

Homocysteine and Depression in Later Life

Oswaldo P. Almeida, MD, PhD, FRANZCP; Kieran McCaul, PhD; Graeme J. Hankey, MD, FRACP; Paul Norman, PhD, FRACS; Konrad Jamrozik, MBBS, DPhil; Leon Flicker, MBBS, FRACP, PhD

Context: The prevalence of depression in later life increases with plasma total homocysteine concentration (tHcy). High tHcy accounts for about 15% of prevalent cases, but observational studies are prone to confounding and bias. Genetic association studies are not prone to the same sources of error and offer an opportunity to explore the consistency and external validity of this association.

Objective: To determine if tHcy is causally related to depression in later life.

Design: Cross-sectional study (Health in Men Study), systematic review, and meta-analysis.

Patients: Community sample of 3752 men aged 70 years or older (Health in Men Study).

Main Outcome Measures: Fifteen-Item Geriatric Depression Scale and self-reported past or current treatment for depression (Health in Men Study).

Results: In the Health in Men Study, the odds ratio (OR) of prevalent depression increased 4% (OR, 1.04; 95% confidence interval [CI], 1.02-1.05) with every unit increase of tHcy (micromoles per liter). The tHcy was 0.19 mg/L higher among participants with the *MTHFR* C677T TT genotype compared with the CC genotype. The meta-analysis showed that older adults with high tHcy had increased risk of depression (OR, 1.70; 95% CI, 1.38-2.08) and TT carriers were 22% more likely than CC carriers to have current depression or a history of depression (OR, 1.22; 95% CI, 1.01-1.47).

Conclusions: The triangular association between the *MTHFR* genotype, tHcy, and depression implies that higher concentrations of tHcy increase the risk of depression and that lowering tHcy by 0.19 mg/L could reduce the odds of depression by about 20%. Confirmatory data from sufficiently powered randomized trials of homocysteine-lowering therapy are now required to test if the relationship between tHcy and depression is truly causal.

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DEPRESSION IS A LEADING source of disability worldwide,¹ with prevalence ranging from 8.8% to 18.3% in people aged 60 years or older.² The causes of depression in later life are likely varied and complex, but current evidence suggests that cardiovascular risk factors and diseases are involved.³ We have recently examined the association of prevalent depression, diabetes, hypertension, coronary heart disease, cerebrovascular disease, smoking, and lipids with plasma total homocysteine (tHcy) in 4204 older men and found that tHcy had the highest population-attributable fraction (15% of cases could be attributed to high tHcy, assuming the relationship is causal).⁴

Homocysteine is a sulfur-containing amino acid derived from the essential amino acid methionine through demethylation.⁵ Homocysteine can be remethylated to methionine via the 5-methyltetra-

hydrofolate pathway, which is mediated by the methyltetrahydrofolate reductase enzyme and requires vitamin B₁₂ and folic acid, or it can be condensed with serine to form cystathionine in a reaction that uses vitamin B₆.⁶ A third metabolic pathway of homocysteine that involves betaine is not present in the central nervous system.⁷ Methionine is the immediate precursor of S-adenosylmethionine in the central nervous system, the methyl donor of numerous methylation reactions in the brain, including DNA, RNA, phospholipids, and proteins.⁶ In addition, the synthesis of neurotransmitters, such as serotonin, noradrenaline, and dopamine, is dependent on 5-methyltetrahydrofolate in a bipterin-dependent process. It seems, therefore, that the link between the folate/methylation cycles and depression is biologically plausible.^{5,6}

The best way to establish a causal relationship between tHcy and depression is to show, by means of sufficiently pow-

Author Affiliations are listed at the end of this article.

ered randomized controlled trials, that lowering tHcy reduces the incidence and severity of depressive symptoms. Taylor et al⁸ performed a systematic review and meta-analysis of studies designed to investigate the effect of folic acid supplementation on mood and found that existing evidence from 2 small trials was consistent with an antidepressant effect of folate.^{9,10}

Traditionally, the next best form of evidence for causality is a systematic review of large observational studies. Currently, no such review of the association between high tHcy and depression in later life is available. The Hordaland Homocysteine Study is the largest population-based survey for which data on this topic have been published. Bjelland et al¹¹ found that 224 of their 5948 participants had clinically significant depressive symptoms. After adjustment for age and sex, they were twice as likely to have a tHcy concentration of 2.03 mg/L (to convert to micromoles per liter, multiply by 7.397) or greater as those without depression at the time of assessment (95% confidence interval [CI], 1.17-3.41). Similar findings were reported by the authors of the Rotterdam Study¹² and others.^{13,14}

More recently, genetic epidemiologists have proposed that mendelian randomization can be used to infer causality between an exposure and a clinical outcome.¹⁵ The rationale, in this case, is that when homocysteine is remethylated, the methyl donor (5-methyltetrahydrofolate) comes from the metabolism of 5,10-methylenetetrahydrofolate by the 5,10-methylenetetrahydrofolate reductase gene (*MTHFR*; OMIM *607093). About 10% of the population is homozygous for the 677 C→T polymorphism of *MTHFR*. The TT polymorphism reduces production of the methyl donor 5-methyltetrahydrofolate (akin to low dietary intake of folic acid) and increases tHcy by about 20% (approximately 0.22 mg/L).¹⁶ Consequently, the 677 C→T polymorphism produces a natural (mendelian) randomization, with individuals allocated to higher (TT) or lower (CC) tHcy according to a random assortment of alleles during gamete production and fertilization. The groups should not differ systematically in any other way. For example, those with the TT genotype should be no more likely to be smokers or of lower socioeconomic class than individuals with the CC genotype. However, because the effect of the *MTHFR* polymorphism on tHcy is modest, studies need large numbers of patients to reliably estimate the risk of depression in those with and without the TT mutation.

Four cross-sectional studies have examined the association between the *MTHFR* C677T genotype and depression in later life.^{11,17-19} Three of these studies found an excess of depression among TT compared with CC carriers,^{11,18,19} but none had sufficient power to demonstrate, unequivocally, an increase in the odds of having depression.

The aim of the present study was to determine if a high concentration of tHcy is causally related to depression in later life. We used 2 complementary approaches to address this question. First, we investigated the associations between tHcy and depression and between the *MTHFR* C677T polymorphism and depression in a large cohort of older adults recruited as part of the Health in Men Study (HIMS). Second, we conducted a systematic

review and meta-analysis of (1) randomized trials of B vitamins to reduce the severity of depressive symptoms, (2) observational studies that investigated the association between a high concentration of tHcy and depression, and (3) the association between the *MTHFR* C677T polymorphism and depression.

METHODS

THE HEALTH IN MEN STUDY

Recruitment of the Study Cohort

The HIMS was an observational study that investigated the association between depression, tHcy concentration, and the *MTHFR* C677T polymorphism. Our analyses are based on a community-derived sample of older male residents of Perth, Australia, who collectively constitute the cohort for HIMS. Details regarding the enrollment of participants have been described elsewhere.²⁰ Briefly, 12 203 men aged 65 years or older were recruited via random sampling from the Australian electoral roll between 1996 and 1998, enrollment to vote being compulsory for all adult Australian citizens (phase 1). Of eligible men who were issued invitations, 70.5% participated. During the years 2001-2004, the men who were still alive (n=9718) were contacted and invited to complete a follow-up assessment (phase 2). This article refers to participants who consented to follow-up and agreed to donate a blood sample for biochemical and genetic analyses (n=4245). The human research ethics committee of the University of Western Australia approved the study protocol.

Procedures and Clinical Assessment

Men who consented to participate in the study were asked to complete a questionnaire that included items assessing demographic and clinical information. Place of birth was coded according to the Australian Bureau of Statistics system. Age was calculated as the difference in years between the date of the assessment (phase 2) and the participant's date of birth. Education was rated as the highest level of education attained: no schooling, primary school, some high school, completed high school, or completed college or another tertiary degree. Participants were asked about their living arrangements and whether they had ever smoked ("Have you ever smoked cigarettes, cigars or a pipe regularly?") or were still smoking at the time of the assessment ("How often do you smoke now?" [every day, not every day, or not at all]). Men were considered current smokers if they answered "every day" or "not every day."

Participants reported whether they had been previously treated for an emotional or nervous illness, such as depression, and whether they were currently undergoing treatment (medication or psychotherapy) for an emotional or nervous illness, such as depression. All men were asked to complete the 15-Item Geriatric Depression Scale (GDS-15); a priori, those with a total score of 7 or higher were considered to display clinically significant depressive symptoms at the time of assessment. This relatively high cut-off point was chosen to ensure high specificity for the diagnosis of depression in this sample.²¹ Men were considered to have depression if they reported having previously received treatment for depression or if they had a GDS-15 score of 7 or higher at the time of assessment. A trained research assistant measured participants' heights (to the 0.5 cm) and weights (to 0.2 kg). Body mass index was calculated as weight in kilograms divided by height in meters squared; men with body mass indexes of 30 or higher were considered obese.

Finally, we used information about physical activity and alcohol consumption that was collected during phase 1 of the study (2.2-5.9 years earlier). Men were considered physically active if they reported 150 minutes or more per week of moderate or vigorous physical activity. Hazardous alcohol use was defined as consumption of more than 4 standard units of alcohol every day or more than 6 standard units any 1 day of the week.

Measurement of Physical Comorbidity

The assessment of physical comorbidity involved 2 components. First, we used the weighted Charlson Comorbidity Index.²² The index takes into account 17 common medical conditions that predict 1-year mortality: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcers, liver disease, diabetes (including diabetes with end organ damage), hemiplegia, renal disease, leukemia, lymphoma, other tumors, metastatic tumors, and AIDS. Charlson and colleagues²² used adjusted relative risks to assign integer weights to these conditions within a composite index score that ranges from 0 to 37. To calculate the index, we obtained administrative medical information from the Western Australian Linked Data System.²³ Briefly, since 1980, the Western Australian Linked Data System has connected records of all hospital admissions (private and public) with the Mental Health Information System, Western Australian Cancer Registry, and Western Australian death register. We retrieved linked data for all participants until the end of 2004, which was the time of the last assessment for HIMS. Coding algorithms to define comorbidities followed the procedures described by Quan et al.²⁴ and scores were calculated using the Stagg Charlson index with Stata, version 9.2 (Stata Corp, College Station, Texas). Second, participating men completed the 36-Item Short-Form Health Survey.²⁵ For the purposes of this study, the analyses were limited to the physical component summary (PCS) measure. The mean PCS score for the Australian population is 50 (standard deviation [SD], 10).²⁶ Participants with a PCS score of 40 or less (ie, at least 1 SD below the population mean) were considered to have poor perceived physical health at the time of the assessment.

Biochemical and Genetic Analyses

Blood samples were collected between 8 and 10:30 AM. Plasma was prepared immediately following phlebotomy and stored at -80°C until assayed. Biochemical assays and genetic analyses were performed in the department of biochemistry at PathWest, Royal Perth Hospital. Total plasma homocysteine was measured by fluorescence polarization immunoassay on an IMx analyzer.²⁷ Genomic DNA was isolated from nucleated blood cells via the Triton X-100 method, and the nt677C→T mutation was determined using polymerase chain reaction and *HinfI* restriction enzyme digestion. *HinfI* digestion (1.5-U/25 µL reaction mixture) was performed directly in the polymerase chain reaction tube at 37°C for 4 hours before analysis of restriction fragments by polyacrylamide gel electrophoresis (12% T, 3.3% C), as previously described.²⁸ Allele frequencies were estimated by gene counting, and observed numbers of each genotype were compared with those expected under Hardy-Weinberg equilibrium.

SYSTEMATIC REVIEW AND META-ANALYSIS

Selection of Studies

We searched the PubMed database for the period 1966 to November 2007 for articles that investigated the association between depression, tHcy, and *MTHFR* genotype. We used the

following keywords: *MTHFR*, *methyltetrahydrofolate reductase*, *homocysteine*, *depression*, *depressive disorder*, *depressive episode*, *affective disorder*, and *dysthymia*. The search was limited to articles published in English. Seven hundred eighty-three publications were reviewed, of which we deemed 93 relevant. Clinical trials were considered suitable for review if they were randomized. The active treatment group received an acceptable form of treatment with folic acid, cyanocobalamin, or pyridoxine hydrochloride (with or without antidepressant medication). The diagnosis of depression was based on acceptable criteria (*DSM-III*, *DSM-III-R*, *DSM-IV*, or *International Statistical Classification of Diseases, 10th Revision [ICD-10]*), or if participants had clinically significant depressive symptoms according to a validated depression rating scale). End points were clearly defined (no longer meeting criteria for depression or change of scores on a depression rating scale). Cohort, case-control, and cross-sectional studies of the association between tHcy and depression were reviewed if the definition of case was based on acceptable criteria for depression (*DSM-III*, *DSM-III-R*, *DSM-IV*, or *ICD-10*, or if participants had clinically significant depressive symptoms according to a validated depression rating scale) and the authors described differences in the concentration of tHcy between the groups or reported information about the proportion of cases and noncases that had high tHcy. Finally, we reviewed studies that reported data on the association between the *MTHFR* C677T genotype and depression (current depression as defined by *DSM-III*, *DSM-III-R*, *DSM-IV*, *ICD-10*, or depression rating scale, or a history of depression—with or without current depressive symptoms). The reviews were limited to studies that included elderly participants to ensure some consistency with the findings of HIMS, which was limited to older adults.

After detailed review of each article, we found 5 trials of adjunctive antidepressant treatment with B vitamins, 9 cross-sectional studies that investigated the association between tHcy and depression or depressive symptoms in later life (including HIMS), and 5 observational studies (including HIMS) of the association between the *MTHFR* C677T genotype and depression, depressive symptoms, or history of depression.

Sample Size Estimation

We based our sample size calculations on data from Lewis et al.¹⁹ as well as HIMS. The expected proportion of older adults who ever had depression was 15%, and Lewis et al showed that the estimated risk of depression (ever) associated with the *MTHFR* C677T TT/CC genotype is 1.3. The proportion of the TT genotype relative to the CC genotype in the population is about 20%. With 80% power and an α level of 5%, we would require a sample of 5760 older adults. As 45% of community-dwelling older adults carry the *MTHFR* CT genotype, the study was expected to require a total of 10 472 participants to establish an odds ratio (OR) of 1.3. As available studies had already contributed 7114 participants, we calculated that HIMS would need to contribute a minimum of 3358 people.

STATISTICAL ANALYSIS

Data were managed and analyzed with Stata, version 9.2 (Stata Corp). Participants were grouped according to whether they were ever depressed (history of treatment for depression or current GDS-15 score ≥ 7). The ORs (and 95% confidence intervals [CIs]) of ever having depression were calculated for measured exposures: age, education, living arrangements, smoking, alcohol use, physical activity, obesity, presence of significant physical health comorbidity, current or past depression treatment, and tHcy concentration. Logistic regression was used to

Table 1. Univariate ORs of Depression According to Demographics, Lifestyle, Health Morbidity Measures, and Plasma tHcy

Characteristic	Total Sample, No. (%) (N=3752)	Ever Depressed (n=513)		Depressed at Assessment (n=186)	
		Participant, No. (%)	OR (95% CI)	Participant, No. (%)	OR (95% CI)
Age ≥75 y	1879 (50.1)	291 (56.7)	1.36 (1.13-1.64)	112 (60.2)	1.54 (1.13-2.11)
Completed high school	1814 (48.4)	247 (48.2)	0.99 (0.82-1.19)	75 (40.3)	0.71 (0.52-0.97)
Living alone	625 (16.7)	101 (19.7)	1.27 (1.00-1.61)	42 (22.6)	1.49 (1.02-2.14)
Ever smoked	2515 (67.0)	379 (73.9)	1.46 (1.19-1.80)	145 (78.0)	1.78 (1.24-2.61)
Current smoker	198 (5.3)	39 (7.6)	1.59 (1.11-2.29)	20 (10.7)	2.93 (1.33-3.76)
History of hazardous alcohol use	673 (18.8) ^a	105 (21.5)	1.21 (0.96-1.53)	52 (28.7)	1.80 (1.26-2.53)
History of being physically active	860 (22.9)	108 (21.1)	0.88 (0.70-1.11)	26 (14.0)	0.53 (0.33-0.82)
BMI ≥30	576 (15.4)	102 (20.2)	1.47 (1.16-1.87)	44 (24.4)	1.84 (1.26-2.64)
Weighted Charlson Comorbidity Index score ≥5	243 (6.5)	51 (9.9)	1.75 (1.27-2.42)	31 (16.7)	3.16 (2.02-4.81)
SF-36 PCS score ≤40	1517 (40.9)	307 (60.9)	2.56 (2.11-3.11)	145 (79.2)	5.98 (4.13-8.84)
tHcy, mean (SD), mg/L	1.81 (0.77)	1.93 (1.05)	1.02 (1.01-1.04)	2.08 (1.51)	1.04 (1.02-1.05)
ln(tHcy), mean (SD)	0.34 (0.04)	0.35 (0.05)	1.67 (1.26-2.22) ^b	0.36 (0.05)	2.52 (1.67-3.82) ^b
tHcy ≥2.03 mg/L	980 (26.1)	171 (33.3)	1.50 (1.23-1.83) ^c	75 (40.3)	1.99 (1.45-2.71) ^d

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CI, confidence interval; OR, odds ratio; SF-36 PCS, 36-Item Short-Form Health Survey physical component summary; tHcy, total homocysteine.

SI conversion factor: To convert tHcy to micromoles per liter, multiply by 7.397.

^aInformation missing for 177 participants.

^bRatio of geometric tHcy (95% CI) relative to men who have never been depressed.

^cAfter adjustment for age group, ever smoking, obesity, and physical morbidity as determined by a SF-36 PCS score of 40 or less and a weighted Charlson Comorbidity Index score of 5 or more, OR, 1.29 (95% CI, 1.04-1.59).

^dAfter adjustment for age group, ever smoking, obesity, and physical morbidity as determined by a SF-36 PCS score of 40 or less and a weighted Charlson Comorbidity Index score of 5 or more, OR, 1.53 (95% CI, 1.10-2.11).

adjust the odds of ever having depression according to the geometric mean of tHcy and included the following variables in the model: age group (<75 vs ≥75 years), education (incomplete high school vs high school or higher), living arrangements (not alone vs alone), ever smoked, currently smoking, history of hazardous alcohol use, history of being physically active, PCS score (>40 vs ≤40), and weighted Charlson Comorbidity Index score (<5 vs ≥5). We did not adjust the analyses for other factors known to affect tHcy (eg, diabetes, liver, and renal disease) because they had already been included in our measurement of comorbidity (Charlson Comorbidity Index). Finally, we investigated the distribution of measured exposures according to participants' *MTHFR* genotype (CC, CT, or TT) and calculated the OR for the TT and CT genotypes relative to CC. The nonparametric Cuzick test for trend was used to determine the associations between *MTHFR* genotypes and tHcy and between *MTHFR* genotypes and depression scores.

We did not conduct a meta-analysis of available clinical trials of B vitamins for the treatment of depression because of significant study heterogeneity and paucity of data. The quantitative summary of observational studies that investigated the association between high tHcy and depression was based on a fixed-effects model of pooled ORs and 95% CIs (test of heterogeneity, $P=.29$). We then conducted a meta-analysis of studies that investigated the association between the *MTHFR* C677T genotype and depression. The meta-analysis focused on the prevalence of the homozygote *MTHFR* C677T variant compared with the wild-type homozygote (ie, TT vs CC) for cases vs controls. In each study, we tested the genotype distribution in the controls for deviations from the Hardy-Weinberg equilibrium using the exact method and the de Finetti diagram and excluded those who returned significant results from the meta-analysis. We combined ORs using a random-effects model. In addition, we conducted a cumulative meta-analysis to examine changes in association over time. Between-study heterogeneity was assessed using the I^2 statistic, and we used funnel plots to ascertain if pooled effects appeared to have been influenced by publication bias.

RESULTS

HEALTH IN MEN STUDY

In total, 4245 community-dwelling men completed the health questionnaire and agreed to donate a blood sample for biochemical analysis and genetic testing. There were GDS-15 data missing for 32 of them and DNA was not accessible for another 461 participants (3 men had missing data for both). Hence, the results reported in this article are limited to 3752 people for whom clinical, biochemical, and genotypic information was available. On average, nonparticipating men were 0.5 years older than participating men at the assessment in phase 1 ($t=5.84$, $P<.001$). In addition, nonparticipating men were more likely than participating men to have reported a history of hypertension (OR, 1.13; 95% CI, 1.04-1.23), stroke (OR, 1.54; 95% CI, 1.27-1.82), and/or diabetes (OR, 1.59; 95% CI, 1.38-1.84) during the phase 1 assessment. They were also more likely to smoke (OR, 1.62; 95% CI, 1.36-1.92), be obese (OR, 1.30; 95% CI, 1.17-1.45), and report less than 150 minutes per week of moderate to vigorous physical activity (OR, 1.62; 95% CI, 1.46-1.80). We also investigated the risk of clinically significant depression among men who agreed to complete the study questionnaire but did not wish to donate a blood sample; they were more likely to be depressed than the 3752 men included in the study (OR, 1.69; 95% CI, 1.33-2.15).

Participants' ages ranged from 69 to 87 years (median, 75 years). The distribution of *MTHFR* C677T genotypes was as expected given Hardy-Weinberg equilibrium (exact test, $P=.91$). **Table 1** shows the demographic, clinical, and biochemical associations of men who were ever

Table 2. Demographic, Clinical, and Biochemical Characteristics of Participants According to Their *MTHFR* C677T Genotype

Characteristic	Participants, No. (%)			Univariate OR (95% CI)	
	<i>MTHFR</i> C677T CC (n=1658)	<i>MTHFR</i> C677T CT (n=1675)	<i>MTHFR</i> C677T TT (n=419)	CT vs CC	TT vs CC
Age ≥75 y	820 (49.5)	851 (50.8)	208 (49.6)	1.06 (0.92-1.21)	1.01 (0.81-1.25)
Living alone	290 (17.3)	275 (16.4)	70 (16.6)	0.93 (0.78-1.12)	0.95 (0.71-1.29)
Ever smoked	1121 (67.0)	1135 (67.5)	279 (65.4)	1.02 (0.88-1.18)	0.94 (0.75-1.17)
Current smoker	91 (5.4)	89 (5.3)	20 (4.7)	0.97 (0.72-1.31)	0.86 (0.53-1.42)
History of hazardous alcohol use	311 (19.5)	303 (18.9)	64 (16.0)	0.96 (0.81-1.15)	0.79 (0.59-1.06)
History of being physically active	405 (24.2)	358 (21.3)	102 (24.1)	0.85 (0.72-1.00)	1.00 (0.78-1.28)
BMI ≥30	229 (13.7)	280 (16.7)	72 (17.0)	1.26 (1.04-1.52)	1.29 (0.96-1.72)
Weighted Charlson Comorbidity Index score ≥5	115 (6.9)	107 (6.4)	27 (6.4)	0.92 (0.70-1.21)	0.92 (0.60-1.43)
SF-36 PCS score ≤40	690 (41.7)	682 (41.0)	157 (38.1)	0.97 (0.85-1.12)	0.86 (0.69-1.07)
tHcy, mean (SD), mg/L	1.77 (0.69)	1.82 (0.66)	1.95 (1.31)	1.02 (1.00-1.03) ^a	1.04 (1.02-1.05) ^b
ln(tHcy), mean (SD)	0.34 (0.04)	0.34 (0.04)	0.35 (0.05)	1.35 (1.10-1.67) ^{c,d}	1.77 (1.28-2.45) ^{c,e}
tHcy ≥2.03 mg/L	394 (23.5)	462 (27.5)	133 (31.4)	1.23 (1.05-1.44) ^f	1.49 (1.18-1.88) ^g
Ever depressed	235 (14.2)	218 (13.0)	60 (14.3)	0.91 (0.74-1.11)	1.01 (0.75-1.37)
GDS-15 score ≥7 at assessment	89 (5.4)	80 (4.8)	17 (4.1)	0.88 (0.65-1.21)	0.75 (0.44-1.27)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CI, confidence interval; GDS-15, 15-Item Geriatric Depression Scale; OR, odds ratio; SF-36 PCS, 36-Item Short-Form Health Survey physical component summary; tHcy, total homocysteine. SI conversion factor: To convert tHcy to micromoles per liter, multiply by 7.397.

^aAfter the exclusion of men with a history of depression (393 men excluded of 3752), OR, 1.02 (95% CI, 1.00-1.04).

^bAfter the exclusion of men with a history of depression, OR, 1.03 (95% CI, 1.01-1.05).

^cRatio of geometric tHcy (95% CI) relative to men with the *MTHFR*677 CC genotype.

^dAfter the exclusion of men with a history of depression, OR of the geometric mean of tHcy, 1.39 (95% CI, 1.10-1.76).

^eAfter the exclusion of men with a history of depression, OR of the geometric mean of tHcy, 1.75 (95% CI, 1.24-1.47).

^fAfter adjustment for age group, ever smoking, obesity, and physical morbidity determined by a SF-36 PCS score of 40 or less and a weighted Charlson Comorbidity Index score of 5 or greater, OR, 1.28 (95% CI, 1.04-1.59). After the exclusion of men with a history of depression, OR, 1.18 (95% CI, 1.00-1.40).

^gAfter adjustment for age group, ever smoking, obesity, and physical morbidity determined by a SF-36 PCS score of 40 or less and a weighted Charlson Comorbidity Index score of 5 or greater, OR, 1.52 (95% CI, 1.23-1.88). After the exclusion of men with a history of depression, OR, 1.45 (95% CI, 1.12-1.86).

depressed. Older age, an incomplete high school education, living alone, smoking, having a history of harmful or hazardous alcohol use, physical inactivity, and obesity were associated with increased odds of depression, as were indexes of greater medical morbidity (weighted Charlson Comorbidity Index and 36-Item Short-Form Health Survey PCS scores). Men who were ever depressed had significantly higher tHcy ($t=3.61$, $P<.001$, after natural logarithmic transformation of tHcy). The odds of being depressed at the time of assessment increased by 4% for every unit (micromoles per liter) increase in tHcy (OR, 1.04; 95% CI, 1.02-1.05) and more than doubled with every unit increase of ln(tHcy) (OR, 2.52; 95% CI, 1.67-3.82). Moreover, participants with high tHcy concentration (≥ 2.03 mg/L) had a 53% increase in the odds of prevalent depression (95% CI, 1.10-2.11; after adjustment for age group, ever smoking, obesity, and physical morbidity). We conducted a series of supplementary analyses to ensure that adjustment for confounding was appropriate in the investigation of the association between tHcy and depression. The 17 medical morbidities derived from the Charlson Comorbidity Index were entered individually in the models as well as age (in years) and number of cigarettes smoked per day among current smokers. The results of these analyses showed that the association between tHcy and depression remained largely unchanged (data not shown) and indicate that the reported models are appropriate and robust.

As expected, *MTHFR* C677T genotypes showed little association with sociodemographic, lifestyle, and morbidity-related measures (Table 2). Compared with the reference CC genotype group, mean tHcy concentra-

tion was 0.05 mg/L (standard error of the mean, 0.03 mg/L) and 0.19 mg/L (standard error of mean difference, 0.04 mg/L) higher among men with CT and TT genotypes, respectively. There was no association between TT homozygosity and ever receiving a diagnosis of depression (OR, 1.01; 95% CI, 0.75-1.37). The Cuzick test for trend showed a dose-effect response of the *MTHFR* C677T genotype on tHcy ($z=3.84$, $P<.001$) but not on GDS-15 scores ($z=0.03$, $P=.98$).

SYSTEMATIC REVIEW AND META-ANALYSIS

Table 3 summarizes the characteristics and results of the 5 clinical trials included in this review. Their results are consistent with an antidepressant effect of folic acid, 5-methyltetrahydrofolate, and cyanocobalamin, but available data were not suitable for meta-analysis.

Table 4 describes the key findings of 9 cross-sectional studies that investigated the association between depression and tHcy among adults (mostly older adults). Six of the studies reported information about the association between high tHcy (≥ 1.69 mg/L) and prevalent depression. Their data were pooled in a meta-analysis that included the findings from HIMS (adjusted OR). High plasma tHcy was associated with an increase in the odds of depression (OR, 1.70; 95% CI, 1.38-2.08; fixed-effects model) (Figure 1). We found no evidence of heterogeneity between study effects ($I^2=0.11\%$, $P=.35$).

Four studies described data on the association between *MTHFR* C677T genotype and depression among older adults (Table 5). These were pooled with the results of HIMS in a meta-analysis that investigated the odds

Table 3. Characteristics of Clinical Trials That Investigated the Outcome of Depression Treated With B Vitamins

Source	Study Design	Participants	Intervention	Outcome Measures	Comments
Coppen et al, 1986 ²⁹	Double-blind, placebo-controlled trial (allocation concealment unclear)	75 Patients with unipolar and bipolar disorder	200 µg daily of folic acid or placebo for 12 mo in addition to lithium	BDI scores	Subgroup analysis of patients with unipolar depression treated with folic acid showed greater reduction in BDI scores
Godfrey et al, 1990 ¹⁰	Double-blind, placebo-controlled, randomized trial (allocation concealment unclear)	24 Patients with major depression according to <i>DSM-III</i>	15 mg daily of methyltetrahydrofolate or placebo in addition to standard antidepressant treatment for 6 mo	HDS and Clinical Outcome Scale scores (clinical impression regarding clinical and social functioning)	Significant decline in clinical outcome scores (but not HDS) in the group treated with methyltetrahydrofolate at 3 and 6 mo
Passeri et al, 1993 ³⁰	Double-blind, controlled trial (allocation concealment unclear)	96 Patients with cognitive impairment (MMSE score <24) and depression (HDS score >17)	50 mg daily of methyltetrahydrofolate or 100 mg of trazodone for 8 wk	HDS scores	Significant decline in HDS scores at 4 and 8 wk in both treatment groups
Coppen and Bailey, 2000 ⁹	Double-blind, placebo-controlled randomized trial (allocation concealment unclear)	127 Patients with a major depressive episode according to <i>DSM-III-R</i>	500 µg daily of folic acid or placebo combined with fluoxetine for 10 wk	HDS scores	Subgroup analysis showed that only women treated with folic acid gained extra benefit from antidepressant treatment; only people completing 6 wk of treatment were included in the analysis
Hvas et al, 2004 ³¹	Double-blind, randomized trial	140 Hospital patients with plasma methylmalonic acid 0.4-2.0 µmol/L; it is unclear if any of the participants were depressed	Intramuscular injections of 1 mg of cyanocobalamin (n=70) or 1 mL of sodium chloride (n=70) per wk for 4 wk	MDI scores	Participants treated with cyanocobalamin showed a mean decrease in MDI scores of 1.8 (SD, 5.9) points compared with 0.2 (SD, 6.8) for placebo; the study had only 32% power to detect a between-group difference of this magnitude

Abbreviations: BDI, Beck Depression Inventory; HDS, Hamilton Depression Scale; MDI, Major Depression Inventory; MMSE, Mini-Mental State Examination.

of ever having depression among those with the TT vs the CC *MTHFR* C677T genotype (**Figure 2**). All of the studies identified showed distributions of genotypes that were consistent with Hardy-Weinberg equilibrium. There was a small increase in the odds of ever having depression among persons with the homozygote TT variant (OR, 1.22; 95% CI, 1.01-1.47; fixed-effects model) (Figure 2). We found no evidence of heterogeneity between study effects ($I^2=0\%$) or publication bias (as assessed by funnel plots). Finally, the pooled estimate showed that there was a nonsignificant increase in the odds of depression among CT relative to CC carriers (OR, 1.07; 95% CI, 0.93-1.24).

COMMENT

The results of this study showed that tHcy is associated with both current depression and a history of depression and that the risk of depression increases between 2% and 5% for every unit increase in the concentration of tHcy (micromoles per liter). The meta-analysis demonstrated that older people with high tHcy concentrations (≥ 1.69 mg/L) have an excess risk of about 70% of prevalent depression (95% CI, 38%-108%), and HIMS extended these findings to older men who were ever depressed (excess risk of 29% after adjustment for confounding). We confirmed that the common *MTHFR* C677T polymorphism increases the concentration of tHcy by 0.19 mg/L, and our meta-analysis found an excess risk of ever having depression of 22% in those with the TT

compared with the CC genotype (95% CI, 1%-47%). Finally, the review of published randomized trials indicated that treatment with folic acid or methyltetrahydrofolate, with or without cyanocobalamin, contributes to a decrease in the severity of depressive symptoms, though these observations are best described as preliminary.

Should these findings be interpreted as proof of a causal relationship between a high tHcy concentration and depression in later life? Not yet. Currently, available evidence from randomized trials is mostly based on subgroup analyses of relatively small studies. The largest available trial included 127 adults with a *DSM-III-R* major depressive episode who were randomly allocated to daily treatment with folic acid (0.5 mg) plus fluoxetine (20 mg) or placebo plus fluoxetine (20 mg) for 10 weeks. Remission of symptoms was observed in 33 of the 51 participants who received folic acid (64.7%) and 28 of the 58 treated with placebo (48.3%); only people who completed at least 6 weeks of treatment were included in the analyses. The study had only 33% power to demonstrate a between-group difference of this magnitude. More recently, Ford et al³⁵ reported that treatment with cyanocobalamin, pyridoxine hydrochloride, and folic acid for 2 years reduced the incidence of depressive symptoms among nondepressed elderly men, though the effect size of the intervention was modest and consistent with the possibility of harm (hazard ratio, 1.24; 95% CI, 0.68-2.26). In addition, the results of observational studies that investigated the association between tHcy and depres-

Table 4. Summary of Observational Studies That Investigated the Association Between Plasma tHcy and Depression in Older Adults

Source	Setting	Participants	Definition of Depression	Results
Penninx et al, 2000 ¹³	Community	700 Women aged ≥ 65 y with physical disability and MMSE scores ≥ 18	No depression: GDS-15 score ≤ 9 (n=478) Mild depression: 9 < GDS-15 score < 14 (n=100) Severe depression: GDS-15 score ≥ 14 (n=122)	tHcy, mean (SD), mg/L: 1.54 (0.66) (no depression), 1.57 (1.05) (mild depression), and 1.66 (0.76) (severe depression) No. of participants with tHcy ≥ 1.89 mg/L: 99/478 (no depression), 20/100 (mild depression), and 34/122 (severe depression) OR of severe depression for women with tHcy ≥ 1.89 mg/L: 1.49 (95% CI, 0.92-2.36)
Bottiglieri et al, 2000 ³²	Hospital	18 Healthy controls (mean age, 34 y) 20 Neurological controls (mean age, 53 y) 46 Patients with depression (mean age, 53 y)	DSM-III major depressive episode and HDS score >17	tHcy, mean (SD), mg/L: 1.03 (0.32) (healthy controls), 0.89 (0.27) (neurological controls), and 1.78 (0.84) (with depression) No. of participants with tHcy ≥ 1.69 mg/L: 0/18 (healthy controls), 0/20 (neurological controls), and 20/46 (with depression)
Tiemeier et al, 2002 ¹²	Community	416 Random controls 278 Participants with CES-D score ≥ 16 112 Participants with a depressive disorder (from among the 278 with CES-D score ≥ 16) All participants were aged ≥ 55 y	DSM-IV dysthymia, minor or major depression	OR of depressive symptoms associated with tHcy ≥ 2.03 mg/L relative to controls: 1.55 (95% CI, 0.95-2.53) OR of depressive disorder relative to controls associated with tHcy ≥ 2.03 mg/L: 2.07 (95% CI, 1.11-3.83) (adjusted for age and sex)
Bjelland et al, 2003 ¹¹	Community	5948 Participants (1 group aged 46-49 y, another aged 70-74 y)	HADS-D score ≥ 8 and HADS-A score <8 (n=224)	OR of depression among participants with tHcy ≥ 2.03 mg/L: 2.00 (95% CI, 1.17-3.41) (adjusted for age and sex)
Tolmunen et al, 2004 ¹⁴	Community	871 Men aged 46-64 y	HPL score ≥ 8 (n=109)	tHcy, mean (SD), mg/L: 1.46 (0.42) (no depression) and 1.61 (0.68) (clinically significant depressive symptoms) OR of depression for men with tHcy ≥ 1.62 mg/L: 1.62 (95% CI, 1.03-2.51)
Ramos et al, 2004 ³³	Community	627 Men and 883 women aged ≥ 60 y	CES-D score ≥ 16 (100 men and 285 women)	tHcy, median (range), mg/L: 1.40 (0.59-17.47) and 1.45 (0.88-15.60) for men; 1.26 (0.63-8.90) and 1.26 (0.57-4.91) for women with and without depression, respectively
Almeida et al, 2005 ¹⁷	Community	240 Women aged ≥ 70 y	BDI score ≥ 13 (n=42)	tHcy, mean (SD), mg/L: 1.47 (0.61) (no depression) and 1.68 (0.61) (depression) OR of depression among women with tHcy ≥ 2.03 mg/L: 3.03 (95% CI, 1.27-7.23) (adjusted for age) ^a
Dimopoulos et al, 2007 ³⁴	Community centers for older adults	33 Participants with depression and 33 nondepressed controls aged ≥ 60 y	DSM-IV dysthymia, minor or major depression, and GDS-15 score ≥ 10	tHcy, mean (SD), mg/L: 1.42 (0.30) (no depression) and 2.89 (1.04) (depression) No. of participants with tHcy ≥ 2.03 mg/L: 27/33 ^b

Abbreviations: BDI, Beck Depression Inventory; CES-D, Center for Epidemiologic Studies Depression Scale; CI, confidence interval; GDS-15, 15-Item Geriatric Depression Scale; HADS-A, Hospital Anxiety and Depression Scale, anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale, depression subscale; HDS, Hamilton Depression Scale; HPL, Human Population Laboratory Depression Scale; OR, odds ratio; MMSE, Mini-Mental State Examination; tHcy, total homocysteine.

SI conversion factor: To convert tHcy to micromoles per liter, multiply by 7.397.

^aData supplied by the authors.

^bInformation not available for controls.

sion could be explained by reverse causality (depression leading to poorer diet, less physical activity, greater alcohol consumption, smoking, and, consequently, higher tHcy concentrations). This possibility cannot be easily dismissed even after careful adjustment of the analyses for confounding, as unmeasured factors associated with depression and raised tHcy (such as changes in diet and alcohol use) may explain the observed associations. Analysis of the potential bias introduced by the self-selection of participants indicates that men who elected to join the study had lower clinical comorbidity and better lifestyles than nonparticipating men. Moreover, men who agreed to complete the questionnaire but who did not consent to the donation of a blood sample were 70% more

likely than participating men to display clinically significant symptoms of depression. In other words, our study sample was biased toward lower concentrations of tHcy and better mood, which would move the results toward the null hypothesis. This shows that the findings from HIMS cannot be easily explained by selection bias.

The estimate of risk obtained from the meta-analysis of genetic observational studies should also be interpreted with caution, as the definition phenotype was inconsistent and subject to misclassification bias. As depression is a recurrent disorder that often includes lengthy periods of remission, investigations of the link between *MTHFR* C677T genotype and prevalent depression are likely to be systematically affected by false-negative cases

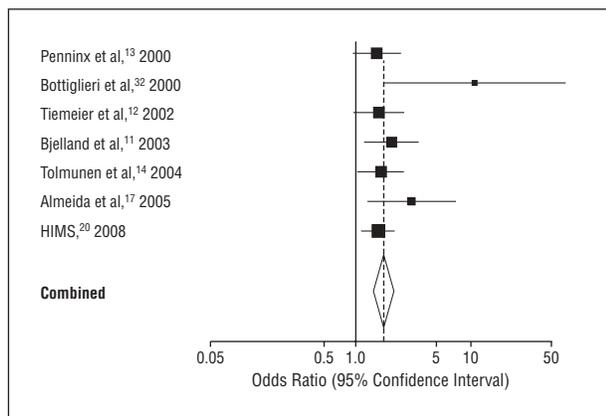


Figure 1. Meta-analysis of cross-sectional studies that investigated the association between homocysteine and depression. The diamond represents the pooled odds ratio of depression among people with high plasma total homocysteine concentrations and its 95% confidence intervals. Combined odds ratio equals 1.70 (95% confidence interval, 1.38-2.08).

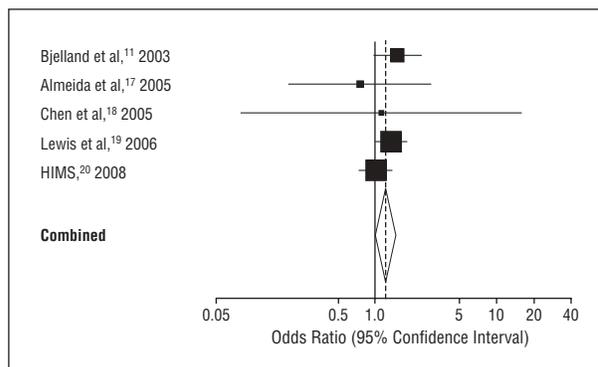


Figure 2. Meta-analysis of studies that investigated the association between *MTHFR* C677T polymorphism and depression. Odds ratios for depression in those with the TT genotype relative to those with the CC genotype. The diamond represents the pooled odds ratio and its 95% confidence intervals. Combined odds ratio equals 1.22 (95% confidence interval, 1.01-1.47).

Table 5. Summary of Studies That Investigated the Association Between the *MTHFR* C677t Genotype and Depression in Later Life

Source	Setting	Definition of Depression	No. of Participants						Univariate OR (95% CI)	
			<i>MTHFR</i> C677t CC		<i>MTHFR</i> C677t CT		<i>MTHFR</i> C677t TT		CT vs CC	TT vs CC
			Cases	Controls	Cases	Controls	Cases	Controls		
Chen et al, 2005 ¹⁸	Hospital patients and community volunteers aged ≥ 60 y	DSM-IV major depressive episode	22	11	15	9	2	0	0.83 (0.24-2.90)	1.13 (0.08- ∞)
Bjelland et al, 2003 ¹¹	Community men and women aged 70-74 y	HADS-D score ≥ 8	102	1554	78	1320	25	249	0.90 (0.66-1.23)	1.53 (0.93-2.44)
Almeida et al, 2005 ¹⁷	Community volunteers and women aged ≥ 70 y	BDI score ≥ 13	13	85	26	87	3	26	1.95 (0.90-4.42)	0.75 (0.13-3.06)
Lewis et al, 2006 ¹⁹	Community women aged 60-79 y	Self-reported history	221	1344	251	1269	73	329	1.20 (0.98-1.47)	1.35 (0.99-1.82)

Abbreviations: BDI, Beck Depression Inventory; CI, confidence interval; HADS-D, Hospital Anxiety and Depression Scale, depression subscale; OR, odds ratio.

and reduced power. The 2 studies that reported an association between history of depression and *MTHFR* C677T genotype (Lewis et al¹⁹ and HIMS) used a definition of phenotype that has uncertain validity (affirmative answer to “Have you ever been told by a doctor that you have depression?” or “Have you ever received treatment for depression?”), which raises doubts as to how best to interpret their results. Furthermore, the common *MTHFR* C677T polymorphism seems to produce a dose-response effect on the concentration of tHcy (lowest, intermediate, and highest concentrations for the CC, CT, and TT genotypes, respectively), but a similar effect of these genotypes on depression was not noticeable in HIMS nor could it be unequivocally established when we pooled the data from existing studies. This suggests that our meta-analysis lacked power to establish a credible dose-response effect of the *MTHFR* C677T polymorphism on depression risk. Finally, the lack of reliable information on ethnicity hampered our ability to explore the issue of population stratification in our study.

Despite these limitations, the triangular association between *MTHFR* genotype, tHcy, and depression implies that higher concentrations of tHcy increase the risk of depression, and that lowering tHcy by 0.19 mg/L could

potentially reduce the odds of depression by approximately 20% in the overall population, independent of individual genotypes. Such an effect is consistent with previously published data.⁴ Moreover, treatment with B vitamins does reduce tHcy by approximately 20%,^{36,37} which raises the possibility that both the prevalence and incidence of depression could be diminished with appropriate homocysteine-lowering treatment. We now require sufficiently powered randomized trials to test these hypotheses.

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Author Affiliations: Western Australian Centre for Health and Ageing, Western Australian Institute for Medical Research, Perth (Drs Almeida, McCaul, and Flicker); Department of Psychiatry (Dr Almeida), Stroke Unit, Department of Neurology (Dr Hankey), and Department of Geriatric Medicine (Dr Flicker), Royal Perth Hospital, Perth, Australia; Schools of Psychiatry and Clinical Neurosciences (Dr Almeida), Medicine and Pharmacology (Drs Hankey and Flicker), and Surgery (Dr Norman), University of Western Australia, Perth; Department of Surgery, Fremantle Hospital, Fremantle, Australia (Dr Nor-

man); and School of Population Health and Clinical Practice, University of Adelaide, Adelaide, Australia (Dr Jamrozik).

Correspondence: Osvaldo P. Almeida, MD, PhD, FRANZCP, Western Australian Centre for Health and Ageing, University of Western Australia, Mail Box M573, 35 Stirling Hwy, Crawley, WA 6009, Australia (osvaldo.almeida@uwa.edu.au).

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