Inherited thrombophilic abnormalities and risk of portal vein thrombosis

A meta-analysis

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Summary
Inherited thrombophilic abnormalities may have a role in the development of portal vein thrombosis (PVT). However, the prevalence of these factors in patients with PVT has been evaluated only in small studies with non-conclusive results. It was the purpose of this study to assess the risk of PVT associated with factor V Leiden (FVL) and G20210A prothrombin mutation (PTM). The MEDLINE, EMBASE, Cochrane Library databases, reference lists of retrieved articles and contact with content experts were used. Studies carried out in Western Europe comparing the prevalence of prothrombotic abnormalities in patients with PVT and in controls without a history of thromboembolic disease were included. Two reviewers independently selected studies and extracted study characteristics, quality and outcomes. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each trial and pooled using a fixed and random-effects model. Statistical heterogeneity was evaluated using the I^2 statistic. Sensitivity analyses were performed examining separately studies according to the etiology of PVT and to control population. Twelve studies involving more than 3,000 patients were included. The pooled OR for PVT was 1.90 (95%CI: 1.25, 2.90) in patients with FVL and 4.48 (95%CI: 3.10, 6.48) in patients with PTM. In conclusion, PVT is associated with the presence of FVL and PTM in Western Europe.

Keywords
Thrombophilia, venous thrombosis, hepatology

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Introduction

Since its first description in 1868 (1), portal vein thrombosis (PVT) has been considered a relatively uncommon but potentially life-threatening disease. The incidence of PVT varied widely in different studies, according to the different study populations and to the different diagnostic procedures used. For instance, Okuda reported a 0.6% incidence of PVT in 708 consecutive cirrhotic patients studied by angiography (2). On the other hand, PVT was diagnosed in 21% of patients who underwent surgery for portal hypertension (3) and in 26% of patients undergoing liver transplantation (4). Several acquired risk factors such as liver cirrhosis, hepatocellular carcinoma (HCC) and myeloproliferative disorders may contribute to the development of PVT. Congenital risk factors may also play a role (5, 6). In the last decade, several inherited factors causing an hypercoagulable state have been studied in patients with deep vein thrombosis (DVT) and pulmonary embolism (PE). Resistance to activated protein C, the most common cause of inherited thrombophilia, was discovered in 1993 (7). One year later, factor V Leiden (FVL) was identified as the most frequent cause of this resistance (8). Finally, in 1996 a mutation in the prothrombin regulatory sequence was found to be another common prothrombotic factor (9). Geographic distribution of these thrombophilic abnormalities varied widely in the general population (10, 11). FVL and G20210A prothrombin mutations are more prevalent in Western Europe and are uncommon in other countries. Several large studies and meta-analyses have confirmed that these thrombophilic abnormalities are associated with an increased risk of a first episode and recurrence of DVT and PE (12–14).

The prevalence and the role of these factors in patients with PVT has been evaluated only in small studies and with conflicting or non-conclusive results.

The aims of our meta-analysis are therefore to obtain the most reliable estimates of the prevalence of FVL and G20210A prothrombin mutations among patients with PVT and to assess

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the risk of PVT associated with these two inherited thrombophilic disorders.

Methods

A protocol was prospectively developed, detailing the specific objectives, criteria for study selection, approach to assess study quality, outcomes, and statistical methods.

Study identification

We tried to identify all published studies that evaluated the prevalence of the two most common inherited thrombophilc abnormalities (FVL and G20210A prothrombin mutation) in patients with PVT using the MEDLINE (1994 to April Week 5, 2006), EMBASE (1994 to April Week 5 2006) and The Cochrane Library (2006, Issue 2) electronic databases. We excluded articles published before 1994, since this was the year when substantial evidence for the importance of activated protein C resistance/FVL began to become available. The search strategy was developed without any language restriction, and used the keywords and subject headings presented in Appendix 1 (see online at www.thrombosis-online.com). We supplemented our search by manually reviewing abstracts books from the Congress of the International Society on Thrombosis and Haemostasis (ISTH) and the reference lists of all retrieved articles, manually searching recent issues of thrombosis and haemostasis journals, and contacting content experts for additional published or unpublished trials.

Study selection

Study selection was performed independently by two reviewers (FD, MGa), with disagreements resolved through discussion and by the opinion of a third reviewer, if necessary. Studies were included if they met the following criteria: i) diagnosis of PVT was objectively confirmed (patients with PVT diagnosed with Doppler ultrasonography, computed tomography, magnetic resonance imaging, angiography or patients in which PVT was diagnosed during laparoscopy or abdominal surgery were considered eligible); ii) patients were 18 years or older; iii) patients were compared to a control group of healthy subjects without a history of thromboembolic disease or genetic relationship with the patients or to subjects with similar characteristics of cases (i.e. patients with liver cirrhosis); iv) FVL and G20210A prothrombin mutation were assesed in patients and in controls; v) other inherited or acquired factors causing an hypercoagulable state were measured in an objectively and commonly accepted manner. We excluded case series of patients and all the studies in which PVT was diagnosed only on the basis of clinical symptoms and not confirmed by an objective imaging. Only studies carried out in Western Europe were included in the analysis since in other countries FVL and G20210A prothrombin mutation are extremely uncommon (10, 11). When multiple papers on a single study had been published, we decided to use the latest publication and to supplement it, if necessary, with data from the earlier publications. Patients with DVT in other sites (e.g. DVT of the legs) without PVT were excluded from the analyses.

To assess the agreement between reviewers for study selection, we used the kappa (*k*) statistic, which measures agreement beyond chance (15).

Study validity assessment

Two unmasked investigators independently completed the assessment of study validity (FD, MGa). Because the use of quality scoring systems or quality scales in observational studies is controversial (16), the internal validity of each study was evaluated considering two potential sources of bias of case-control studies (17). Studies were considered of low quality when subjects were arbitrary excluded from either the case or control groups, and when baseline characteristics of the control group (age, gender) were not matched with the characteristics of the patients group. Otherwise, studies were considered of higher quality.

Data extraction

Two reviewers (MGa, MGi) independently extracted the following data: i) study characteristics (year of publication, design, study center); ii) patients and controls characteristics (number of subjects studied, mean age, variation in age, gender and race); iii) prevalence of FVL and of G20210A prothrombin mutation in cases and in controls.

The risk associated with coexistence of FVL and G20210A prothrombin mutation could not be evaluated because individual patient-data could not be obtained.

Disagreement was resolved by consensus and by opinion of a third reviewer (FD), if necessary. If the required data could not be located in the published report, we contacted by mail the corresponding author, with a reminder e-mail sent after fifteen days.

Statistical analysis

We used Review Manager (RevMan; version 4.2 for Windows; Oxford, England; The Cochrane Collaboration, 2003) to pool data for each risk factor. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a fixed-effects model (Mantel-Haenszel method) (18) and comparing these findings with results obtained using a random-effects model (DerSimonian and Laird method) (19). Statistical heterogeneity was evaluated using the I^2 statistic, which assesses the appropriateness of pooling the individual study results (20). The I^2 value provides an estimate of the amount of variance across studies due to heterogeneity rather than chance. $P < 0.05$ was considered to denote statistically significant heterogeneity. When heterogeneity was present, we repeated the analyses removing 1 study at time to assess the source of heterogeneity.

The proportion of PVT in the population that could be attributed to FVL or to G20210A prothrombin mutation (population attributable risk [PAR]) was estimated as follows:

PAR= 100 x [prevalence (OR-1)/ prevalence (OR-1)+1]

For this calculation, we used the fixed effects model, and we estimated the prevalence of exposure as the genotype frequency among control subjects .

Sensitivity analyses

Since our meta-analysis was not performed on individual patient data and we were unable to adjust results of principal analyses for other established risk factors for PVT (myeloproliferative disorders, liver cirrhosis), we performed a sensitivity analysis considering only patients with idiopathic PVT. We accepted the re-

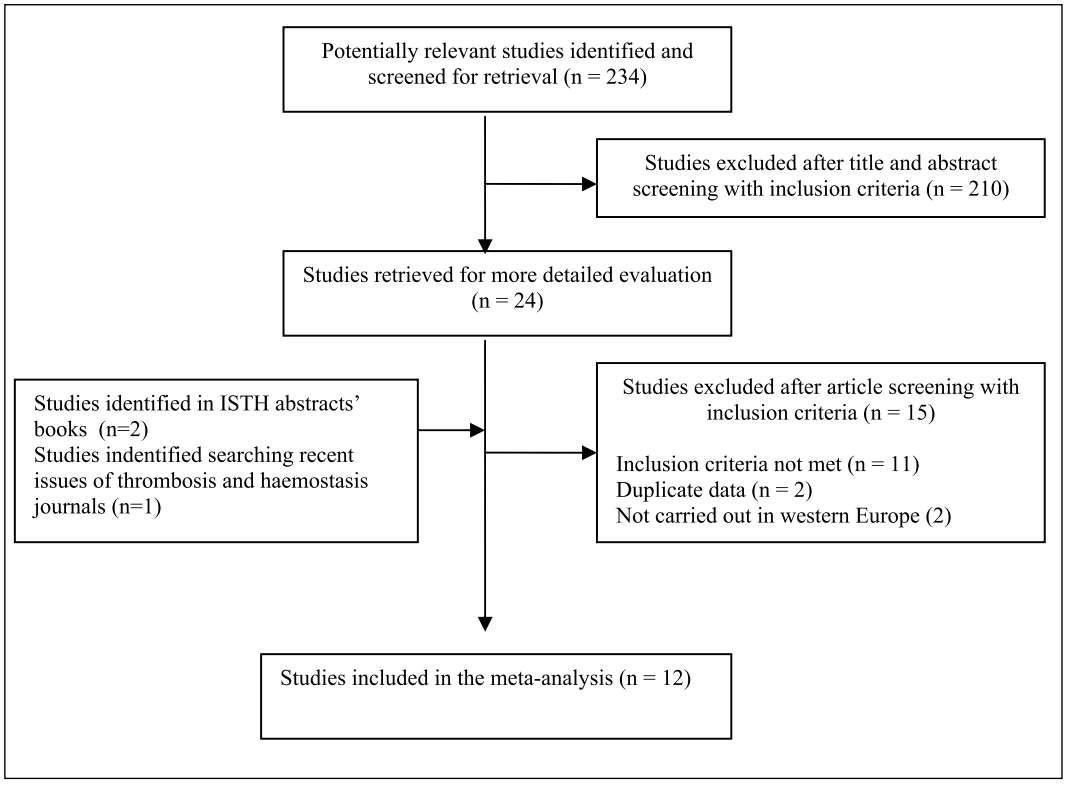

Figure 1: Studies selection progression.

ported definitions of idiopathic PVT and did not attempt to reclassify these events.

Furthermore, we performed a sensitivity analysis by examining separately patients with liver disease (i.e. patients with liver cirrhosis or HCC).

Publication bias

Presence of publication bias was explored using funnel plots of effect size against standard error (21).

Results

Study identification and selection

Three hundred studies were identified using our search strategy: 105 from Medline, 184 from Embase and 11 from The Cochrane Library (Fig. 1); 66 studies were identified in duplicate. We could exclude 210 studies after title and abstract screening using the predefined inclusion and exclusion criteria, and 24 studies were retrieved for more detailed evaluation (22–45) (Fig. 1). The inter-observer agreement for the study selection was excellent, with *k* of 0.91. Additionally, two studies were identified by manually reviewing abstracts books from the Congresses of the ISTH (46, 47) and one searching recent issues of thrombosis and haemostasis journals (48). Contact with the experts and manual review of references did not reveal any additional studies. Fifteen of the 27 studies were subsequently excluded for the following reasons: 11 did not meet inclusion criteria, two were not carried out in Western Europe, and two contained duplicate data; 12 studies were therefore included in our systematic review (22, 23, 27, 28, 30, 33, 38–40, 42, 44, 46). Supplementary data were provided by the authors of seven studies (22, 28, 30, 33, 38, 39, 42).

Studies characteristics

Baseline characteristics of included studies are summarized in Table 1. All the 12 studies included were written in English language. The number of subjects studied ranged from 30 (27) to 765 (42). Seven studies used healthy subjects as controls (23, 28, 30, 33, 38, 42, 46), five studies used subjects with similar characteristics of cases but without PVT as controls (patients with liver cirrhosis, HCC or other liver disease) (22, 27, 39, 40, 44). Seven studies provided adequate information on the prevalence of inherited thrombophilia in the subgroup of patients with idiopathic PVT (23, 28, 30, 33, 38, 42, 46).

Study quality

Only few patients were arbitrary excluded from either the case or control groups. On the other hand, only in three of 12 studies control subjects were matched with patients according to age and gender (30, 39, 46).

Factor V Leiden (FVL)

Eleven studies evaluated the role of FVL mutation in the risk for PVT (22, 23, 27, 28, 30, 33, 39, 40, 42, 44, 46). These studies included 437 cases and 2,555 control subjects. In eight studies the method used to determine the presence of FVL mutation was described (22, 23, 28, 33, 39, 40, 42, 44): in these studies detection of mutation was carried out according to the method used by Bertina et al. (5). In this population, FVL mutation was associated with an increased risk of PVT (OR 1.90; 95%CI 1.25, 2.90) (Fig. 2). There was no heterogeneity between the studies ($I^2 = 0\%$). The analysis repeated using random effect model yielded similar results (OR 2.02; 95% CI 1.32, 3.10).

Funnel plot evaluated 10 of 11 studies included in our meta-analysis, since in one study there were neither patients nor con-

Table 1: Baseline characteristics.

Study, year (reference)	Study design	Presence of thrombophilic abnormalities				Cases' mean or median age (range), years	Cases, description	Controls' mean or median age (range), years	Controls, description	Excluded patients
		RF	Cases n/N	Controls n/N	P					
Primignani, 2005 (42)	Case-control	FV FII	2/65 14/65	17/700 23/700	NS <0.001	39 (15–66)	Patients with PVT confirmed by Doppler ultrasound, CT, MRI, arteriography or during abdominal surgery	44 (12–84)	Healthy individuals	Patients with overt neoplastic diseaseliver cirrhosis.
Mangia, 2005 (44)	Case-control	FV FII	1/43 2/43	6/176 6/176	NS NS	61.6 (33–84)	Consecutive cirrhotic patients with PVT diagnosed with Doppler ultrasound and confirmed by CT or angiography	58.7 (21–84)	Consecutive cirrhotic patients without PVT	Patients with cancer of the liver, bile ducts or pancreas
Amitrano,° 2000, 2004 (24,39)	Case-control	FV FII	8/74 14/74	26/510 24/510	NS <0.001	59.3 (ND) 60 (36–75)	Cirrhotic patients with PVT diagnosed with Doppler ultrasound and confirmed by CT or MR Cirrhotic patients with PVT detected by Doppler ultrasound, confirmed by CT or angiography	59.7 (ND) 36 (10–75)	Age, gender and Child-Pugh score matched cirrhotic controls Healthy subjects	ND HCC
Samonakis, 2004 (40)	Case-control	FV FII	1/4 2/4	2/78 1/78	NS <0.001	ND	HCC patients with PVT diagnosed with Doppler ultrasound, CT or MRI	ND	Cirrhotic patients and Healthy controls without PVT	ND
Maakaroun, 2003 (46)	Case-control	FV FII	3/34 8/34	4/68 3/68	NS 0.004	48 (23–70)	Patients with idiopathic PVT	ND	Healthy age and sex- matched subjects	Patients with digestive cancer, cirrhosis, myeloproliferative disorder, local precipitating factor, recent abdominal surgery
Barcellona, 2003 (38)	Case-control	FII	0/7	24/464	NS	ND	Patients with PVT	48 (ND)	Healthy controls	ND
Bombeli 2002 (33)	Case-control	FV FII	6/42 1/23	8/120 2/80	NS NS	46.3 (27–70)	Patients with PVT diagnosed with Doppler ultrasound, CT, MRI, arteriography	37.4 (19–62)	Healthy volunteers without a history of venous or arterial thromboembolic diseases	Unknown circumstances of the thrombotic event
Madonna, 2001 (30)	Case-control	FV FII	2/21 5/21	19/313 18/313	NS 0.002	ND	Consecutive patients with PVT confirmed by Doppler ultrasound or MRI	ND	Age and sex matched healthy individuals	ND

Table 1: Continued

Janssen, 2000 (28)	Case-control	FV FII	7/92 3/92	14/474 11/474	0.003 NS	51 (19–83)	Patients with PVT diagnosed with Doppler ultrasound, CT, venography	47 (16–73)	Healthy individuals whith no history of VTE, no use of coumarin derivates within 3 months, no myeloproliferative or malignant disease	Patient with veno-occlusive disease, congestive heart failure or < 15 years of age
Gomez, 2000 (27)	Case-control	FV	0/10	0/20	NS	60 (ND)	Cirrhotic patients with PVT diagnosed with Doppler ultrasound, confirmed with CT or angiography	55 (ND)	Cirrhotic patients without PVT	ND
Chamouard, 1999 (23)	Case-control	FV FII	2/20 4/20	2/42 2/60	NS 0.001	50.4 (34–74)	Patients with idiopathic PVT documented with CT or angiography	ND	Patients without history of thrombotic disease treated for colorectal carcinoma	ND
Mahmoud, 1997 (22)	Case-control	FV	1/32	3/54	NS	47 (19–68)	Patients with PVT	43 (17–69)	Patients chosen at random from those attending the liver unit with hepatic disorders and no history of VTE	ND

RF, risk factor; n, number of patients/controls with thrombophilic abnormalities; N, total number of included patients/controls; FV, factor V Leiden mutation; FII, G20210A prothrombin mutation; PVT, portal vein thrombosis; CT, computed tomography; MR, magnetic resonance; ND, not declared; MRI, magnetic resonance imaging; HCC, hepatocarcinoma; VTE, venous thromboembolism; NS, not statistically significant.
° Data from two studies.

trols with FVL mutation (27). The plot appeared symmetric suggesting the absence of publication bias (see Appendix 2 online at www.thrombosis-online.com).

The estimated attributable risk of PVT conferred by FVL mutation was 3.44 % in this pooled cohort.

Five studies provided data on the prevalence of FVL mutation in patients with idiopathic PVT (23, 28, 30, 42, 46). In these patients the presence of FVL mutation resulted to be associated with an increased risk of PVT (OR 2.73; 95%CI 1.25, 5.96). The estimated attributable risk of PVT conferred by FVL mutation was 5.72% in this subgroup.

Five studies provided data on the prevalence of FVL mutation in patients with PVT associated with liver disease (22, 27, 39, 40, 44). In these patients, the presence of FVL mutation did not result to be statistically significant associated with an increased risk of PVT (OR 1.52; 95%CI 0.64, 3.60). The estimated attributable risk was 1.99 %.

Mutation G20210A of factor II

Ten studies evaluated the role of G20210A prothrombin mutation in the risk of PVT. In the pooled analysis, 383 patients and 2,925 controls were studied (23, 28, 30, 33, 38, 39, 40, 42, 44, 46). Seven of 10 studies described the method to detect the G20210A mutation of factor II (23, 28, 33, 38–40, 44). In these studies the method described by Poort et al. was used (6). In this population, G20210A prothrombin mutation was associated with an increased risk of PVT (OR 4.48; 95%CI 3.10, 6.48) (Fig. 3). The heterogeneity between the studies was not significant (I^2 = 33.8%). The analysis repeated using random effect model yielded similar results (OR 4.56; 95%CI 2.70, 7.70). The estimated attributable risk of PVT conferred by G20210A mutation of factor II was 11.94% in this pooled cohort.

Funnel plot appeared symmetric suggesting the absence of publication bias (see Appendix 2 online at www.thrombosis-online.com).

Six studies provided data on the prevalence of G20210A prothrombin mutation in patients with idiopathic PVT (23, 28, 30, 38, 42, 46). In these patients, the presence of the mutation resulted to be associated with an increased risk of PVT (OR 4.19; 95%CI 2.12, 8.30). The estimated attributable risk of PVT conferred by G20210A mutation of factor II was 11.50 % in this subgroup.

Three studies provided data on the prevalence of G20210A prothrombin mutation in patients with PVT associated with liver disease (39, 40, 44). In these patients, the presence of the mutation resulted to be associated with an increased risk of PVT (OR 3.68; 95%CI 1.58, 8.57), with an estimated attributable risk of PVT of 9.37%.

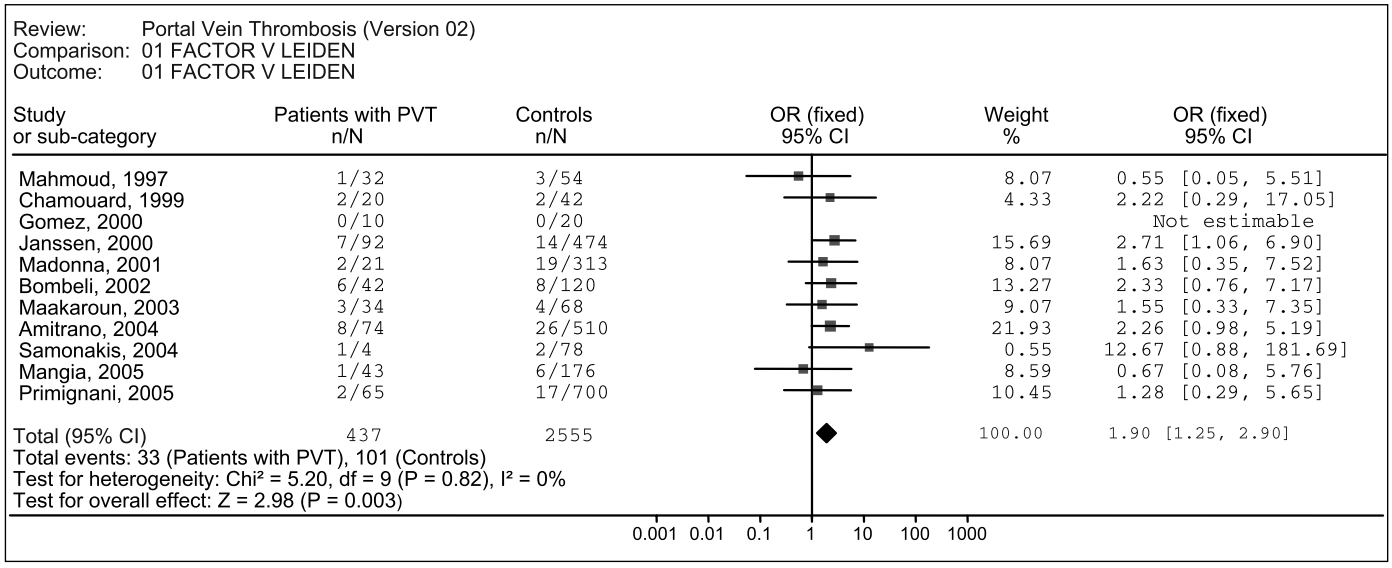

Figure 2: Odds ratio for portal vein thrombosis in factor V Leiden carriers. n, number of positive; N, total number.

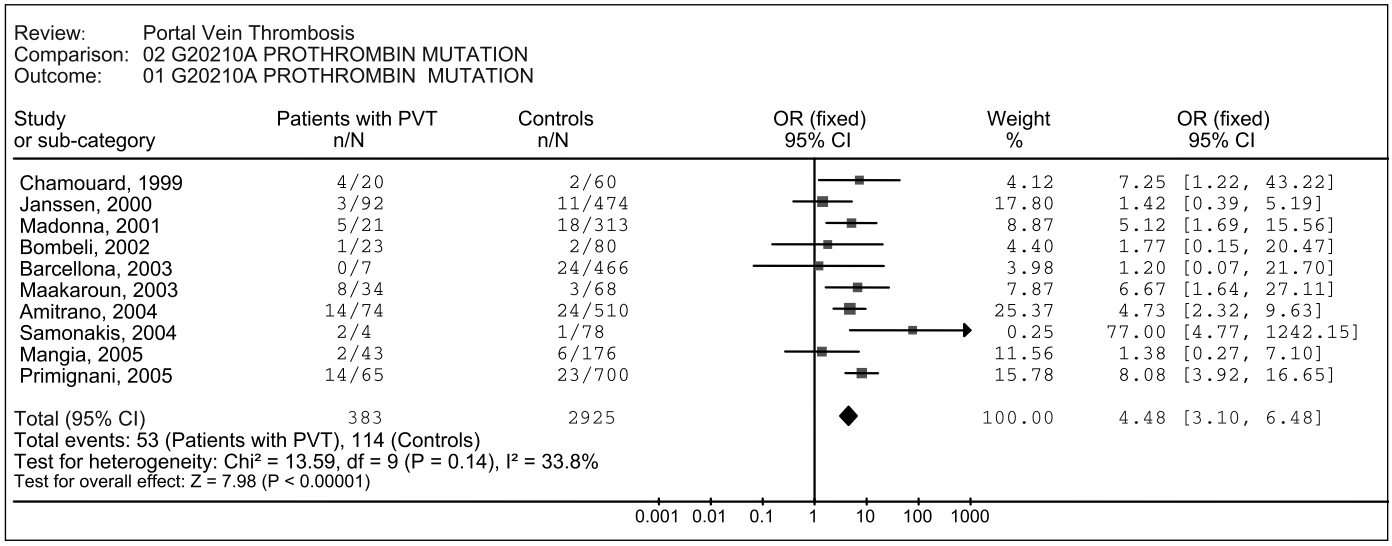

Figure 3: Odds ratio for portal vein thrombosis in G20210A factor II mutation carriers. n, number of positive; N, total number.

Discussion

This is the first meta-analysis to assess the role of inherited thrombophilia in patients with PVT.

The results of this study indicate that, considering population from Western Europe, G20210A prothrombin and the FVL mutations are more common in patients with PVT than in controls, and suggest a strong association between PVT and the G20210A prothrombin mutation and a moderate association between PVT and the FVL mutation. Our conclusions are strengthened by the uniform nature of our results, and by the narrow confidence intervals of the resulting ORs. Furthermore, results of the principal analyses were confirmed by sensitivity analyses considering only idiopathic patients.

Myeloproliferative disorders, splenectomy, and liver cirrhosis, especially if decompensated or if complicated by HCC, are established risk factors for PVT (49). Other reports suggested an association between PVT and pancreatitis or sepsis (50). Finally, other major risk factors for venous thromboembolism such as the use of oral contraceptives, pregnancy or puerperium may contribute to the development of PVT (42). However, venous thromboembolic complications may be the result of an interaction between congenital and acquired risk factors. Based on the results of our study, G20210A prothrombin mutation is not only associated with an increased risk of idiopathic PVT, but is also associated with a significantly increased risk of PVT in patients with concomitant known predisposing factors, such as the presence of liver disease. On the other hand, FVL mutation did not result to be statistically significant associated with an increased risk of PVT in patients with concomitant known predisposing factors. However, result of this analysis may be due to the small number of included patients. Testing for most common thrombophilic factors in young patients presenting with unprovoked serious

thrombotic events is currently recommended. Results of this meta-analysis suggest that the presence of G20210A prothrombin mutation should be evaluated in patients with PVT, whether or not other potential precipitating factors have been identified. On the other hand , the role of FVL in patients with precipitating factors is less clear.

Our meta-analysis has limitations. First, it was restricted to case-control studies, and the application of formal meta-analytic methods to observational studies is controversial, since bias implicit in the study design may misrepresent the strength of associations within the data (16). To minimize this potential bias, we selected only studies in which the diagnosis of PVT was objectively confirmed. Second, studies included in our meta-analysis have different size and different inclusion and exclusion criteria, and to combine results across studies may be inappropriate. However, the heterogeneity among the studies was generally low. Furthermore, after repeating the analysis using a random-effects model, an approach which accounts for some of the variance between studies, we found similar results. Third, our results should be extrapolated with caution in patients with liver cirrhosis, since only a few patients with cirrhosis were included in our meta-analysis and a separate analysis was not performed. Fourth, despite a careful review of references and contact with content experts we failed to identify any published or unpublished study not found in our initial literature search. Because it is recognized that publication bias can affect the results of meta-analyses, we attempted to assess this potential bias using a funnel plot. However, funnel plot that considered FVL and G20210A prothrombin mutation appeared symmetric suggesting the absence of publication bias. Fifth, other inherited thrombophilic abnormalities potentially associated with an increased risk of PVT, such as antithrombin, protein C, and protein S, were not considered in our meta analysis (51). However, since PVT is often associated with hepatic impairment giving rise to acquired deficiency of these factors, we considered that the real prevalence of hereditary deficiency of these coagulation factors cannot be adequately estimated in these patients. Finally, although patients with PVT not associated with clinically evident myeloproliferative disorders were considered idiopathic, presence of non clinically evident disorders may not definitively be excluded since sensitive diagnostic methods were not used in all studies.

In conclusion, our meta-analysis shows that, in the Western European population, PVT is associated with the presence of FVL and G20210A prothrombin mutation. Whether or not PVT patients should be routinely tested for thrombophilia remains to be assessed in specifically addressed studies.

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