Meta analysis of the relationship between methylenetetrahydrofolate reductase C677T and A1298C polymorphism and Venous thromboembolism in the Caucasian and Asian

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ABSTRACT

Recent years, it is a highly debated topic that whether methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and A1298C polymorphism could increase susceptibility to venous thromboembolism (VTE) in the Asian and Caucasian. Therefore, we expect to settle that controversy evidentially. Basic methods: Electronic databases (Pubmed, embase, Cochrane library, scopus, OvidSP, Wiley Online library, Springer link, EBSCO, Elsevier Science Direct, Google scholar) without date limitation were searched. Crude odds ratio (OR) along with 95% confidence interval (95% CI) was calculated to assess the association quantitatively. Finally, a total of 32 eligible studies were included, containing 31 for MTHFR C677T polymorphism and 6 for MTHFR A1298C polymorphism. The pooled results suggested that, MTHFR C677T mutation may increase susceptibility to VTE in reverse recessive model (CC+CT vs TT) : OR=0.68(0.56, 0.83), reverse dominant model (CC vs CT +TT) : OR=0.82(0.72, 0.94), heterozygote model (CT vs TT) : OR=0.65(0.52, 0.81), homozygote model (CC vs TT) : OR=0.73(0.60, 0.89) and allele model (C vs T) : OR=0.80(0.71, 0.90). Subgroup analysis about Asian also support that results, but Caucasian group not. In addition, MTHFR A1298C polymorphism may be not related to VTE in all genetic model. The results of meta-analysis indicated that MTHFR C677T polymorphism might increase the risk of VTE, especially in Asian population.

Keywords: Methylenetetrahydrofolate reductase; polymorphism; venous thromboembolism; meta-analysis
Introduction

Venous thromboembolism (VTE) is a common clinical vascular syndrome, consisting of deep vein thrombosis (DVT) and pulmonary embolism (PE), which are two different forms of the same disease [1]. At present, venous thrombosis has become the third cause of cardiovascular disease and common complications of cancer, such as lung cancer [2]. VTE is a complex multi-factor disease, in which gene mutation plays an important role [3]. However, there are great ethnic and regional characteristics of gene mutation in VTE. Therefore, exploring the susceptible genes to provide the basis for the prevention and treatment of VTE will be one of the important research directions of comprehensive therapy for vascular diseases and cancer.

Methylenetetrahydrofolate reductase (MTHFR) is homocysteine (Hey) metabolic regulatory enzyme. It could reduce N5, N10-methylene tetrahydro folic acid to N5-methyl tetrahydro folic acid, and the latter has the ability to maintain the stability of plasma Hey. The decrease of MTHFR activity will give rise to impaired Hey methylation and further hyperhomocysteinemia, which could destroy vascular endothelium and change platelet function as well as blood coagulation state, finally participating in the pathogenesis of VTE. Both the mutation of MTHFR gene at 677 site from base cytosine (C) to thymine (T) and the mutation of MTHFR gene at 1298 site from adenine (A) to cytosine (C) could cause amino acid mistranslation, further decrease MTHFR activity and increase Hey level.

In recent years, many studies about the relationship between MTHFR gene polymorphism and the risk of VTE have been reported, but with inconsistent conclusions. Some hold the view that MTHFR/C677T was a significant risk factor of VTE, which demonstrates the association of MTHFR C677T polymorphism with the susceptibility to VTE [4], but some not [5]. In addition to MTHFR C677T polymorphism, it is also a highly debated issue that whether MTHFR A1298C polymorphism could increase the susceptibility to VTE. Therefore, we conducted this meta-analysis to explore the correlation between MTHFR C677T polymorphism as well as MTHFR A1298C polymorphism and the risk of VTE, providing theoretical basis for the prevention and treatment of VTE.

Materials and methods

Search strategy and selection criteria

This systematic review and meta-analysis is reported in accordance with the Preferred Items for Systematic Reviews and Meta-analysis (PRISMA) Statement. Literature was retrieved by formal search of electronic databases (Pubmed, embase, Cochrane library, scopus, OvidSP, Wiley Online library, Springer link, EBSCO, Elsevier Science Direct, Google scholar) without date limitation. To achieve the maximum sensitivity of the search strategy, we used appropriated free text and thesaurus terms including “methyltetrahydrofolate reductase or MTHFR”, “Venous thromboembolism or VTE”, “polymorphism or mutation or variant”. We also search reference lists of related articles by hand to obtain more studies. The retrieval strategy of Pubmed is as follows: (((polymorphism[Title/Abstract] OR mutation[Title/Abstract] OR variant[Title/Abstract]) OR "Polymorphism, Genetic"[Mesh])) AND ((deep venous...
thrombosis[Title/Abstract]) OR "Venous thromboembolism"[Mesh]) AND (((Methylenetetrahydrofolate Reductase (NADPH) OR Methylene-THF Reductase (NADPH) OR Methylene tetrahydrofolate Reductase or 5,10-Methylenetetrahydrofolate Reductase (NADPH) or Methylene Tetrahydrofolate Reductase or Tetrahydrofolate Reductase, Methylene)) OR "Methylenetetrahydrofolate Reductase (NADPH2)"[Mesh])

Inclusion criteria: (1) Patients with VTE, including venous thrombosis and deep venous thrombosis; (2) Methylenetetrahydrofolate reductase C677T polymorphism and A1298C polymorphism; (3) Sufficient genotype data; (4) P value for Hardy–Weinberg equilibrium test > 0.05; (5) Case-control design.

**Data extraction and quality assessment**

Two authors independently extracted the original data. As recommended by the Cochrane Non-Randomized Studies Methods, newcastle-ottawa scale (NOS) was used to assess the quality of included researches and a total score of included studies ranging from 7 to 9 was deemed high quality. Disagreement was resolved by discussion. The extracted data were consisted of the follow items: the first author’s name, publication year, country, race, genotype distribution data, total number of cases and controls.

**Statistical analysis**

Meta-analysis was performed to calculate pooled ORs (Odds ratios) with 95% CI (Confidence interval) by using Review manager 5.3. Heterogeneity among studies was assessed by I^2 statistic. I^2>50% is indicative of heterogeneity [6], random effects model will be used. Otherwise, fixed effect will be implemented. Chi-square distribution was employed to measure the deviation of genotype distribution from Hardy–Weinberg equilibrium in control group. Subgroup analysis was conducted to explore the sources of heterogeneity and the differences between races. We also perform sensitive analysis by changing effect models. Finally, funnel plots were carried out to evaluate publication bias. The p-value <0.05 in all tests was considered significant.

**Results**

**Flowchart and Characteristic of Including Studies**

There are 32 eligible studies meeting to our inclusion criteria [4, 5, 7-36], including 31 papers for MTHFR C677T polymorphism [4, 5, 7-25, 27-36] and 6 papers for MTHFR A1298C polymorphism [12, 15, 19, 21, 26, 30]. The details of flow diagram for literature selection were shown in Figure 1. Among included studies, a total of 15 for the Asian [5, 8, 9, 11, 13, 14, 17, 18, 21-24, 27, 34, 36], mainly in China, and 17 for the Caucasian [4, 7, 10, 12, 15, 16, 19, 20, 25, 26, 28-33, 35]. Due to the comprehensive search, the publication year is from 1999 to 2019. The total sample size is nearly 20,000, containing 8223 patients and 10859 controls. The main features of eligible studies are summarized in Table 1 and table 2.
Figure 1. Flow diagram for literature selection.

Table 1. Characteristics of include studies about MTHFR C677T polymorphism.

| Author, year | Country     | Race   | Case group | Control group | pHWE NOS |
|--------------|-------------|--------|------------|---------------|----------|
| Jang, 2013   | South Korea | Asian  | 74         | 203           | 0.62     |
| Xu, 2019     | China       | Asian  | 42         | 101           | 0.10     |
| Yin, 2012    | China       | Asian  | 171        | 440           | 0.30     |
| Kailibinuer, 2012 | China   | Asian  | 22         | 88            | 0.65     |
| Wang, 2004   | China       | Asian  | 13         | 58            | 0.51     |
| Qiu, 2002    | China       | Asian  | 23         | 69            | 0.97     |
| Hsu, 2001    | China       | Asian  | 60         | 107           | 0.98     |
| Lu, 2002     | China       | Asian  | 18         | 90            | 0.73     |
| Guo, 2002    | China       | Asian  | 4          | 63            | 0.66     |
| Zheng, 2000  | China       | Asian  | 12         | 53            | 0.34     |
| Lin, 2000    | China       | Asian  | 53         | 112           | 0.75     |
| He, 2010     | China       | Asian  | 15         | 63            | 1.00     |
| Li, 2015     | China       | Asian  | 71         | 246           | 0.21     |
| Gao, 2008    | China       | Asian  | 16         | 64            | 0.21     |
| Dong, 2013   | China       | Asian  | 16         | 68            | 0.23     |
| Hsu TS, 2001 | China       | Asian  | 48         | 83            | 1.00     |
| Karmadonova, 2014 | Russia | Caucasian | 76 | 174 | 226 | 0.50 |
| Spiroski, 2008 | Macedonia | Caucasian | 20 | 63 | 11 | 0.92 |
| Bezemer, 2007 | Netherlands | Caucasian | 2044 | 4375 | 2245 | 4856 | 0.68 |
| Almawi, 2005 | America     | Caucasian | 80 | 198 | 350 | 0.07 |
| Miranda, 2002 | Netherlands | Caucasian | 67 | 233 | 186 | 0.86 |
| Zalavras, 2002 | Greece      | Caucasian | 70 | 176 | 153 | 0.30 |
| Amparo, 2010 | Spain       | Caucasian | 14 | 42 | 14 | 0.79 |
| Tawfik, 2012 | Egypt       | Caucasian | 20 | 49 | 11 | 0.01 |
| Hanson, 2001 | America     | Caucasian | 58 | 130 | 41 | 0.80 |
| Ray, 2001    | Canada      | Caucasian | 49 | 72 | 13 | 0.30 |
| Gerald, 2000 | Australia   | Caucasian | 67 | 155 | 122 | 0.84 |
| Phillip, 2000 | Canada    | Caucasian | 25 | 65 | 35 | 0.53 |
| Ben, 2012    | Tunisia     | Caucasian | 20 | 26 | 101 | 0.96 |
| Kupeli, 2011 | Turkey      | Caucasian | 49 | 80 | 26 | 0.35 |
| Lupi-Herrera, 2018 | Mexico | Caucasian | 77 | 212 | 33 | 0.45 |

pHWE: p values for Hardy–Weinberg equilibrium test

Table 2. Characteristics of include studies about MTHFR A1298C polymorphism.

| Author, year | Country     | Race   | Case group | Control group | pHWE NOS |
|--------------|-------------|--------|------------|---------------|----------|
| Hanson, 2001 | America     | Caucasian | 60 | 137 | 164 | 0.90 |
| Karmadonova, 2014 | Russia | Caucasian | 67 | 174 | 204 | 0.98 |
| Martine,1999 | France      | Caucasian | 65 | 168 | 215 | 0.49 |
| Li, 2015     | China       | Asian   | 163        | 246           | 0.92     |
| Ray, 2001    | Canada      | Caucasian | 68 | 129 | 49 | 0.86 |
| Spiroski, 2008 | Macedonia | Caucasian | 32 | 63 | 39 | 0.18 |

pHWE: p values for Hardy–Weinberg equilibrium test

Meta-analysis results

MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism

The pooled results suggested that there were significant differences in all models of MTHFR C677T polymorphism, including CC+CT vs TT: OR=0.68(0.56, 0.83)(Figure 2), CC vs CT +TT: OR=0.82(0.72, 0.94) (Figure 3), CT vs TT: OR=0.65(0.52, 0.81) (Figure 4), CC vs TT: OR=0.73(0.60, 0.89) (Figure 5) and C vs T: OR=0.80(0.71, 0.90) (Figure 6). Due to significant heterogeneity, random effect models were used in all the comparisons. Subgroup analysis showed that, for the Asian, there was no heterogeneity in all the comparisons. But for the Caucasian, no significant association was observed, which tells us the source of heterogeneity and the difference between races (Table 3).
Table 4 detailed the results of sensitive analysis, which demonstrated no significant change appeared in all pooled results after the transformation of random effect models into fixed effect models. The funnel plots showed good symmetry bias in all comparisons.

Figure 2. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (CC+CT vs TT)

Figure 3. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (CC vs CT+TT)

Figure 4. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (CT vs TT)

Figure 5. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (CC vs TT)

Figure 6. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (C vs T)

Table 3. Subgroup analysis of the relationship between MTHFR C677T polymorphism and the susceptibility to VTE
| Comparison       | Group    | OR(95%CI)       | I squares, P |
|------------------|----------|-----------------|--------------|
| CC+CT vs TT      | Total    | 0.68(0.56, 0.83) | 69%, <0.0001 |
|                  | Asian    | 0.54(0.46, 0.65) | 10%, 0.34    |
|                  | Caucasian| 0.85(0.63, 1.14) | 69%, <0.0001 |
| CC vs CT+TT      | Total    | 0.82(0.74, 0.93) | 62%, <0.0001 |
|                  | Asian    | 0.79(0.66, 0.95) | 38%, 0.06    |
|                  | Caucasian| 0.88(0.72, 1.07) | 71%, <0.0001 |
| CC vs TT         | Total    | 0.65(0.52, 0.81) | 69%, <0.0001 |
|                  | Asian    | 0.52(0.42, 0.65) | 19%, 0.24    |
|                  | Caucasian| 0.81(0.58, 1.12) | 72%, <0.0001 |
| CT vs TT         | Total    | 0.73(0.60, 0.89) | 61%, <0.0001 |
|                  | Asian    | 0.56(0.46, 0.68) | 13%, 0.31    |
|                  | Caucasian| 0.93(0.73, 1.19) | 50%, 0.01    |
| C vs T           | Total    | 0.80(0.71, 0.90) | 76%, <0.0001 |
|                  | Asian    | 0.74(0.65, 0.83) | 41%, 0.04    |
|                  | Caucasian| 0.88(0.74, 1.04) | 81%, <0.0001 |

OR, odds ratio.

MTHFR A1298C polymorphism and the susceptibility to VTE

Similar to C677T polymorphism, the comparisons of five models were conducted. As shown in Figure 7, none of any comparison exhibited significant difference statistically, with AA+AC vs CC: OR= 0.97(0.71, 1.32) (Figure 7A), AA vs AC +CC: OR=0.91(0.77, 1.08) (Figure 7B), AA vs CC: OR= 0.90(0.66, 1.23) (Figure 7C), AC vs CC: OR= 1.01(0.67, 1.52) (Figure 7D) and A vs C: OR=0.95(0.83,1.07) (Figure 7E). Because of none heterogeneity, fixed effects were adapted. Sensitive analysis also suggested our results were stable (Table 4). Publication test failed to be conducted due to small sample included.

Figure 7. MTHFR A1298C polymorphism and the susceptibility to Venous thromboembolism (A: AA+AC vs CC, B: AA vs AC +CC, C: AA vs CC, D: AC vs CC, E: A vs C)

Table 4. Sensitive analysis about MTHFR C677T and A1298C Polymorphism and VTE susceptibility
| Comparison         | Effect model | OR(95%CI)        |
|-------------------|--------------|-----------------|
| MTHFR C677T       |              |                 |
| CC+CT vs TT       | Random       | 0.68(0.56, 0.83)|
|                   | Fixed        | 0.80(0.73, 0.87)|
| CC vs CT+TT       | Random       | 0.82(0.74, 0.93)|
|                   | Fixed        | 0.92(0.87, 0.98)|
| CC vs TT          | Random       | 0.65(0.52, 0.81)|
|                   | Fixed        | 0.80(0.73, 0.88)|
| CT vs TT          | Random       | 0.73(0.60, 0.89)|
|                   | Fixed        | 0.83(0.75, 0.91)|
| C vs T            | Random       | 0.80(0.71, 0.90)|
|                   | Fixed        | 0.90(0.87, 0.95)|
| MTHFR A1298C      |              |                 |
| AA+AC vs CC       | Random       | 0.97(0.71, 1.32)|
|                   | Fixed        | 0.99(0.74, 1.33)|
| AA vs AC+CC       | Random       | 0.91(0.77, 1.08)|
|                   | Fixed        | 0.91(0.77, 1.08)|
| AA vs CC          | Random       | 0.90(0.66, 1.23)|
|                   | Fixed        | 0.91(0.67, 1.25)|
| AC vs CC          | Random       | 1.01(0.67, 1.52)|
|                   | Fixed        | 1.05(0.77, 1.43)|
| A vs C            | Random       | 0.95(0.83, 1.07)|
|                   | Fixed        | 0.95(0.83, 1.07)|

Discussion

Although some meta-analysis about the relationship between the risk of VTE and MTHFR mutation have been reported, but the objects are mainly limited to C677T and Chinese. We not only expanded the population, including the Asian and Caucasian, but also explored the association of A1298C polymorphism and VTE susceptibility. Our results showed that, in all the comparisons of the gene phenotypic model, MTHFR C677T mutation could increase the risk of VTE in the Asian, but not in the Caucasian. In addition, there may be no association between MTHFR A1298C mutation and VTE susceptibility. Sensitive analysis and publication test suggested our results were stable and reliable.

The human MTHFR gene, located on lp36.3 and with a cDNA length of 2.2kb. is composed of 11(12) exons and 10(11) introns. MTHFR plays a key role in folic acid metabolism. The gene sequence of MTHFR is high conserved. If the gene sequence of 677 base cytosine C is mutated to thymine T, the valine generated by the mutation will replace the conserved alanine, which will lead to a serious decrease in the binding ability of MTHFR to flavin adenine dinucleotide [37]. The increased risk of many diseases caused by MTHFR mutation has been reported, such as congenital heart diseases [38], coronary artery disease [39], systemic lupus erythematosus [40] and cancer [41]. MTHFR's thermal stability and enzyme activity were reduced due to the mutant T allele, resulting in hyperhomocysteine, which is an independent risk factor for VTE [42].
Zhang et al. [43] reported T allele, CT genotype, and TT genotype were associated with the risk of VTE in the Chinese population. Similar to our results, a pooled study of 3 Asian populations also showed the TT homozygous genotype could increase and the susceptibility to VTE [17]. Den et al [44] reported that, in non-north American populations, the mutant T allele increased the risk of VTE compared with the wild-type C allele, but not in north American populations. The reason may be that higher intake of folic acid and riboflavin in north American populations reduces the risk of high homocysteine in carriers of the mutant T allele. Our study demonstrated that, regardless of gene models, C677T mutation couldn’t increase VTE susceptibility in the Caucasian.

1298 site of MTHFR is located in the exon 7 and encodes regulatory region of s-adenosine methionine. Likewise, the mutation of adenine (A) to cytosine (C) in this site causes glutamate to be replaced by alanine, decreasing the phosphorylation of serine and cysteine and thus affecting the expression of MTHFR as well [45]. As another MTHFR gene mutation, the relationship between MTHFR A1298C polymorphism and the risk of disease is also explored, such as Alzheimer's disease [46] and lung cancer [47]. Our study is the first meta-analysis to explore the relationship between MTHFR A1298C polymorphism and VTE susceptibility. 6 studies were included, in which only 1 paper was from the Asian, so we didn’t conduct subgroup analysis and publication test. Finally, no significant association was observed in any comparison of all gene models.

Because of the comprehensive search, large samples were included. Subgroup analysis suggested race is the source of heterogeneity and there exists great difference between the Asian and Caucasian. Of course, there were some limitation we need point out. Owing to insufficient data provided, confounding factors, including age, gender, body mass index, smoking status, drink abuse, and other environmental factors, are difficult to fully be adjusted. Then, the controls were not uniformly defined, such as population-based controls and hospital-based controls, and the latter may not necessarily be representative of the underlying source population.

In conclusions, our study uncovered that MTHFR C677T polymorphism may increase susceptibility to VTE in the Asian, but not in the Caucasian. There may be no association between MTHFR A1298C polymorphism and VTE. Our conclusion requires further focus on the effect of gene–gene and gene–environment interaction as well as different VTE types.

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Author contribution

M Gao and N Feng conceived and designed the methods, extracted the original data, and drafted the manuscript. M Gao, MX Zhang and N Feng performed statistical analysis and interpreted results. XY Ti and XP Zuo revised the manuscript and had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of data analysis.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

OR, Odds ratio; CI, confidence interval; NOS, Newcastle-Ottawa Scale; MTHFR, Methylene-tetrahydrofolate reductase; Venous thromboembolism, VTE; pHWE: p values for Hardy–Weinberg equilibrium test; PRISMA, Preferred Items for Systematic Reviews and Meta-analysis.

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Figure 1. Flow diagram for literature selection
Figure 2. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (CC+CT vs TT)
| Study or Subgroup       | Experimental Events | Total Events | Control Events | Total | Weight | M-H, Random, 95% CI | Odds Ratio | M-H, Random, 95% CI |
|-------------------------|---------------------|--------------|----------------|-------|--------|---------------------|------------|---------------------|
| Almawi, 2005            | 80                  | 198          | 350            | 697   | 4.9%   | 0.67 [0.49, 0.93]   |            |                     |
| Amparo, 2010            | 14                  | 42           | 23             | 79    | 2.0%   | 1.22 [0.54, 2.72]   |            |                     |
| Ben, 2012               | 20                  | 26           | 101            | 197   | 1.6%   | 3.17 [1.22, 8.23]   |            |                     |
| Bezemer, 2007           | 2044                | 4375         | 2245           | 4856  | 6.6%   | 1.02 [0.94, 1.11]   |            |                     |
| Dong, 2013              | 16                  | 68           | 15             | 68    | 2.0%   | 1.09 [0.49, 2.42]   |            |                     |
| Gao, 2008               | 16                  | 64           | 14             | 64    | 2.0%   | 1.19 [0.52, 2.70]   |            |                     |
| Gerald, 2000            | 67                  | 155          | 122            | 298   | 4.3%   | 1.10 [0.74, 1.63]   |            |                     |
| Guo, 2002               | 4                   | 63           | 16             | 80    | 1.2%   | 0.27 [0.09, 0.86]   |            |                     |
| Hanson, 2001            | 58                  | 137          | 130            | 329   | 4.2%   | 1.12 [0.75, 1.68]   |            |                     |
| He, 2010                | 15                  | 63           | 26             | 75    | 2.2%   | 0.59 [0.28, 1.25]   |            |                     |
| Hsu TS, 2001            | 48                  | 83           | 43             | 82    | 2.9%   | 1.24 [0.67, 2.30]   |            |                     |
| Hsu, 2001               | 60                  | 107          | 55             | 107   | 3.3%   | 1.21 [0.70, 2.07]   |            |                     |
| Jiang, 2013             | 74                  | 203          | 140            | 403   | 4.7%   | 1.08 [0.76, 1.53]   |            |                     |
| Kalihinuer, 2012        | 22                  | 88           | 30             | 86    | 2.6%   | 0.62 [0.32, 1.20]   |            |                     |
| Karmadonova, 2014       | 76                  | 174          | 226            | 461   | 4.7%   | 0.81 [0.57, 1.15]   |            |                     |
| Kupeli, 2011            | 49                  | 80           | 78             | 104   | 2.8%   | 0.53 [0.28, 0.99]   |            |                     |
| Li, 2015                | 71                  | 246          | 97             | 292   | 4.5%   | 0.82 [0.56, 1.18]   |            |                     |
| Lin, 2000               | 53                  | 112          | 76             | 125   | 3.4%   | 0.58 [0.35, 0.97]   |            |                     |
| Liu, 2002               | 18                  | 90           | 31             | 143   | 2.7%   | 0.90 [0.47, 1.73]   |            |                     |
| Lupi-Herrera, 2018      | 77                  | 212          | 33             | 122   | 3.6%   | 1.54 [0.94, 2.51]   |            |                     |
| Miranda, 2002           | 67                  | 171          | 233            | 461   | 4.6%   | 0.63 [0.44, 0.90]   |            |                     |
| Phillip, 2000           | 25                  | 65           | 21             | 64    | 2.3%   | 1.28 [0.62, 2.64]   |            |                     |
| Qiu, 2002               | 23                  | 69           | 42             | 101   | 2.7%   | 0.70 [0.37, 1.33]   |            |                     |
| Ray, 2001               | 49                  | 129          | 72             | 129   | 3.6%   | 0.48 [0.29, 0.80]   |            |                     |
| Spiroski, 2008          | 20                  | 63           | 34             | 80    | 2.5%   | 0.63 [0.32, 1.26]   |            |                     |
| Tawfik, 2012            | 20                  | 49           | 22             | 24    | 0.7%   | 0.06 [0.01, 0.30]   |            |                     |
| Wang, 2004              | 13                  | 58           | 19             | 58    | 1.9%   | 0.59 [0.26, 1.35]   |            |                     |
| Xu, 2019                | 42                  | 101          | 70             | 120   | 3.3%   | 0.51 [0.30, 0.87]   |            |                     |
| Yin, 2012               | 171                 | 440          | 182            | 440   | 5.4%   | 0.90 [0.69, 1.18]   |            |                     |
| Kalavras, 2002          | 70                  | 176          | 117            | 300   | 4.4%   | 1.03 [0.71, 1.51]   |            |                     |
| Zheng, 2000             | 12                  | 53           | 62             | 122   | 2.3%   | 0.28 [0.14, 0.59]   |            |                     |

Total (95% CI)           | 7960                | 10567        | 100.0%         |       | 0.82 [0.72, 0.94]   |            |                     |

Total events             | 3394                | 4725         |                |       |                     |            |                     |

Heterogeneity: Tau² = 0.07; Chi² = 80.71, df = 30 (P < 0.00001); I² = 63%
Test for overall effect: Z = 2.81 (P = 0.005)

Figure 3. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (CC vs CT+TT)
| Study or Subgroup       | Experimental Events | Control Events | Total | Total Weight | Odds Ratio M-H. Random, 95% CI |
|------------------------|---------------------|----------------|-------|--------------|--------------------------------|
| Almawi, 2005           | 80                  | 121            | 350   | 427          | 4.9% 0.43 [0.27, 0.67]          |
| Amparo, 2010           | 14                  | 23             | 23    | 37           | 2.6% 0.95 [0.33, 2.76]          |
| Ben, 2012              | 20                  | 20             | 101   | 118          | 0.6% 7.07 [0.41, 122.32]        |
| Bezemer, 2007          | 2044                | 2484           | 2245  | 2762         | 5.9% 1.07 [0.93, 1.23]          |
| Dong, 2013             | 16                  | 31             | 15    | 27           | 2.7% 0.85 [0.30, 2.40]          |
| Gao, 2008              | 16                  | 30             | 14    | 25           | 2.6% 0.90 [0.31, 2.61]          |
| Gerald, 2000           | 67                  | 82             | 122   | 157          | 3.9% 1.28 [0.65, 2.51]          |
| Guo, 2002              | 4                   | 37             | 16    | 45           | 2.2% 0.22 [0.07, 0.73]          |
| Hanson, 2001           | 58                  | 74             | 130   | 171          | 4.0% 1.14 [0.59, 2.20]          |
| He, 2010               | 15                  | 36             | 26    | 39           | 2.9% 0.36 [0.14, 0.91]          |
| Hsu TS, 2001           | 48                  | 55             | 43    | 49           | 2.3% 0.96 [0.30, 3.07]          |
| Hsu, 2001              | 60                  | 67             | 55    | 63           | 2.5% 1.25 [0.42, 3.67]          |
| Jiang, 2013            | 74                  | 121            | 140   | 200          | 4.8% 0.67 [0.42, 1.08]          |
| Kailibinuer, 2012      | 22                  | 57             | 30    | 41           | 3.2% 0.23 [0.10, 0.55]          |
| Karmadonova, 2014      | 76                  | 95             | 226   | 260          | 4.2% 0.60 [0.32, 1.12]          |
| Kupeli, 2011           | 49                  | 56             | 78    | 78           | 0.6% 0.04 [0.00, 0.75]          |
| Li, 2015               | 71                  | 139            | 97    | 137          | 4.7% 0.43 [0.26, 0.71]          |
| Lin, 2000              | 53                  | 62             | 76    | 84           | 2.7% 0.62 [0.22, 1.71]          |
| Lu, 2002               | 18                  | 48             | 31    | 77           | 3.7% 0.89 [0.42, 1.87]          |
| Luki-Herrera, 2018     | 77                  | 106            | 33    | 68           | 4.1% 2.82 [1.49, 5.34]          |
| Miranda, 2002          | 67                  | 81             | 233   | 275          | 4.0% 0.86 [0.44, 1.67]          |
| Phillip, 2000          | 25                  | 37             | 21    | 29           | 2.6% 0.79 [0.27, 2.31]          |
| Qiu, 2002              | 23                  | 37             | 42    | 54           | 3.0% 0.47 [0.19, 1.18]          |
| Ray, 2001              | 49                  | 68             | 72    | 85           | 3.5% 0.47 [0.21, 1.03]          |
| Sporiski, 2008         | 20                  | 30             | 34    | 45           | 2.7% 0.65 [0.23, 1.79]          |
| Tawfik, 2012           | 20                  | 45             | 22    | 23           | 1.0% 0.04 [0.00, 0.29]          |
| Wang, 2004             | 13                  | 30             | 19    | 26           | 2.4% 0.28 [0.09, 0.87]          |
| Xu, 2019               | 42                  | 73             | 70    | 94           | 4.0% 0.46 [0.24, 0.90]          |
| Yin, 2012              | 171                 | 283            | 182   | 250          | 5.2% 0.57 [0.40, 0.82]          |
| Kalavras, 2002         | 70                  | 94             | 117   | 147          | 4.2% 0.75 [0.41, 1.38]          |
| Zheng, 2000            | 12                  | 22             | 62    | 77           | 2.7% 0.29 [0.11, 0.80]          |

| Total (95% CI)         | 4544                | 5970           | 100.0%| 0.65 [0.52, 0.81] |
| Total events           | 3394                | 4725           |       |               |

Heterogeneity: $\tau^2 = 0.22$; $\chi^2 = 96.25$, df = 30 ($P < 0.00001$); $I^2 = 69$

Test for overall effect: $Z = 3.82$ ($P = 0.0001$)

Figure 4. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (CT vs TT)
| Study or Subgroup | Experimental | Control | Odds Ratio | Odds Ratio |
|------------------|--------------|---------|------------|------------|
|                  | Events       | Total   | Events     | Total      | M-H. Random. 95% CI | M-H. Random. 95% CI |
| Almawi, 2005     | 77           | 118     | 270        | 347        | 5.0% | 0.54 [0.34, 0.84] | |
| Amparo, 2010     | 19           | 28      | 42         | 56         | 2.4% | 0.70 [0.26, 1.91] | |
| Ben, 2012        | 6            | 6       | 79         | 96         | 0.4% | 2.86 [0.15, 53.20] | |
| Bezemer, 2007    | 1891         | 2331    | 2094       | 2611       | 6.7% | 1.06 [0.92, 1.22] | |
| Dong, 2013       | 37           | 52      | 41         | 53         | 2.9% | 0.72 [0.30, 1.74] | |
| Gao, 2008        | 34           | 48      | 39         | 50         | 2.7% | 0.68 [0.27, 1.71] | |
| Geral, 2000      | 73           | 88      | 141        | 176        | 3.8% | 1.21 [0.62, 2.36] | |
| Guo, 2002        | 26           | 59      | 35         | 64         | 3.6% | 0.65 [0.32, 1.33] | |
| Hanson, 2001     | 63           | 79      | 158        | 199        | 3.9% | 1.02 [0.53, 1.95] | |
| He, 2010         | 27           | 48      | 36         | 49         | 3.0% | 0.46 [0.20, 1.09] | |
| Hsu TS, 2001     | 28           | 35      | 33         | 39         | 1.9% | 0.73 [0.22, 2.42] | |
| Hsu, 2001        | 40           | 47      | 44         | 52         | 2.1% | 1.04 [0.35, 3.12] | |
| Jiang, 2013      | 82           | 129     | 203        | 263        | 5.0% | 0.52 [0.33, 0.82] | |
| Kailbinuer, 2012 | 31           | 66      | 45         | 56         | 3.1% | 0.22 [0.10, 0.49] | |
| Karmadonova, 2014| 79           | 98      | 201        | 235        | 4.1% | 0.70 [0.38, 1.31] | |
| Kupeli, 2011     | 24           | 31      | 26         | 26         | 0.4% | 0.06 [0.00, 1.14] | |
| Li, 2015         | 107          | 175     | 155        | 195        | 5.0% | 0.41 [0.26, 0.64] | |
| Lin, 2000        | 50           | 59      | 41         | 49         | 2.3% | 1.08 [0.38, 3.06] | |
| Lu, 2002         | 42           | 72      | 66         | 112        | 4.2% | 0.98 [0.53, 1.78] | |
| Lupi-Herrera, 2018| 106         | 135     | 54         | 89         | 4.2% | 2.37 [1.31, 4.28] | |
| Miranda, 2002    | 90           | 104     | 186        | 228        | 3.9% | 1.45 [0.75, 2.79] | |
| Phillip, 2000    | 28           | 40      | 35         | 43         | 2.4% | 0.53 [0.19, 1.48] | |
| Qiu, 2002        | 32           | 46      | 47         | 59         | 2.8% | 0.58 [0.24, 1.42] | |
| Ray, 2001        | 61           | 80      | 44         | 57         | 3.2% | 0.95 [0.42, 2.12] | |
| Spiroski, 2008   | 33           | 43      | 35         | 46         | 2.5% | 1.04 [0.39, 2.76] | |
| Tawfik, 2012     | 4            | 29      | 1          | 2          | 0.4% | 0.16 [0.01, 3.11] | |
| Wang, 2004       | 28           | 45      | 32         | 39         | 2.4% | 0.36 [0.13, 1.00] | |
| Xu, 2019         | 28           | 59      | 26         | 50         | 3.4% | 0.83 [0.30, 2.29] | |
| Yin, 2012        | 157          | 269     | 190        | 258        | 5.3% | 0.50 [0.35, 0.72] | |
| Kalavras, 2002   | 82           | 106     | 153        | 183        | 4.2% | 0.67 [0.37, 1.22] | |
| Zheng, 2000      | 31           | 41      | 45         | 60         | 2.7% | 1.03 [0.41, 2.60] | |

Total (95% CI) 4566 5842 100.0% 0.73 [0.60, 0.89]

Total events 3416 4597

Heterogeneity: Tau² = 0.14; Chi² = 75.93, df = 30 (P < 0.00001); I² = 60%

Test for overall effect: Z = 3.12 (P = 0.002)

Figure 5. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (CC vs TT)
| Study or Subgroup | Experimental Events | Control Events | Total Events | Total Weight | M-H, Random, 95% CI | M-H, Random, 95% CI |
|------------------|---------------------|----------------|--------------|--------------|----------------------|----------------------|
| Almawi, 2005     | 237                 | 396            | 970          | 1394         | 4.3%                 | 0.65 [0.52, 0.82]    |
| Aman, 2010       | 47                  | 84             | 88           | 158          | 2.5%                 | 1.01 [0.59, 1.72]    |
| Ben, 2012        | 46                  | 52             | 281          | 394          | 1.3%                 | 3.08 [1.28, 7.42]    |
| Bezemer, 2007    | 5979                | 8750           | 6584         | 9712         | 5.1%                 | 1.03 [0.96, 1.09]    |
| Dong, 2013       | 69                  | 136            | 71           | 136          | 2.8%                 | 0.94 [0.59, 1.52]    |
| Gao, 2008        | 66                  | 128            | 67           | 128          | 2.7%                 | 0.97 [0.59, 1.58]    |
| Gerald, 2000     | 207                 | 310            | 385          | 596          | 3.9%                 | 1.10 [0.82, 1.47]    |
| Guo, 2002        | 34                  | 126            | 67           | 160          | 2.6%                 | 0.51 [0.31, 0.85]    |
| Hanson, 2001     | 179                 | 274            | 418          | 658          | 3.9%                 | 1.08 [0.81, 1.45]    |
| He, 2010         | 57                  | 126            | 88           | 150          | 2.8%                 | 0.58 [0.36, 0.94]    |
| Hsu, 2001        | 124                 | 166            | 119          | 164          | 2.7%                 | 1.12 [0.68, 1.82]    |
| Hsu, 2001        | 160                 | 214            | 154          | 214          | 3.1%                 | 1.15 [0.75, 1.77]    |
| Jiang, 2013      | 230                 | 406            | 483          | 806          | 4.2%                 | 0.87 [0.69, 1.11]    |
| Kallibinuer, 2012| 75                  | 176            | 105          | 172          | 3.1%                 | 0.47 [0.31, 0.73]    |
| Karmadonova, 2014| 231                 | 348            | 653          | 922          | 4.1%                 | 0.81 [0.62, 1.06]    |
| Kupeli, 2011     | 122                 | 160            | 182          | 208          | 2.4%                 | 0.46 [0.26, 0.79]    |
| Li, 2015         | 249                 | 492            | 349          | 584          | 4.2%                 | 0.69 [0.54, 0.88]    |
| Lin, 2000        | 156                 | 224            | 193          | 250          | 3.2%                 | 0.68 [0.45, 1.02]    |
| Lu, 2002         | 78                  | 180            | 128          | 286          | 3.4%                 | 0.94 [0.65, 1.37]    |
| Lui-Herrera, 2018| 260                 | 424            | 120          | 244          | 3.7%                 | 1.64 [1.19, 2.25]    |
| Miranda, 2002    | 224                 | 342            | 652          | 922          | 4.1%                 | 0.79 [0.60, 1.02]    |
| Phillip, 2000    | 78                  | 130            | 77           | 128          | 2.7%                 | 0.99 [0.60, 1.64]    |
| Qiu, 2002        | 78                  | 138            | 131          | 202          | 3.0%                 | 0.70 [0.45, 1.10]    |
| Ray, 2001        | 159                 | 258            | 188          | 258          | 3.4%                 | 0.60 [0.41, 0.87]    |
| Sprioski, 2008   | 73                  | 126            | 103          | 160          | 2.8%                 | 0.76 [0.47, 1.23]    |
| Tawfik, 2012     | 44                  | 98             | 45           | 48           | 0.8%                 | 0.05 [0.02, 0.19]    |
| Wang, 2004       | 54                  | 116            | 70           | 116          | 2.6%                 | 0.57 [0.34, 0.96]    |
| Xu, 2019         | 112                 | 202            | 166          | 240          | 3.3%                 | 0.55 [0.38, 0.82]    |
| Yin, 2012        | 499                 | 880            | 554          | 880          | 4.6%                 | 0.77 [0.64, 0.93]    |
| Kalavras, 2002   | 222                 | 352            | 387          | 600          | 4.6%                 | 0.94 [0.71, 1.24]    |
| Zheng, 2000      | 55                  | 106            | 169          | 244          | 2.8%                 | 0.48 [0.30, 0.76]    |

Total (95% CI) 15920 21134 100.0% 0.80 [0.71, 0.90]

Heterogeneity: Tau² = 0.07; Chi² = 126.39, df = 30 (P < 0.00001); I² = 76%
Test for overall effect: Z = 3.80 (P = 0.0001)

Figure 6. MTHFR C677T polymorphism and the susceptibility to Venous thromboembolism (C vs T)
Figure 7. MTHFR A1298C polymorphism and the susceptibility to Venous thromboembolism (A: AA+AC vs CC, B: AA vs AC+CC, C: AA vs CC, D: AC vs CC, E: A vs C)
