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Homocysteine, Methylenetetrahydrofolate Reductase C677T Polymorphism, and Risk of Retinal Vein Occlusion: A Meta-analysis

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Objective: To assess the role of plasma total homocysteine (tHcy) concentrations and homozygosity for the thermolabile variant of the methylenetetrahydrofolate reductase (MTHFR) C677T gene as risk factors for retinal vascular occlusive disease.

Design: Retinal vein occlusion (RVO) is an important cause of vision loss. Early meta-analyses showed that tHcy was associated with an increased risk of RVO, but a significant number of new studies have been published.

Participants and/or Controls: RVO patients and controls.

Methods: Data sources included MEDLINE, Web of Science, and PubMed searches and searching reference lists of relevant articles and reviews. Reviewers searched the databases, selected the studies, and then extracted data. Results were pooled quantitatively using meta-analytic methods.

Main Outcome Measures: tHcy concentrations and MTHFR genotype.

Results: There were 25 case-control studies for tHcy (1533 cases and 1708 controls) and 18 case-control studies for MTHFR (1082 cases and 4706 controls). The mean tHcy was on average 2.8 μmol/L (95% confidence interval [CI], 1.8–3.7) greater in the RVO cases compared with controls, but there was evidence of between-study heterogeneity ($P<0.001$, $I^2 = 93\%$). There was funnel plot asymmetry suggesting publication bias. There was no evidence of association between homozygosity for the MTHFR C677T genotype and RVO (odds ratio [OR] 1.20; 95% CI, 0.84–1.71), but again marked heterogeneity ($P = 0.004$, $I^2 = 53\%$) was observed.

Conclusions: There was some evidence that elevated tHcy was associated with RVO, but not homozygosity for the MTHFR C677T genotype. Both analyses should be interpreted cautiously because of marked heterogeneity between the study estimates and possible effect of publication bias on the tHcy findings.

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Retinal vein occlusion (RVO) is an important cause of vision loss[1,2] and has been linked to an increased risk of cardiovascular mortality and stroke.[3–5] Central RVO, even when unilateral, may be associated with reduced vision-related quality of life.[6] It has been hypothesized that elevated total plasma homocysteine (tHcy) is a risk factor in RVO,[7] as in atherosclerotic disease.[8,9] Although elevated tHcy has been implicated in vascular occlusions in the retina, a number of studies have failed to demonstrate such a relationship.[10–12]

Homocysteine, a sulphur-containing amino acid, is an intermediary product in methionine metabolism.[13] It is metabolized by 2 major pathways. When methionine is in excess, tHcy follows the transsulphuration pathway, where it is irreversibly conjugated to serine by cystathionine β-synthase in a process requiring vitamin B_6 as a cofactor. Under conditions of low methionine, tHcy is primarily metabolized through the methionine-conserving remethylation pathway. In most tissues, tHcy is remethylated in a process requiring methionine synthase, vitamin B_{12} as cofactor, and methyltetrahydrofolate as cosubstrate. The pathway requires the enzyme methylene tetrahydrofolate reductase (MTHFR) and an adequate supply of folic acid. Genetic and acquired abnormalities in the function of any of these enzymes or deficiencies in folic acid, vitamin B_6, or vitamin B_{12} cofactors can lead to elevated tHcy levels.[13] One important cause of elevated tHcy is a polymorphism in the MTHFR C677T gene, which is common in Western populations (10%–15% of individuals homozygous for this polymorphism).[14,15] Individuals homozygous for the thermolabile TT variant of MTHFR show greater levels of tHcy, particularly when serum folate levels are low.[14,15]

Two meta-analyses[7,16] that were previously performed concluded that elevated plasma tHcy was probably associated with RVO. Neither of these meta-analyses revealed a significant association between the thermolabile TT genotype and RVO risk.[7,16] Because there has been a significant number of further studies published,[10–12,17–24] we undertook an updated meta-analysis of published data on the relationships among tHcy, MTHFR C677T polymorphism, and RVO. The finding

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of a conclusive link between tHcy and RVO risk could lead to preventative measures because folic acid supplementation can reduce serum tHcy levels[25] even in those with the thermolabile MTHFR C677T polymorphism.[26]

Materials and Methods

Search Strategy and Data Extraction

Eligible studies were identified by searching MEDLINE through OVID ONLINE using this strategy ("explode 'Retinal Vein Occlusion'/all subheadings") and ("explode 'homocysteine' or 'homocysteine' keyword") or ("explode 'MTHFR' or 'MTHFR' keyword"). A similar strategy was used in searches on Web of Science and PubMed. On each database, the search was limited to studies on humans published up to and including October 2007.

Abstracts were screened independently by 2 investigators (SMcG, JW) to establish whether studies were likely to provide relevant data based on the following criteria: (1) included laboratory assessment of serum or plasma tHcy concentrations; or (2) assessed the C677T MTHFR polymorphism; and (3) compared human subjects with and without RVO.

If abstracts were considered relevant, full articles were obtained and examined. The reference lists of all relevant articles were reviewed for citations to articles.

Eligible studies were assessed independently by 3 reviewers (SMcG, JVW, CC) using a structured form to extract information about the study (country and year of publication), study subjects (number of cases and controls, selection of cases and controls, age), and tHcy and MTHFR data. A number of possible indicators of study quality were also extracted, including closeness of age-matching of cases and controls, use of fasting glucose samples, and pre-statement of exclusion criteria. The extracted datasets were cross-checked before analysis was performed.

Initially, corresponding authors from each study were contacted to ask for the original dataset used for study analysis, but this approach had limited success, because the investigators either did not respond to our requests for data or were unable to provide access to original datasets.

Statistical Methods

Homocysteine. The difference in mean tHcy between the RVO and control groups and 95% confidence intervals were calculated for each study. In 3 studies,[17,27,28] the mean and standard deviation were estimated from formulae using the median and range.[29] In one study,[30] the mean and standard deviation were estimated from formulae using the median and interquartile range.[31] In another study,[32] the standard deviation estimates were not available within groups, so the standard error of the difference in means was estimated from the P value of a t test.[33]

Because the distribution of tHcy is typically slightly skewed, sensitivity analyses were conducted on log-transformed data where patient-level data were available, and including only those studies with more than 35 patients in each group (in which non-normality would be less problematic). An additional sensitivity analysis was conducted removing studies for which formulae were applied to calculate the mean and standard deviations.

Methylenetetrahydrofolate Reductase. Odds ratios (OR) and 95% CIs were calculated for the MTHFR TT genotype exposure and RVO. In one study,[34] no TT genotype was observed in the RVO group, so a correction was added to provide an estimate of the OR[35] and standard error.

The I^2 statistic was used to quantify the inconsistency between study estimates, and chi-square tests were used to formally test for heterogeneity. Publication/selection bias was investigated by checking for asymmetry in funnel plots.[36]

Where appropriate, random-effect models were used to calculate pooled estimates.[35] Study-specific weights in the random-effects model were calculated and scaled to percentages. The analysis was repeated in European and non-European studies, in studies in which fasting samples were taken, in studies in which the cases and controls were closely age matched (to within 3 years), and in studies in which greater than 50% of the cases had CRVO or BRVOs. The analysis was also repeated, broadly grouping studies by control source (details in Tables 1 and 2).

Publication bias/funnel plot asymmetry was formally tested using Begg's[37] and Egger's test.[38] A further sensitivity analysis was conducted using the Trim and Fill method to calculate pooled estimates after adjustment for suspected publication bias/funnel plot asymmetry.[39] Finally, meta-regression techniques were used to investigate the association between the study characteristics and the observed estimates. All statistical analyses were performed using STATA 9.0 (Stata Corporation, College Station, TX).

Results

Total Homocysteine

By using the above search criteria, we found 47 articles for tHcy and RVO. On individual examination of each of these, 36 fitted the inclusion criteria for tHcy.[10–12,17–24,27,28,30,32,40–60] The remaining 11 articles were meta-analyses,[7,16] review articles,[61] commentaries/letters,[62,63] case reports,[64–69] or series. On closer examination, 2 articles were duplicate publications of the same dataset,[48,57] whereas a further 9 articles did not present the data in a suitable format that could be used for meta-analysis,[41,42,46,47,49–51,55,56] for example, presenting the tHcy data as percentage of the population with elevated levels[41,42,50] or not reporting tHcy concentrations or variance in a control groups.[47]

Original data were available from only a limited number of studies (n = 7).[10,27,40,44,45,59,60] Thus, the patient-level data were used only for sensitivity analyses.

For the meta-analysis of tHcy we used 25 case-control studies, and the characteristics of these are summarized in Tables 1 and 2. A total of 1533 RVO cases and 1708 control subjects were included.

The forest plot for tHcy and RVO is shown in Figure 1. There was evidence of a greater mean tHcy in the cases compared with the controls in the majority of studies. Overall, the mean tHcy in the cases was 2.8 μmol/L (95% CI, 1.8–3.7) greater than in the controls, but there was strong evidence of between-study heterogeneity ($P<0.001$, $I^2 = 93\%$), suggesting the need for cautious interpretation.

Heterogeneity persisted despite reanalysis in the following subsets of studies: (a) European studies (European n = 14, $P<0.001$, $I^2 = 90\%$); (b) non-European studies (non-European studies = 11, $P<0.001$, $I^2 = 91\%$); (c) studies with similar cases and control ages (number of studies = 18, $P<0.001$, $I^2 = 94\%$); (d) studies in which both case and control groups contained more than 35 participants (number of studies = 19, $P<0.001$, $I^2 = 93\%$); (e) studies in which no approximations were used to impute means or standard errors (number of studies = 20, $P<0.001$, $I^2 = 92\%$); (f) studies for which we had individual patient data (number of studies = 7, $P<0.001$, $I^2 = 85\%$); (g) studies using only fasting samples (number of studies = 18, $P<0.001$, $I^2 = 93\%$); (h) studies with more than 50% CRVO cases (number of studies = 17, $P<0.001$, $I^2 = 93\%$) and studies with less than 50% CRVO cases (number of studies = 7, $P<0.001$, $I^2 = 90\%$). A further analysis by type of controls was performed on 3 groups of studies (Table

Table 1. Characteristics of Studies Examining Plasma Homocysteine and Retinal Vein Occlusion Used in Meta-analysis: Cases

First Author (Reference)	Publication Year	Country of Origin	Fasting	Cases				
				n	*BRVO*	*CRVO*	*Age, yr Mean (SD)*	*Ascertainment (Time Since Diagnosis)*
Cahill[45]	2000	Ireland	yes	61	21	40	69 (—)	Hospital records (3–12 mos)
Vine[58]	2000	US	no	74	0	74	70 (range 40–88)	Hospital records (<10 yrs)
Larsson[52a]	2000	Sweden	no	37	0	37	41 (range 20–49)	Hospital records (<4 yrs)
				79		79	70 (range 50–91)	
Pianka[54]	2000	Israel	no	21	0	21	59 (12)	Consecutive patients
Martin[53]	2000	UK	yes	60	24	36	66 (13)	Consecutive patients
Marcucci[27]	2001	Italy	yes	100	0	100	Median 59 (range 18–77)	Consecutive patients (<2 yrs)
Boyd[30]	2001	UK	no	63	0	63	60 (16)	Consecutive patients (<3 mos)
Brown[44]	2002	US	yes	20[b]	15	3	69 (11)	Hospital records (<6 mos)
Weger[59]	2002	Austria	yes	84	84	0	68 (11)	Consecutive patients
Weger[60]	2002	Austria	yes	78	0	78	69 (11)	Consecutive patients
El-Asrar[40]	2002	Saudi Arabia	no	48	12	36	45 (11)	Consecutive patients ("recently diagnosed")
Blondel[43]	2002	France	—	101	16	85	54 (14)	Consecutive patients
Marcucci[28]	2003	Italy	yes	55	29	26	Median 57 (range 18–82)	Consecutive patients (<2 yrs)
Parodi[32]	2003	Italy	yes	31	0	31	45 (—)	Consecutive patients (<1 wk)
Yildirim[17]	2004	Turkey	yes	33[b]	20	9	61 (range 37–79)	Consecutive patients (<6 mos)
Yaghoubi[18]	2004	Iran	yes	25[b]	10	14	61 (12)	Hospital records
Atchaneeyasakul[11]	2005	Thailand	yes	32[b]	15	11	54 (13)	Consecutive patients (<2 wks)
Terrazzi[19]	2005	Italy	yes	69	—	—	64 (15)	Consecutive patients (<1 wk)
McGimpse[10]	2005	UK	no	100	43	57	68 (14)	Hospital records
Lattanzio[20]	2006	Italy	no	58	0	58	40 (10)	Consecutive patients (<1 yr)
Gao[23]	2006	China	yes	64	0	64	60 (4)	Consecutive patients (<3 mos)
Gumus[24]	2006	Turkey	yes	82	56	26	58 (9)	Consecutive patients/hospital records
Pinna[12]	2006	Italy	yes	75	42	33	64 (15)	Consecutive patients
Narayanasamy[21]	2007	India	yes	29	0	29	30 (6)	Consecutive patients
Moghimi[22]	2007	Iran	yes	54	0	54	60 (13)	(<1 mo)

BRVO = branch retinal vein occlusion; CRVO = central retinal vein occlusion; SD = standard deviation.
[a]Data presented in 2 age groups: <50 yrs and >50 yrs.
[b]Includes others (e.g., hemi-retinal, hemispheric, macular).

2): Controls who were hospital staff or healthy volunteers and people with cardiovascular disease were excluded (number of studies = 9, $P<0.001$, $I^2 = 93\%$): studies with controls who were patients attending hospital (usually ophthalmology outpatients clinics but without known retinal disease) (number of studies = 9, $P = 0.36$, $I^2 = 8\%$) and studies in which the control group was unclear (number of studies = 7, $P<0.001$, $I^2 = 89\%$). The estimates from studies in which control patients had mostly other diseases were fairly homogenous.[18,10,22,24,30,45,58,59,60] The mean tHcy was 1.83 μmol/L (95% CI, 1.3–2.3; $P<0.001$) greater in cases than in the controls in this group.

The funnel plot in Figure 2 did not conform to the expected funnel shape. There is some evidence that the larger studies observed smaller differences in mean between the cases and controls than the smaller studies. The shape of the observed funnel plot could have arisen from the non-publication of small studies, which observed no difference in mean, or a slight reduction in mean, in the cases compared with the controls, although other explanations are possible. Formal tests revealed evidence of funnel plot asymmetry perhaps due to publication bias (Begg's test $P = 0.012$, Egger's test $P = 0.08$). However, the pooled estimate was unaltered (overall difference in mean = 2.8 μmol/L) after conducting the Trim and Fill method to attempt to adjust for funnel plot asymmetry/publication bias, suggesting that any such bias had little effect on the overall findings.

Finally, meta-regression techniques demonstrated little evidence of an association ($P = 0.39$) between the difference in mean tHcy between cases and controls and the difference in mean ages between the cases and controls.

Methylenetetrahydrofolate Reductase

We found 25 published journal articles on MTHFR and RVO, of which 18 fitted the inclusion criteria.[10,19,27,28,30,32,35,41,47,49,52,59,60,70–74] Exclusions were 3 case reports/series,[68,75,76] 2 meta-analyses,[7,16] 1 review,[77] and 1 article reporting duplicate data.[48]

A total of 1082 RVO cases and 4706 controls were included in the analysis. The Forest plot for thermolabile MTHFR polymorphism and RVO is shown in Figure 3. Overall there was no evidence of any association between MTHFR and RVO (OR = 1.20; 95% CI, 0.84–1.71), but there was marked heterogeneity ($P = 0.004$, $I^2 = 53\%$) between studies.

For MTHFR, the funnel plots demonstrate the expected pattern without significant departure from symmetry (Fig 4). There was also no evidence of publication bias after formal testing (Begg's test $P = 0.43$, Egger's test = 0.30).

Discussion

We performed a meta-analysis to determine the association among tHcy, MTHFR C677T genotype, and RVO risk. We found a statistically significant association between

Table 2. Characteristics of Studies Examining Plasma Homocysteine and Retinal Vein Occlusion Used in Meta-analysis: Controls

First Author (Reference)	Publication Year	Country of Origin	Fasting	Controls		
				n	*Age, yr Mean (SD)*	*Source (Matching Criteria)*
Cahill[45]	2000	Ireland	yes	87	70 (—)	Hospital patients, primarily cataract extraction (age)[d]
Vine[58]	2000	US	no	74	65 (range 37–90)	Hospital patients with non-retinal vascular diagnosis[d]
Larsson[52a]	2000	Sweden	no	65	41 (range 20–49)	Unknown, "randomly selected" (age)[e]
				88	70 (range 50–88)	
Pianka[54]	2000	Israel	no	81	66 (18)	Unknown, "healthy adults"[c]
Martin[53]	2000	UK	yes	85	52 (15)	Laboratory staff/hospital patients, no cardiovascular disease[c]
Marcucci[27]	2001	Italy	yes	100	Median 56 (range 18–84)	Friends/partners, no cardiovascular disease (age, sex)[c]
Boyd[30]	2001	UK	no	63	61 (16)	Clinic patients[d]
Brown[44]	2002	US	yes	20	70 (7)	Unknown, "normal subjects" with no diabetes[be]
Weger[59]	2002	Austria	yes	84	68 (11)	Hospital patients, no anterior ischemic optic neuropathy or vasculitis (age, sex)[d]
Weger[60]	2002	Austria	yes	78	69 (11)	Hospital patients, no anterior ischemic optic neuropathy or vasculitis (age, sex)[d]
El-Asrar[40]	2002	Saudi Arabia	no	59	46 (12)	Healthy blood donors (age, sex)[c]
Blondel[43]	2002	France	—	29	51 (10)	Source not given, (age)[e]
Marcucci[28]	2003	Italy	yes	61	Median 56 (range 20–80)	Friends/partners, no cardiovascular disease or venous thromboembolism (age, sex)[c]
Parodi[32]	2003	Italy	yes	31	44 (—)	Unknown, "volunteers" (age, sex)[be]
Yildirim[17]	2004	Turkey	yes	25	58 (range 47–72)	Unknown (age, sex)[e]
Yaghoubi[18]	2004	Iran	yes	24	63 (14)	Clinic patients, no glaucoma, uveitis or intraocular trauma[d]
Atchaneeyasakul[11]	2005	Thailand	yes	88	54 (13)	Unknown, "volunteers" (age, sex)[e]
Terrazzi[19]	2005	Italy	yes	50	58 (12)	Unknown, "volunteers" (age)[e]
McGimpse[10]	2005	UK	no	91	68 (14)	Clinic patients (primarily cataract surgery)/friends/relatives (age, sex)[d]
Lattanzio[20]	2006	Italy	no	103	40 (13)	Hospital staff[c]
Gao[23]	2006	China	yes	64	60 (4)	Volunteers undergoing routine physical examination (age, sex, hypertension, smoking, drinking)[c]
Gumus[24]	2006	Turkey	yes	78	57 (10)	Clinic patients with refractive errors, presbyopia, or cataract (age, sex)[d]
Pinna[12]	2006	Italy	yes	72	64 (8)	Friends/partners/hospital staff[c]
Narayanasamy[21]	2007	India	yes	57	27 (5)	Hospital staff/students (age, sex)[c]
Moghimi[22]	2007	Iran	yes	51	63 (9)	Clinic patients, no retinal disease (age, sex, hypertension, diabetes, smoking)[d]

SD = standard deviation.
[a]Data presented in 2 age groups: <50 yrs and >50 yrs.
[b]Study included 2 controls; group information represents control group used in meta-analysis.
[c]Classified as healthy controls, volunteers, or excluding cardiovascular disease.
[d]Studies using mainly controls with other diseases and not excluding cardiovascular risk factors.
[e]Studies in which the control group was unclear.

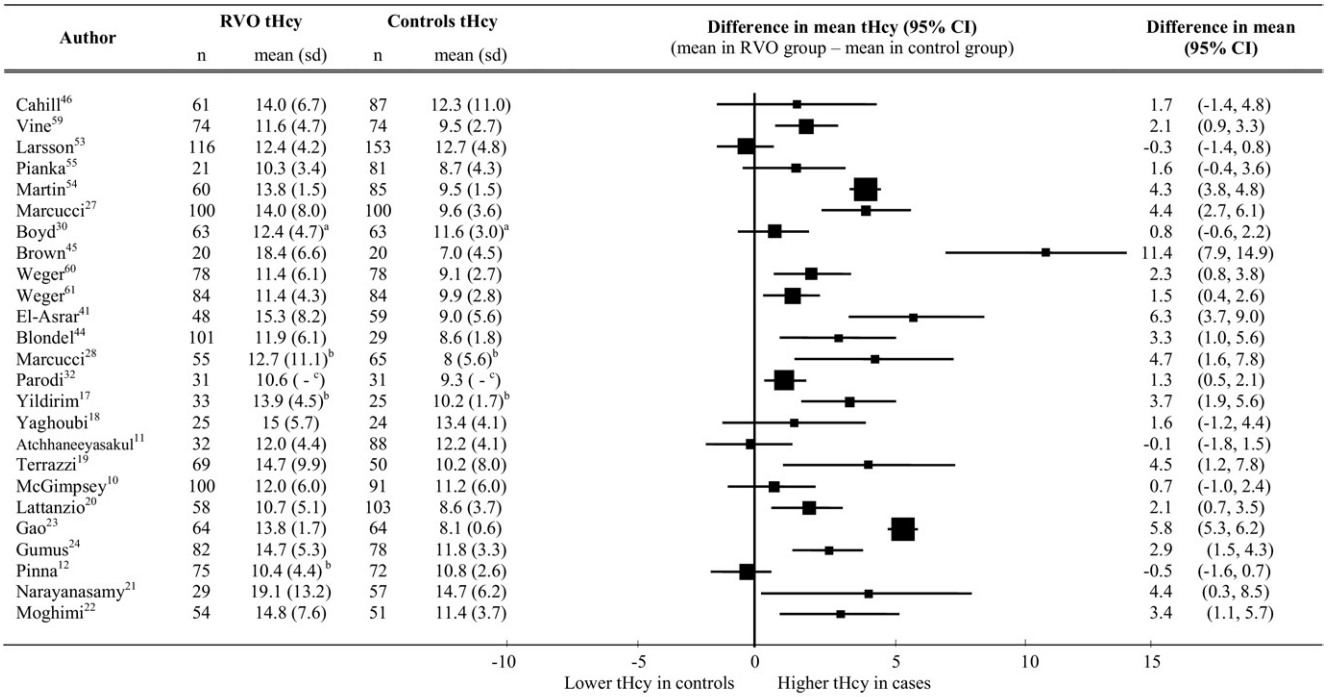
increased tHcy serum levels and RVO risk. We did not find any association between MTHFR C677T genotype and RVO risk. Although our combined estimates are similar to 2 previous meta-analyses, the inclusion of 15 additional studies have highlighted marked heterogeneity in the associations between studies from unidentified sources. Also, in contrast with these previous meta-analyses, funnel plots indicated that the association between tHcy and RVO seems less marked in larger studies, perhaps because of publication bias.[7,16]

Study Heterogeneity

One of the strengths of this analysis was the exploration of the heterogeneity of the association between tHcy and RVO. However, our attempts to identify homogeneous subsets were largely unsuccessful, except for a subgroup of studies in which the control group comprised hospital patients, making any firm conclusions difficult.

We attempted to reduce heterogeneity by considering the geographic origin of studies, classifying them as European and non-European, because a previous meta-analysis of MTHFR C677T polymorphism and coronary heart disease risk showed that heterogeneity disappeared when studies were stratified by geographic region.[78] However, this was not observed in the current study.

Homocysteine concentrations are greater in the non-fasting than fasting state, and therefore it is recommended that blood samples are acquired after a period of fasting.[13] This is not always possible when opportunistic sampling methods are used and only two thirds of the studies included in the meta-analysis had used fasting glucose samples. However, exclusion



*Test for heterogeneity χ^2= 327.77, df=24, P<0.001; I^2 (95% CI) = 93% (90%, 94%).

[a] Mean and standard deviation (sd) estimated from the median and the interquartile range.[31]
[b] Mean and standard deviation (sd) estimated from the median and range.[29]
[c] Standard deviation estimates not available within groups, standard error of difference in means estimated from P-value of t-test.[33]
Confidence interval (CI).

Figure 1. Forest plot of studies of tHcy (μmol/L) and RVO, ordered by date of publication. CI = confidence interval; RVO = retinal vein occlusion; SD = standard deviation; tHcy = total homocysteine.

of studies that did not use fasting samples did not reduce heterogeneity.

Summary statistics published by the authors were used for the meta-analysis because patient-level data were not available to us in the majority of the studies. However, when we carried out a subgroup analysis that was limited to those studies for which we had patient-level data, the estimates of risk increase were similar, and heterogeneity was not reduced.

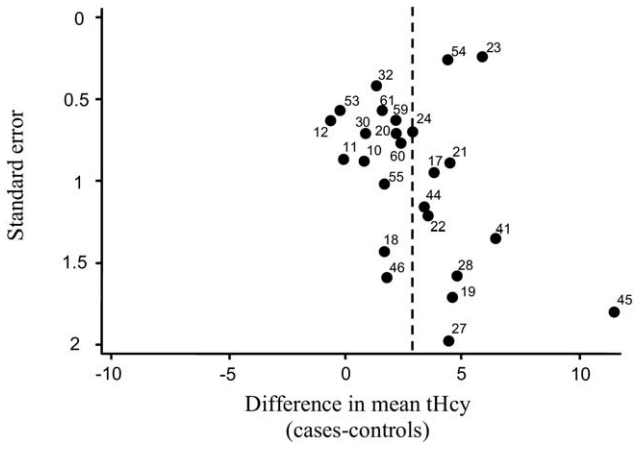
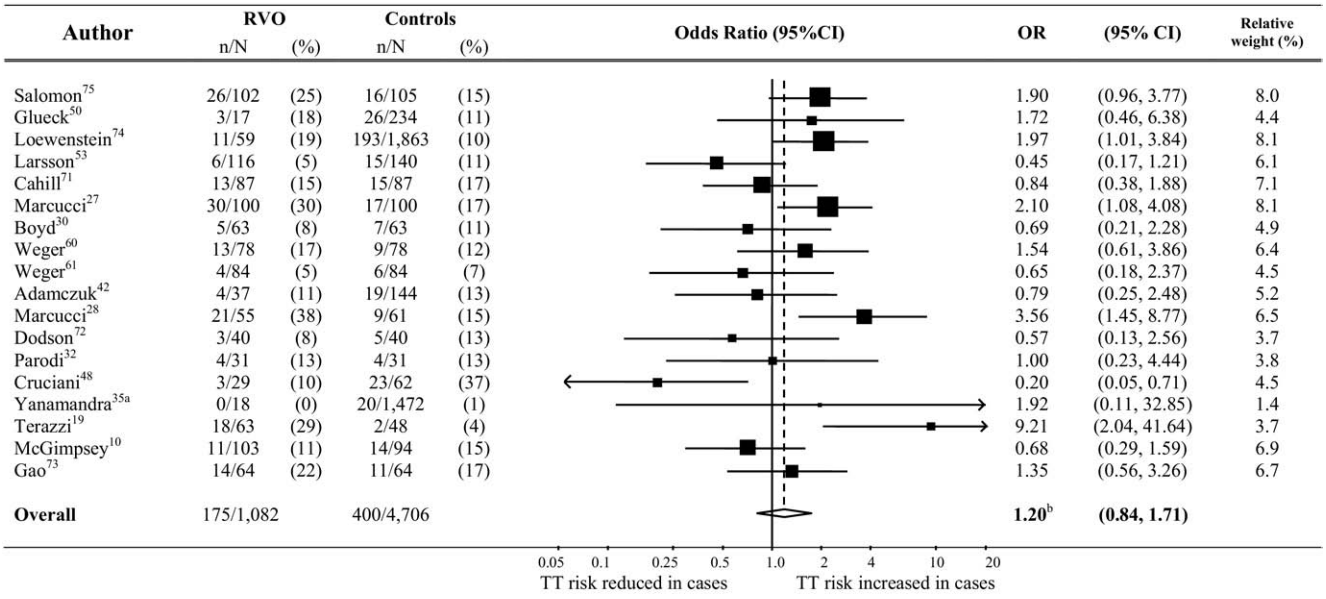


Figure 2. Funnel plot of studies of tHcy and RVO, labeled by reference number. Dashed line denotes combined mean. tHcy = total homocysteine.

A further possible reason for heterogeneity in this meta-analysis is that we included both branch and central RVO, because in most of the studies a distinction was not made between these 2 disease groups. It has been suggested that, although central RVO is associated with cardiovascular risk factors, branch RVO may be more influenced by local factors such as atherosclerotic retinal arteries compressing retinal veins at arteriovenous crossings, and therefore the cause of these conditions may be different.[45] We did attempt to address this and repeated the analysis in studies in which more than 50% of cases had CRVO and in studies in which more than 50% of cases had BRVO, but in both subgroups there was a high level of heterogeneity and the difference in tHcy between cases and controls in these 2 groups was similar, indicating that the role of tHcy is unlikely to differ in the cause of these 2 conditions.

A crucial consideration in case-control studies is the selection of the control group. An ideal control group in a case-control study will be as similar as possible to the cases, except that they would not have the disease being investigated. We found wide variability in the source of control group between the studies that could have lead to the observed heterogeneity of our results. The controls in



n, number with MTHFR TT allele; N, total number in RVO and control groups.
[a] Correction added to calculate odds ratio (OR) and 95% confidence interval (CI) [36].
[b] Test for heterogeneity $\chi^2 = 36.5$, df=17, P=0.004; I^2 (95% CI) = 53% (20%, 73%).

Figure 3. Forest plot of studies investigating the association between thermolabile MTHFR genotype (comparing TT heterozygotes with heterozygotes [CT] and wild-type homozygotes [CC]) and RVO using the random effects model, ordered by date of publication. CI = confidence interval; OR = odds ratio; RVO = retinal vein occlusion.

the studies usually comprised hospital staff, healthy volunteers, or patients attending hospital (usually ophthalmology outpatient clinics, but without known retinal disease), whereas in some studies the source of controls was not clearly stated. There are strengths and weaknesses in the use of either hospital- or community-based controls. tHcy has been shown to be elevated in several diseases, including cardiovascular disease, and the use of healthy controls or volunteers (e.g., excluding those with cardiovascular disease) may enhance mild associations with a phenomenon referred to as the "healthy participant effect." Therefore, the use of hospital-based controls may be more appropriate here because these patients' characteristics may be closer to that of the case group.

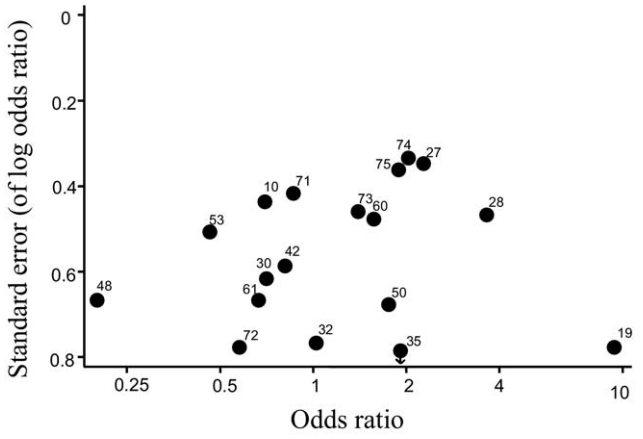


Figure 4. Funnel plot of studies investigating the association between thermolabile MTHFR genotype (comparing TT genotype with heterozygous CT and homozygous CC genotypes) and RVO.

An observed greater tHcy concentration in RVO cases than in hospital-based controls is then likely to be due to RVO rather than the presence of other conditions. We observed less heterogeneity in studies that recruited their control patients from those attending a hospital. However, even within this group of studies there was variability in how controls were selected. Although this subgroup did demonstrate a significant association between tHcy and RVO, the difference between cases and controls was lower than that observed within the overall meta-analysis. Thus, we recommend that our results be interpreted cautiously, because many subsets of studies were investigated. Nonetheless, it is reassuring that the overall findings were similar within these studies.

The difference in mean ages between the cases and controls could account for some of the between-study variability because there is evidence showing that tHcy increases with age.[13] For example, in one study[53] cases were on average 14 years older than their controls. However, there was no evidence of more homogenous findings in the group of studies with an age difference between cases and controls of less than 3 years. Furthermore, meta-regression showed no evidence of a correlation between the difference in mean tHcy between cases and controls and the difference in mean age between cases and controls. These analyses, although rather crude, suggest that a difference in age between cases and controls does not account for much of the variability in the study estimates.

Limitations of the Meta-analysis

The case-control study design means that the assessment of tHcy in patients at varying time intervals after the

occlusive vascular event is methodologically weak. The vascular occlusive event itself could increase the tHcy concentration, and this type of reverse causality is supported by studies of tHcy and coronary heart disease in which evidence from longitudinal cohorts demonstrate weaker associations than those found in the case-control scenario.[8]

We were also unable assess the contribution of confounding factors to the observed results. tHcy is elevated in several conditions, including cardiovascular disease,[8,9] and is positively associated with renal function,[13] There are a number of dietary determinants of tHcy, including B-vitamin status[13] and fruit and vegetable intake,[79,80] whereas certain drugs and cigarette smoking can interfere with folate/tHcy metabolism.[13] Imbalances in these parameters between cases and controls have the potential to influence the results.

For example, we were unable to account for folate status in this meta-analysis because it was only examined in a small number of studies. It is therefore a possibility that differences in folate intake between studies explain the observed heterogeneity.

Effect of Publication Bias

For obvious reasons it is recognized that studies yielding significant results are more likely to be published leading to publication bias. The funnel plot (Fig 2) indicated that larger studies observed smaller differences in mean between the cases and controls than smaller studies. This observation is consistent with publication bias because studies with smaller sample sizes that may have been conducted and that did not detect a difference between cases and controls were not published. If this is the case, then our combined estimate would exaggerate the true difference in mean tHcy between the cases and controls. Alternatively, it is possible that the funnel plot shape could have arisen because the larger studies differed from the smaller studies with respect to other study characteristics, such as quality. By ignoring the funnel plot and significant heterogeneity, it would be easy to conclude that an association exists between tHcy and RVO. However, the marked heterogeneity in the studies, except for the subgroup of studies that used hospital patients as controls, and the evidence of publication bias are strong reasons for questioning the finding that tHcy and RVO are related.

Thermolabile Methylenetetrahydrofolate Reductase and Retinal Vein Occlusion Risk

Because of the weaknesses in the case-control approach outlined above (residual confounding and reverse causality), it has been proposed that a more robust method for detecting association would be to examine the relationship between the genetic variant MTHFR C677T and elevated tHcy concentrations,[14,15] because this will not be subject to reverse causation or the confounding that exists in observational studies of disease risk in relation to directly measured tHcy concentrations.[78] This approach follows the principle of Mendelian randomization.[81] By examining the association between MTHFR C677T genotype and RVO risk, we found no association between homozygosity for the TT genotype and RVO risk. Heterogeneity between studies was high, but there was no indication of publication bias.

There are several possible explanations for this lack of association between MTHFR and RVO risk, other than a true lack of association. Differences in tHcy concentration by MTHFR C677T genotype have been shown to be greater at lower levels of folate intake and status, and are reduced after folate supplementation.[26] We did not have information on folate status in the majority of studies and thus were unable to account for folate status in our analysis.

Second, with 1082 cases and 4706 controls, this is still a modestly sized meta-analysis in terms of genetic polymorphisms, and the power to detect a small increase in risk may be limited.

Overall, the findings from our meta-analysis on the lack of an association between MTHFR C677T genotype and RVO risk are consistent with the possibility that weaknesses in the study design may have contributed to the observation of a positive association between tHcy and RVO in the individual studies and the overall meta-analysis.

Despite the inclusion of 15 additional case-control studies after initial meta-analyses,[7,16] we were still unable to draw firm conclusions on the relationship among tHcy, MTHFR, and RVO. The majority of studies published have tended to conclude that an association between tHcy and RVO exists, and our meta-analysis supports this view through the finding of a significantly increased pooled estimate, suggesting that reduction of tHcy with folate supplementation may reduce RVO risk. However, the presence of marked heterogeneity and clear evidence of publication bias makes direct comparison and pooling of the results unreliable. The pooled increase in risk due to tHcy is also not in accord with the absence of an association between RVO and MTHFR TT genotype. Therefore, the association between tHcy and RVO remains tentative and requires high-quality, well-designed epidemiologic studies, preferably of cohort design, before firm conclusions on the putative role of elevated tHcy and MTHFR status on RVO occlusion can be made.

In conclusion, there is evidence to suggest an association between tHcy and RVO. Because of the presence of heterogeneity and publication bias, no recommendation can be made with regard to routine investigation and treatment of elevated tHcy in the setting of RVO (rating CIII). There is no evidence to suggest an association between MTHFR C677T genotype and RVO. There was heterogeneity between groups but no evidence of publication bias. There is no evidence to suggest routine testing of MTHFR C677T genotype in clinical practice (rating CII).

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Footnotes and Financial Disclosures

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This study conforms to the following Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[82]

Appendix 1 available online (http://aaojournal.org).

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Appendix 1. Proposed Reporting Checklist for Authors, Editors, and Reviewers of Meta-analyses of Observational Studies*

- Reporting of background should be
  - Problem definition
  - Hypothesis statement
  - Description of study outcome(s)
  - Type of exposure or intervention used
  - Study population
- Reporting of search strategy should include
  - Qualifications of searchers (e.g., librarians and investigators)
  - Search strategy, including time period included in the synthesis and keywords
  - Effort to include all available studies, including contact with authors
  - Databases and registries searched
  - Search software used, name and version, including special features used (e.g., explosion)
  - Use of hand searching (e.g., reference lists of obtained articles)
  - List of citations located and those excluded, including justification
  - Method of addressing articles published in languages other than English
  - Method of handling abstracts and unpublished studies
  - Description of any contact with authors
- Reporting of methods should include
  - Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested
  - Rationale for the selection and coding of data (e.g., sound clinical principles or convenience)
  - Documentation of how data were classified and coded (e.g., multiple raters, blinding and interrater reliability)
  - Assessment of confounding (e.g., comparability of cases and controls in studies where appropriate)
  - Assessment of study quality, including blinding of quality assessors; stratification of regression on possible predictors of study results
  - Assessment of heterogeneity
  - Description of statistical methods (e.g., complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated
  - Provision of appropriate tables and graphics
- Reporting of results should include
  - Graphic summarizing individual study estimates and overall estimate
  - Table giving descriptive information for each study included
  - Results of sensitivity testing (e.g., subgroup analysis)
  - Indication of statistical uncertainty of findings
- Reporting of discussion should include
  - Quantitative assessment of bias (e.g., publication bias)
  - Justification for exclusion (e.g., exclusion of non-English language citations)
  - Assessment of quality of included studies
- Reporting of conclusions should include
  - Consideration of alternative explanations for observed results
  - Generalization of the conclusions (i.e., appropriate for the data presented and within the domain of the literature review)

*This study conforms to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.