authors reported no potential conflicts of interest.

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