THROMBOPHILIC ABNORMALITIES, ORAL CONTRACEPTIVES AND RISK OF CEREBRAL VEIN THROMBOSIS: A META-ANALYSIS.

SHORT TITLE: Thrombophilic abnormalities and oral contraceptives in cerebral vein thrombosis.

Francesco Dentali, MD¹, Mark Crowther, MD, MSc¹, Walter Ageno, MD²
¹ Department of Medicine, McMaster University, Hamilton, Ontario, Canada
² Department of Clinical Medicine, University of Insubria, Varese, Italy

Word count: Text 3192, Abstract 195.

Acknowledgements: Dr Crowther is a Career Investigator of the Heart and Stroke Foundation of Canada.

Corresponding author:

Dr Mark A Crowther
Room L208, St Joseph’s Hospital
50 Charlton Ave East
Hamilton, Ontario, L8N 4A6
P: 905 521 6024
F: 905 540 6568
Email:crowthrm@mcmaster.ca
ABSTRACT

Background: Recent studies suggest that thrombophilic abnormalities and the use of oral contraceptives (OC) are the leading causes of cerebral vein thrombosis (CVT).

Purpose: To assess the association between CVT and thrombophilic states, OC and their interaction.

Data Sources: The MEDLINE, EMBASE, Cochrane Library databases (January 1994 to March 2005), reference lists of retrieved articles and contact with content experts.

Study Selection: Studies comparing the prevalence of OC use and the prevalence of prothrombotic abnormalities in patients with CVT compared with healthy controls.

Data Extraction: Two reviewers independently selected studies and extracted study characteristics, quality and outcomes. Odds Ratios were calculated for each trial and pooled using the Mantel-Haenszel method.

Data Synthesis: Seventeen studies were included. There was an increased risk of CVT in patients using OC (OR, 5.59; 95% CI: 3.95 to 7.91; p<0.0001), and in patients with factor V Leiden (OR; 3.38; 95% CI: 2.27 to 5.05; p<0.00001), with mutation G20210A of prothrombin (OR 9.27; 95% CI: 5.85 to 14.67; p<0.00001) and with hyperhomocysteinemia (OR, 4.07; 95% CI: 2.54 to 6.52; p<0.00001).

Conclusion: OC-users, and patients with factor V Leiden, the prothrombin G20120A mutation and hyperhomocysteinemia are at significantly increased risk of CVT.
INTRODUCTION

Cerebral veins thrombosis (CVT) is a relatively uncommon but potentially life threatening disease. Recent series showed that as many as 8% of patients with CVT die\(^1\). In previous studies, the majority of CVT were found to be secondary to local or systemic infections and in more than 30% of cases of CVT were considered idiopathic.\(^2,\,3\) However, more recent trials reported other risk factors, such as thrombophilia or the use of oral contraceptives (OC) to be associated with CVT.\(^1,\,4\) In the last decade several inherited or acquired factors causing 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.\(^5\) One year later, factor V Leiden was identified as the most frequent cause of this resistance.\(^6\) Finally in 1996 a mutation in the prothrombin regulatory sequence was found to be a common prothrombotic factor.\(^7\) Several large studies and meta-analyses have confirmed that Factor V Leiden and G20210A of mutation of prothrombin are associated with an increased risk of DVT and PE.\(^8-10\) Large epidemiological studies have also confirmed that OC-users, particularly users of third generation OCs, are at increased risk of venous thromboembolism (VTE).\(^11\) Furthermore, the absolute risk of venous thrombosis in women who use OC and have a thrombophilic state is higher than expected from the addition of these risks suggesting a possible enhancement in their individual procoagulant effect.\(^12\)

Unfortunately, due to the rarity of this pathology, the prevalence of these factors in patients with CVT has been evaluated only in small studies and the results are often conflicting or not conclusive.

The aims of our systematic review and meta-analysis are therefore to assess the prevalence and the risk of CVT associated with different inherited and acquired thrombophilic states, to evaluate the prevalence and the risk of CVT associated with use of OC and the possible interaction between these different prothrombotic factors.

In particular, we considered the following factors: use of OC, factor V Leiden, prothrombin mutation G20210, hyperhomocysteinemia, antithrombin III, protein C, or protein S deficiency and antiphospholipid syndrome either anticardioliipin antibodies and/or lupus anticoagulants.

METHODS

STUDY IDENTIFICATION
We tried to identify all published studies that evaluated the presence of inherited or acquired risk factors for CVT using the MEDLINE (1994 to March Week 5 2005), EMBASE (1994 to 2005 Week 15) and The Cochrane Library (2005, Issue 2) electronic databases. We excluded articles published before 1994 since this was the year that substantial evidence for the importance of activated protein C resistance/factor V Leiden began to become available. The search strategy was developed in collaboration with a professional librarian, had no language restrictions, and used the keywords and subject headings presented in Appendix 1. We supplemented our search by manually reviewing the reference list of all retrieved articles and contacted content experts for additional published or unpublished trials.

STUDY SELECTION

Study selection was performed independently by two reviewers with disagreements resolved through discussion and by opinion of a third reviewer, if necessary. Studies were included if they met the following criteria: 1) diagnosis of CVT was objectively confirmed; 2) patients were 18 years or older 3) patients were compared to a control group of healthy subjects without a history of thromboembolic disease or genetic relationship with the patients 4) prevalence of at least one of the following risk factors was measured in patients and in control group: use of OC, factor V Leiden, prothrombin mutation G20210, hyperhomocysteinemia, antithrombin III, protein C, or protein S deficiency and antiphospholipid syndrome (anticardiolipin antibodies and/or lupus anticoagulant positive); 5) inherited or acquired factors causing hypercoagulable state were measured in a objectively and commonly accepted manner. We excluded case series of patients and all the studies in which CVT was diagnosed only on the basis of clinical symptoms and not confirmed by an objective imaging. When multiple papers for 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.

To assess the agreement between reviewers for study selection, we used the kappa (k) statistic, which measures agreement beyond chance. According to Maclure et al a k values higher than 0.6 are considered to represent a substantial agreement and values higher than 0.8 an almost perfect agreement.

STUDY VALIDITY ASSESSMENT

Two unmasked investigators independently completed the assessment of study validity (FD, WA). Because the use of quality scoring systems or quality scales in observational studies is
controversial, the internal validity of each study was evaluated considering two potential sources of bias of case-control studies. 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 characteristics of patient group. Otherwise studies were considered of higher quality.

DATA EXTRATION
Two reviewers independently completed data extraction. Disagreement was resolved by consensus and by opinion of a third reviewer, if necessary. The following baseline characteristics for cases and control groups were collected: number of subjects studied, mean age, variation in age, gender and race. One or more of the following risk factors were collected in each study: 1) number and proportion of patients and controls with, factor V Leiden, prothrombin mutation G20210, hyperhomocisteinemia, antithrombin III, protein C, or protein S deficiency and antiphospholipid syndrome either anticardiolipin antibodies and lupus anticoagulant positive; 2) number and proportion of women of reproductive age using oral contraceptives (pregnant, postpartum and postmenopausal women were excluded). If the required data could not be located in the published report, we contacted the corresponding author, with a reminder e-mail sent in 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 by using the Mantel-Haenszel method and a fixed-effects model. Pooled results are reported as odds ratio (OR) and are presented with 95% confidence interval (CI) and with two sided p values. A p value of 0.05 or less was considered statistically significant. Statistical heterogeneity was evaluated using the I² statistic, which assesses the appropriateness of pooling the individual study results. The I² value provides an estimate of the amount of variance across studies due to heterogeneity rather than chance. When heterogeneity was found, the analysis was repeated using the random-effects model, which includes also a measure of variance between studies. To further clarify the role of thrombophilic states and oral contraceptives as risk factors for CVT, a priori secondary analysis was planned. We decided to assess, whenever possible, the prevalence and the risk of CVT associated with different inherited and acquired thrombophilic states and associated with the use of OC, in patients without any other risk factor known to
predispose to thrombosis. Finally, funnel plots of effect size against standard error were completed, whenever possible, to assess for the presence of publication bias.20

RESULTS

STUDY IDENTIFICATION AND SELECTION
Four hundred and thirty six studies were identified using our search strategy: 187 from Medline, 346 from Embase and 20 from The Cochrane Library (Figure 1). Ninety-seven studies were identified in duplicate. We could exclude four hundred and ten studies after title and abstract screening using the predefined inclusion and exclusion criteria, and twenty-six studies were retrieved for more detailed evaluation.21-46 The inter-observer agreement for the study selection was excellent, with $k$ of 0.99. Contact with the experts and manual review of references did not reveal any additional studies. Nine of the twenty-six studies were subsequently excluded for the following reasons: eight did not meet inclusion criteria27, 28, 33, 34, 36, 40, 42, 44 and one contained duplicate data.25 Seventeen studies were therefore included in our systematic review.21-24, 26, 29-32, 35, 37-39, 41, 43, 45, 46

STUDIES CHARACTERISTICS
Of the seventeen studies included, sixteen were written in English language21-24, 26, 29-31, 35, 37-39, 41, 43, 45 and one in Chinese language.32 All included studies were case-control studies. The number of subjects studied ranged from 48 to 2285. Baseline characteristics of included studies are summarized in Table 1. Eight studies evaluated the frequency of oral contraceptives use in patients with CVT and in a control population.21-23, 29, 30, 39, 41, 45 Sixteen studies evaluated the role of different thrombophilic factors in the CVT pathogenesis21-23, 25, 29-32, 35, 37-39, 43, 45, 46: thirteen studies considered Leiden mutation of factor V22-24, 26, 29-32, 34, 38, 43, 45, 46, nine mutation G20210 of factor II22-24, 29-31, 35, 39, 46, four hyperhomocysteinemia21-23, 31, two antithrombin III, protein C and protein S deficiency23, 24, one antiphospholipid syndrome23 and one anticardiolipin antibodies37. No studies specifically studied patients with more than one hereditary or acquired prothrombotic defect, except as discussed below.

Of the four studies that evaluated the presence of hyperhomocysteinemia, two measured only fasting levels of homocysteine22, 31 and two also postmethionine load levels21, 23. Therefore we decided to pool only fasting levels of homocysteine in our meta-analysis.

STUDY QUALITY
The quality of included studies was generally high. Only few patients were arbitrary excluded from either the case or control groups. Furthermore in ten of seventeen studies control subjects were matched with patients according to age and gender.\textsuperscript{21-23, 26, 29, 35, 37, 41, 43, 45}

**ORAL CONTRACEPTIVES**

Eight studies evaluated the role of oral contraceptives in CVT.\textsuperscript{21-23, 29, 30, 39, 41, 45} Two hundred and sixty-three women with CVT and 2862 women without CVT were included in the pooled analysis. In women using OC the summary OR for developing CVT was 5.59 in comparison to controls (95% CI: 3.95 to 7.91; p<0.0001), Figure 2. Heterogeneity between the studies was significant ($I^2 = 68.4\%$; $P = .002$) and was maintained after the exclusion all the possible outliers from the analysis (data not shown). However similar OR was obtained using the random-effects model (OR, 4.79; 95% CI: 2.40 to 9.58; p<0.0001). This result probably reflects the difference among the studies in the selection of the population analyzed. Funnel plot of OR versus standard error appeared asymmetric with an absence of studies in the bottom right hand corner suggesting that smaller, unpublished studies which demonstrate an increased OR of CVT in patients taking OC were not included in our meta-analysis.

**FACTOR V LEIDEN**

Thirteen studies included 469 case and 3023 control subjects.\textsuperscript{22-24, 26, 29-32, 35, 37, 43, 45, 46} In twelve studies the method used to determine the presence of the factor V Leiden mutation was described \textsuperscript{22-24, 26, 29, 31, 32, 35, 38, 43, 45, 46}: in all studies detection of Leiden mutation was carried out by amplification of a fragment of exon 10 of the factor V and posterior digestion with an endonuclease, according to the method used by Bertina et al (6). Compared to controls the pooled OR for CVT in patients with FV Leiden mutation was 3.38 (95% CI: 2.27 to 5.05; p<0.00001), Figure 3. Heterogeneity between the studies was extremely low ($I^2 = 27.7\%$; $P = .18$).

Funnel plot of OR versus standard error evaluated 12 of 13 studies included in our meta-analysis since in one study there neither patients nor controls with Leiden mutation of factor V.\textsuperscript{32} The plot appeared symmetric suggesting absence of publication bias.

**MUTATION G20210A OF FACTOR II**

Nine studies evaluated the role of mutation G20210A in the risk of CVT.\textsuperscript{22-24, 29-31, 35, 39, 46} In the pooled analysis 360 patients and 2688 controls were studied. Eight out of nine studies described the method to detect the G20210A mutation of factor II.\textsuperscript{22-24, 29, 31, 35, 39, 46} All the
studies used the method described by Poort et al.\textsuperscript{7} The pooled OR of developing CVT was 9.27 (95% CI: 5.85 to 14.67; \( p < 0.00001 \)) in patients with mutation G20210A of factor II compared to controls, Figure 4. There was no significant between-study heterogeneity (\( I^2 = 0\% \); \( P = .49 \)). The funnel plot appeared symmetric suggesting absence of publication bias.

**HYPERHOMOCYSTEINEMIA**

Four case-control studies evaluated hyperhomocisteinemia in patients with CVT.\textsuperscript{21-23, 31} Fasting hyperhomocisteinemia was defined as above the 95\textsuperscript{th} percentile of normal population value in two studies\textsuperscript{23, 31}, as above the 90\textsuperscript{th} percentile of normal population value in one study\textsuperscript{21}, and as higher than 12 \( \mu \text{mol/L} \) in one study.\textsuperscript{22} Compared to controls the pooled OR for CVT in patients with hyperhomocisteinemia was 4.07 (95% CI: 2.54 to 6.52; \( p < 0.00001 \)), Figure 4. There was no heterogeneity between the studies (\( I^2 = 0\% \); \( P = .48 \)). In the two studies that evaluated also postmethionine load levels of homocysteine, patients with fasting or post load hyperhomocysteinemia had an OR of developing CVT of 4.3 and 4.2, respectively, compared to controls.\textsuperscript{21, 23} Due to the low number of studies, funnel plot analysis could not be done. Therefore, the presence of publication bias could not be excluded.

**OTHER RISK FACTORS**

Two studies analyzed the role of the deficiency of antithrombin III, protein C and protein S\textsuperscript{23, 24} as risk factors for CVT. Only one study considered antiphospholipid syndrome\textsuperscript{23} and one anticardiolipin antibodies.\textsuperscript{37} Antithrombin levels were measured using functional assay and then confirmed by an immunological assay. The combined OR of the two studies was 2.69 (95% CI: 0.66 to 10.96; \( p = 0.19 \)). Protein C and protein S were measured by functional and immunological assays. The combined OR of the two studies was 11.10 (95% CI: 1.87 to 66.05; \( p = 0.009 \)) for protein C and 12.49 for protein S (95% CI: 1.45 to 107.29; \( p = 0.03 \)). Due to the low number of eligible patients, the confidence interval is very wide and it is impossible to draw any major conclusion from these results.

The only study that considered the antiphospholipid antibodies syndrome\textsuperscript{23} found a higher incidence of antiphospholipid antibodies in patients with CVT (9/121) compared to controls (0/242).

Finally, Christopher et al\textsuperscript{37} evaluated the role of anticardiolipin antibodies in CVT. They found a significantly higher incidence of anticardiolipin antibodies in patients (7/31) in comparison to controls (1/31) (OR 8.75, 95% CI: 1.01 to 75.64).
OC AND THROMBOPHILIC FACTORS INTERACTIONS

Few studies provided separate analyses of OR of CVT in thrombophilic women taking OC. Therefore, it was not possible to pool these data. In their recent study, Martinelli et al stratified patients for the presence of hyperhomocysteinemia, factor V Leiden or prothrombin mutation and the intake of OC. They found that the presence of both risk factors gave an OR of 19.5 (95% CI: 5.7 to 67.3) for hyperhomocysteinemia, of 30.0 (95% CI: 3.4 to 263.0) for factor V Leiden and of 79.3 (95% CI: 10.0 to 629.4) for prothrombin mutation in comparison to controls.

The multivariate analysis performed in the study conducted by Gadelha et al confirmed the independent association between CVT, prothrombin mutation and OC use.

DISCUSSION

In the first meta-analysis published on this particular topic, we have reviewed 17 publications that analyzed the association between CVT and the most frequent prothrombotic states. We found a strong association between CVT and use of OC, the factor V Leiden mutation, the G20210A mutation of prothrombin and hyperhomocysteinemia. Our conclusions are strengthened by the uniform nature of our results, and the narrow confidence interval about the resulting ORs. Only one study failed to detect factor V Leiden mutation in both patient and control groups, but this study was carried out in Chinese patients within whom the prevalence of factor V Leiden is far lower than is seen in Caucasians. Data on antiphospholipid antibodies syndrome, deficiencies of antithrombin III, protein C, and protein S deficiency were inadequate to allow a reliable statistical analysis. However, a non statistically significant trend toward an association with the disease for antithrombin, protein C and protein S was observed. Finally, only two studies have evaluated the effect of a concomitant thrombophilia in women of reproductive age using OC. These data suggest a possible interaction between OC and some prothrombotic states (hyperhomocysteinemia, factor V Leiden, prothrombin G20210A). In particular, the risk of CVT in women on OC who are carriers of the mutation G20210A of prothrombin seemed to be much higher than in controls.

Several well-designed large trials, reviews and meta-analyses have demonstrated that use of OC and the presence of one or more thrombophilic factors is associated with an increased risk of DVT and PE. Testing for most common thrombophilic factors in young patients presenting with unprovoked serious thrombotic events is currently recommended.
recommendation supported by our observation of a strong association between thrombophilic states and CVT.

Our meta-analysis has limitations. First our systematic review 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. To minimize this potential bias, we selected only studies in which the diagnosis of CVT was objectively confirmed and in which prothrombotic factors were measured with objectively and widely accepted methods. Second, studies included in our meta-analysis have different inclusion and exclusion criteria, and to combine results across studies may be inappropriate. However, the heterogeneity between the studies, calculated using the $I^2$ statistic, was generally low. Only when we pooled studies that evaluated OC in patients with CVT was the heterogeneity between studies was remarkable and it remained high after adjustment for possible outliers. These results suggest that baseline characteristics of patients included in the analysis may be different and that results should be interpreted cautiously. However, 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, 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. Finally, despite several efforts, it was not possible to have information about the methods used to diagnose CVT in one study, in which the authors stated that CVT was objectively confirmed in all patients. However, since only 42 patients were included in this study, it is very unlikely that even in the worst case scenario (the diagnosis of CVT was wrong in all patients) this could influence the results of our meta-analysis. 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 plots appeared symmetric suggesting the absence of publication bias in the three analyses that considered factor V Leiden, mutation G20210A of the prothrombin and hyperhomocysteinemia. In the funnel plot analysis of the association of OC with CVT we observed asymmetry, with an absence of studies in the bottom right hand corner of the plot. This suggests that smaller, unpublished studies likely to demonstrate an increased risk of CVT with use of OC were not included in our meta-analysis. However, inclusion of these studies and elimination of this bias, if it really existed, would increase the observed association between OC and CVT.

In conclusion, our meta-analysis shows that CVT is strongly associated with factor V Leiden mutation, with mutation G20210A of prothrombin and with hyperhomocysteinemia. The role of
the other thrombophilic abnormalities in the pathogenesis of CVT is less clear. The use of OC significantly increases the risk of CVT in women and preliminary data suggest that such risk is further increased in OC-using women with prothrombotic abnormalities. Therefore, our observations suggest that women with CVT should avoid use of OC, and that the use of OC in women with known thrombophilic states should be evaluated on the individual basis assessing the risk of CVT for different thrombophilic abnormalities. Future studies, including careful prospective studies of patients with CVT, are now warranted to better delineate the risk of recurrent thrombosis in patients with, and without, thrombophilic defects. However, due to the rarity of the disease, it is very unlikely that these studies will be performed.
References


Table 1: baseline characteristics

<table>
<thead>
<tr>
<th>Study, Year (Reference)</th>
<th>Risk Factors</th>
<th>Cases, n</th>
<th>Mean or median age (range), years</th>
<th>Cases, description</th>
<th>Controls, n</th>
<th>Mean or median age (range), years</th>
<th>Controls, description</th>
<th>Excluded patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gadelha, 2005 (29)</td>
<td>FV, FII, OC</td>
<td>26</td>
<td>28.5 (3-46)</td>
<td>Patients &lt; 50 aa with CVT confirmed by DA or MRA</td>
<td>217</td>
<td>29 (15-62)</td>
<td>Age and racial matched healthy individuals with no history of thrombosis or genetic relationship brought by patients and volunteering physicians and health care workers</td>
<td>Patients with major systemic diseases known to predisposes to thrombosis or with any positive antiphospholipid test</td>
</tr>
<tr>
<td>Boncoraglio, 2004 (31)</td>
<td>FV, FII, Hyper-Hcy</td>
<td>26</td>
<td>43 (21-73)</td>
<td>Consecutive patients with 1st episode of CVT confirmed by DA or MRA</td>
<td>100</td>
<td>42 (21-72)</td>
<td>Healthy hospital employees</td>
<td>-</td>
</tr>
<tr>
<td>Ventura, 2004 (22)</td>
<td>FV, FII, Hyper-Hcy, OC</td>
<td>30</td>
<td>35 (16-49)</td>
<td>Patients with CVT confirmed by CT DA or MRA</td>
<td>40</td>
<td>34 (18-51)</td>
<td>Age and sex matched healthy individuals with no history of thrombosis or vascular disease</td>
<td>CVT related to other risk factors</td>
</tr>
<tr>
<td>Rodrigues, 2004 (30)</td>
<td>FV, FII, OC</td>
<td>42</td>
<td>28 (2-68)</td>
<td>Patients with objectively confirmed CVT</td>
<td>134</td>
<td>34 (-)</td>
<td>Healthy subjects with no history of thrombosis or genetic relationship with patients</td>
<td>-</td>
</tr>
<tr>
<td>Cantu, 2004 (21)</td>
<td>Hyper-Hcy, OC</td>
<td>45</td>
<td>28 (14-55)</td>
<td>Patients with CVT confirmed by DA or MRA</td>
<td>90</td>
<td>28 (16-53)</td>
<td>Age and sex matched healthy individuals with no history of thrombosis or vascular disease</td>
<td>Dead or lost to follow up patients</td>
</tr>
<tr>
<td>Martinelli, 2003 (23)</td>
<td>FV, FII, OC, Hyper-Hcy, AT III, PC, PS, APL</td>
<td>121</td>
<td>33 (12-64)</td>
<td>Patients with 1st episode of CVT confirmed by CT DA or MRA</td>
<td>242</td>
<td>36 (13-62)</td>
<td>Age and sex matched healthy individuals with no history of thrombosis</td>
<td>Patients with incomplete thrombophilia screening, uncertainty in diagnosis or previous</td>
</tr>
<tr>
<td>Study</td>
<td>FV/Other</td>
<td>Age/Range</td>
<td>Diagnosis</td>
<td>Patients with CVT confirmed by DA or MRA</td>
<td>Healthy individuals with no history of thrombosis or vascular disease and without any medication</td>
<td>Patients with unclear diagnosis of acquired or hereditary protein C or S deficiency</td>
<td>Patients &lt; 14 aa or with previous thromboembolic episodes</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Meng, 2002 (32)</td>
<td>FV</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31 (20-48)</td>
<td>50</td>
<td>Healthy individuals with no history of thrombosis or vascular disease and without any medication</td>
<td>Patients with CVT confirmed by DA or MRA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bombeli, 2002 (24)</td>
<td>FV, FII, AT III, PC, PS</td>
<td>51</td>
<td>36.7 (17-61)</td>
<td>Patients with CVT confirmed by DA or MRA</td>
<td>120</td>
<td>37.4 (19-62)</td>
<td>Patients with unclear diagnosis of acquired or hereditary protein C or S deficiency</td>
<td></td>
</tr>
<tr>
<td>Margaglione, 2001 (46)</td>
<td>FV, FII</td>
<td>28</td>
<td>-</td>
<td>Patients with CVT confirmed by DA or MRA</td>
<td>1304</td>
<td>- (22-66)</td>
<td>Healthy hospital employees</td>
<td>Patients &lt; 14 aa or with previous thromboembolic episodes</td>
</tr>
<tr>
<td>Voetsch, 2000 (35)</td>
<td>FV, FII</td>
<td>14</td>
<td>24.8 (16-31)</td>
<td>Patients with CVT confirmed by DA or MRA considered idiopathic</td>
<td>225</td>
<td>34.1 (16-50)</td>
<td>Age and sex matched volunteer physicians, students and laboratory staff</td>
<td>Patients with deficiency of natural anticoagulants or antiphospholipid antibodies. Underlying venous malformation</td>
</tr>
<tr>
<td>Reuner, 1998 (39)</td>
<td>FII, OC</td>
<td>45</td>
<td>37 (3-69)</td>
<td>Patients with CVT confirmed by CT, DA or MRA</td>
<td>354</td>
<td>39 (18-65)</td>
<td>Healthy blood donors</td>
<td>-</td>
</tr>
<tr>
<td>De Bruijn, 1998 (41)</td>
<td>OC</td>
<td>37</td>
<td>- (18-49)</td>
<td>Women with CVT confirmed by DA or MRA</td>
<td>2248</td>
<td>- (18-49)</td>
<td>Age matched selected randomly women</td>
<td>Women &lt; 18 aa or pregnant</td>
</tr>
<tr>
<td>Weih, 1998 (26)</td>
<td>FV</td>
<td>12</td>
<td>33.8 (21-60)</td>
<td>Consecutive patients with CVT confirmed by DA or MRA</td>
<td>36</td>
<td>34.2 (-)</td>
<td>Age and sex matched subjects with no history of thrombosis</td>
<td>Patients with septic venous thrombosis, neoplasm or non German descendent</td>
</tr>
<tr>
<td>Cristopher, 1998 (37)</td>
<td>ACL</td>
<td>31</td>
<td>27.6 (-)</td>
<td>Patients with CVT confirmed by CT or DA</td>
<td>31</td>
<td>26.6 (-)</td>
<td>Age and sex matched asymptomatic subjects</td>
<td>Patients with CVT related to infection or trauma</td>
</tr>
<tr>
<td>Ludemann,</td>
<td>FV</td>
<td>55</td>
<td>40</td>
<td>Patients with CVT confirmed by DA or MRA</td>
<td>272</td>
<td>-</td>
<td>Adult healthy subjects</td>
<td>-</td>
</tr>
<tr>
<td>Year</td>
<td>Study</td>
<td>Age</td>
<td>Diagnosis</td>
<td>Matched</td>
<td>Condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
<td>-----</td>
<td>-----------</td>
<td>---------</td>
<td>-----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>Zuber (43)</td>
<td>38 (20-72)</td>
<td>Patients with CVT confirmed by DA or MR imaging</td>
<td>57</td>
<td>Age and sex matched subjects with no history of cerebrovascular disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>Martinelli (45)</td>
<td>32 (21-64)</td>
<td>Patients with CVT confirmed by CT, DA or MR imaging</td>
<td>75</td>
<td>Age and sex matched subjects with no history of thrombosis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend for Table 1: ACL, anticardiolipin antibodies; ATIII deficiency of antithombin III; CT, computerized tomography; DA, digital angiography; FII mutation 20210 factor II; FV, mutation1691 factor V (Leiden); Hyper-Hcy, hyperhomocysteinemia; MR, magnetic resonance; MRA, magnetic resonance angiography; OC, oral contraceptives; PC, protein C deficiency; PS, protein S deficiency.
Figure 1: studies selection progression.

Potentially relevant studies identified and screened for retrieval (n = 436)

Studies excluded after article screening with inclusion criteria (n = 9)
- Inclusion criteria not met (n = 8)
- Duplicate data (n = 1)

Studies retrieved for more detailed evaluation (n = 26)

Studies excluded after title and abstract screening with inclusion criteria (n = 410)

Studies included in the meta-analysis (n = 17)
Figure 2: odds ratio for cerebral vein thrombosis for oral contraceptive users.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OC n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martinelli I</td>
<td>12/20</td>
<td>25/60</td>
<td>17.14 [2.10, 5.89]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>De Bruijn</td>
<td>33/37</td>
<td>1007/2248</td>
<td>12.08 [10.17, 28.79]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rouxner</td>
<td>18/31</td>
<td>15/148</td>
<td>6.47 [14.38 [5.77, 35.81]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martinelli</td>
<td>57/80</td>
<td>43/148</td>
<td>29.74 [6.05 [3.32, 11.03]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cantu</td>
<td>2/37</td>
<td>4/66</td>
<td>9.32 [0.89 [0.15, 5.08]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodrigues</td>
<td>15/225</td>
<td>5/40</td>
<td>5.27 [10.50 [3.06, 36.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventura</td>
<td>2/14</td>
<td>5/18</td>
<td>12.85 [0.43 [0.07, 2.67]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gadelha</td>
<td>16/19</td>
<td>53/134</td>
<td>7.12 [8.15 [2.26, 29.34]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>263</td>
<td>2862</td>
<td>100.00 [5.59 [3.95, 7.91]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 155 (OC), 1155 (Control)
Test for heterogeneity: Chi² = 22.12, df = 7 (P = 0.002), I² = 68.4%
Test for overall effect: Z = 9.74 (P < 0.00001)

Legend: n, number of positive; N, total number

Figure 3: odds ratio for cerebral vein thrombosis in factor V Leiden carriers.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>CVT n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martinelli I</td>
<td>5/25</td>
<td>2/75</td>
<td>3.64 [9.13 [1.65, 50.59]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zuber</td>
<td>4/19</td>
<td>1/57</td>
<td>1.80 [14.93 [1.55, 143.71]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ludemann</td>
<td>8/55</td>
<td>17/272</td>
<td>32.23 [2.55 [1.04, 6.26]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weih</td>
<td>3/12</td>
<td>1/187</td>
<td>0.41 [62.00 [5.85, 656.61]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voetsch</td>
<td>1/14</td>
<td>8/225</td>
<td>3.06 [2.09 [0.24, 17.96]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margagnone</td>
<td>0/26</td>
<td>60/1304</td>
<td>11.76 [0.36 [0.02, 5.98]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bombelli</td>
<td>7/51</td>
<td>8/120</td>
<td>18.73 [2.23 [0.76, 6.51]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meng</td>
<td>0/20</td>
<td>0/50</td>
<td>Not estimable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martinelli</td>
<td>15/121</td>
<td>7/242</td>
<td>18.60 [4.75 [1.88, 11.99]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boncoraglio</td>
<td>0/26</td>
<td>3/100</td>
<td>6.59 [0.53 [0.03, 10.50]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodrigues</td>
<td>2/42</td>
<td>3/134</td>
<td>6.20 [2.18 [0.35, 13.53]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventura</td>
<td>4/30</td>
<td>1/40</td>
<td>3.38 [6.00 [0.63, 56.74]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gadelha</td>
<td>2/26</td>
<td>3/217</td>
<td>2.70 [5.94 [0.95, 37.36]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>469</td>
<td>3023</td>
<td>100.00 [3.38 [2.27, 5.05]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 51 (CVT), 114 (Control)
Test for heterogeneity: Chi² = 15.21, df = 11 (P = 0.002), I² = 27.7%
Test for overall effect: Z = 5.96 (P < 0.00001)

Legend: n, number of positive; N, total number
Figure 4: odds ratio for cerebral vein thrombosis in G20210A factor II mutation carriers.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>CVT n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reuner</td>
<td>4/45</td>
<td>8/346</td>
<td>16.45 [4.12, 64.29]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voetsch</td>
<td>2/14</td>
<td>5/225</td>
<td>4.92 [1.29, 18.77]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margaglione</td>
<td>6/28</td>
<td>56/1304</td>
<td>14.14 [2.37, 15.58]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bombelli</td>
<td>2/28</td>
<td>2/80</td>
<td>9.44 [3.00, 22.38]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martelli</td>
<td>26/121</td>
<td>5/242</td>
<td>26.67 [9.84, 74.78]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boncoraglio</td>
<td>3/26</td>
<td>3/100</td>
<td>10.74 [4.22, 22.26]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodrigues</td>
<td>7/42</td>
<td>1/134</td>
<td>3.60 [1.73, 2.41]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventura</td>
<td>9/30</td>
<td>1/40</td>
<td>16.71 [1.98, 141.07]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 360/2688 100.00 [5.85, 14.67]

Test for heterogeneity: Chi² = 7.49, df = 8 (P = 0.49), I² = 0%
Test for overall effect: Z = 9.50 (P < 0.00001)

Legend: n, number of positive; N, total number.

Figure 5: odds ratio for cerebral vein thrombosis in patients with hyperhomocysteinemia.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>CVT n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martinelli</td>
<td>14/121</td>
<td>12/242</td>
<td>14.07 [1.12, 5.61]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boncoraglio</td>
<td>10/26</td>
<td>13/100</td>
<td>20.57 [1.57, 11.15]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cantu</td>
<td>17/45</td>
<td>9/90</td>
<td>23.26 [2.19, 13.64]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventura</td>
<td>13/30</td>
<td>4/40</td>
<td>12.10 [1.95, 24.27]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 222/472 100.00 [2.54, 6.52]

Test for heterogeneity: Chi² = 2.46, df = 3 (P = 0.48), I² = 0%
Test for overall effect: Z = 5.84 (P < 0.00001)

Legend: n, number of positive; N, total number.
APPENDIX 1: Medline search strategy

1 (intracranial embolism and thrombosis).mp. [mp=title, original title, abstract, name of substance word, subject heading word] (7436)
2 exp Cerebral Veins/ (1672)
3 exp Intracranial Thrombosis/ or exp Sinus Thrombosis, Intracranial/ (2032)
4 1 or 2 or 3 (10728)
5 exp Factor V/ (4134)
6 Antithrombin III/ (5505)
7 Protein C/ (3904)
8 Protein S/ (1375)
9 HOMOCYSTEINE/ or 5-METHYLTETRAHYDROFOLATE-HOMOCYSTEINE S-METHYLTRANSFERASE/ (6860)
10 exp Antibodies, Antiphospholipid/ (5050)
11 exp Contraceptives, Oral/ (33169)
12 Prothrombin/ (6075)
13 cerebral vein thrombosis.tw. (79)
14 cerebral venous thrombosis.tw. (548)
15 HYPERHOMOCYSTEINEMIA/ (1470)
16 4 or 13 or 14 (10833)
17 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 15 (61761)
18 16 and 17 (495)
19 limit 18 to yr=1994 - 2005 (236)
20 limit 19 to "review articles" (28)
21 19 not 20 (208)
22 limit 21 to "all infant (birth to 23 months)" (21)
23 21 not 22 (187)
Thrombophilic abnormalities, oral contraceptives and risk of cerebral vein
thrombosis: a meta-analysis

Francesco Dentali, Mark Crowther and Walter Ageno