Systematic Review and Meta-Analysis of L-Methylfolate Augmentation in Depressive Disorders

Authors
Abdullah Al Maruf[1, 2, 3*], Ethan A. Poweleit[4, 5*], Lisa C. Brown[6], Jeffrey R. Strawn[7], Chad A. Bousman[2, 8, 9, 10]

Affiliations
1 College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba, Manitoba, Canada
2 Mathison Centre for Mental Health Research & Education, Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Alberta, Canada
3 Departments of Psychiatry and Physiology & Pharmacology, University of Calgary, Alberta, Canada
4 Divisions of Biomedical Informatics, Clinical Pharmacology & Research in Patient Services, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
5 Department of Biomedical Informatics, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA
6 Tempus Labs, Chicago, IL, USA
7 Departments of Psychiatry and Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA
8 Department of Medical Genetics, University of Calgary, Alberta, Canada
9 Department of Community Health Sciences, University of Calgary, Alberta, Canada
10 Alberta Children's Hospital Research Institute, University of Calgary, Alberta, Canada

Key words
L-methylfolate, depression, adjunct therapy

received 02.06.2021
revised 17.09.2021
accepted 18.10.2021
published online 18.11.2021

Bibliography
Pharmacopsychiatry 2022; 55: 139–147
DOI 10.1055/a-1681-2047
ISSN 0176-3679

Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence
Abdullah Al Maruf, M.Pharm, PhD
College of Pharmacy
Rady Faculty of Health Sciences
University of Manitoba
Winnipeg, Manitoba,
Canada
abdullah.maruf@umanitoba.ca

ABSTRACT

Objectives Partial response to pharmacotherapy is common in major depressive disorder (MDD) and many patients require alternative pharmacotherapy or augmentation, including adjunctive L-methylfolate. Given that L-methylfolate augmentation is rarely included in major clinical practice guidelines, we sought to systematically review evidence for L-methylfolate augmentation in adults with MDD and to examine its efficacy meta-analytically.

Methods We systematically searched PubMed for articles up to December 31, 2020, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations. Included studies were published in peer-reviewed, English-language journals and examined L-methylfolate adjunctive therapy in depressive disorders or its effect on antidepressant response. A fixed- and random-effects meta-analysis and risk of bias assessment using the Cochrane Risk of Bias Tool were conducted.

Results Qualitative assessment of nine articles (N = 6,707 patients) suggests that adjunctive L-methylfolate improved antidepressant response. In the meta-analysis of categorical Hamilton Rating Scale for Depression-17 response, (three studies, $N = 483$) adjunctive L-methylfolate was associated with a small effect versus antidepressant monotherapy (relative risk: 1.25, 95 % confidence interval [CI] = 1.08 to 1.46, $p = 0.004$). A meta-analysis of four studies ($N = 507$) using a continuous measure of depressive symptoms showed a similar effect of adjunctive L-methylfolate (standardized mean difference = − 0.38, 95 % CI = − 0.59 to − 0.17, $p = 0.0003$).

Conclusion Adjunctive L-methylfolate may have modest efficacy in antidepressant-treated adults with MDD.

* Co-first authors.

Introduction

Almost one in five people experience one episode of major depressive disorder (MDD) at some point in their life that severely impacts psychosocial functioning and diminishes their quality of life. In fact, depressive disorders represent an increasing cause of disability worldwide and account for more disability-adjusted life-years than substance use, stroke, diabetes, and chronic kidney disease in individuals under 50 years of age [1]. Antidepressants represent the first-line pharmacotherapies for moderate-to-severe MDD [2]. However, treatment response varies considerably from person to person, with only 50 % benefitting from their first trial [3, 4]. Among the potential causes of non-response to antidepressant medications is the deficit in folate metabolism and cycling/transport (▶ **Fig. 1**) [5].

The folate cycle is important for the synthesis of L-methylfolate, which is utilized in the methylation cycle during DNA synthesis, homocysteine metabolism, and neurotransmitter production [6, 7]. Specifically, the enzyme methylenetetrahydrofolate reductase (MTHFR) converts folic acid to L-methylfolate. According to the 1000 Genomes Project Phase 3, between 9–47 % of the North American population have variants (e. g., C677T, A1298C) in the *MTHFR* gene that reduce its enzymatic activity and have been proposed as biomarkers for risk of depression [8–11]. Therefore, adjunctive L-methylfolate in antidepressant-treated patients may increase the efficiency of these cycles by bypassing the MTHFR-dependent conversion of folate to L-methylfolate, putatively enhancing antidepressant response [12]. (▶ **Tables 1, 2**)

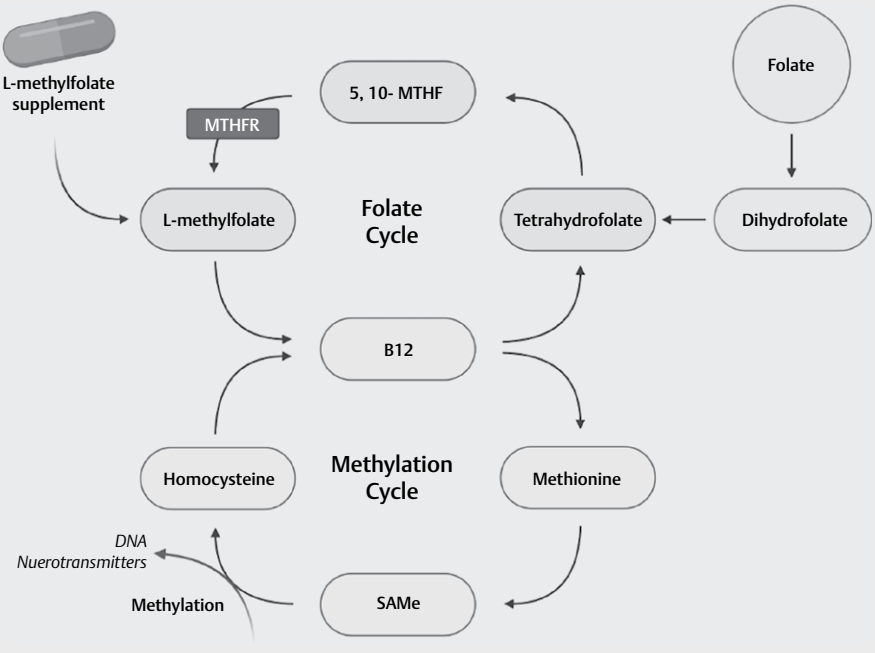
L-methylfolate, the active form of folate that crosses the blood-brain barrier, serves as a methyl donor in processes such as the folate and methylation cycles (▶ **Fig. 1**) [13]. Recent reviews and meta-analyses have recommended L-methylfolate augmentation for depression and emphasized the role of L-methylfolate supplementation in the biochemical and physiological processes that are relevant to the pathophysiology of mood disorders [13, 14]. However, most clinical practice guidelines (i. e., Canadian Network for Mood and Anxiety Treatments, American Psychiatric Association, National Institute for Health and Care Excellence, and World Federation of Societies of Biological Psychiatry) do not provide recommendations on L-methylfolate augmentation [15–18]. One exception is the British Association for Psychopharmacology guidelines, which recommend L-methylfolate augmentation in patients who are not responsive to selective serotonin reuptake inhibitors (SSRIs) [19]. Further, the recent increased use of pharmacogenomic tests for MTHFR and L-methylfolate augmentation makes it important to understand what overall evidence exists for its use in MDD.

To aid efforts to determine the benefits of L-methylfolate augmentation to antidepressants and its appropriateness in clinical practice, we systematically searched the peer-reviewed literature and conducted meta-analyses on L-methylfolate augmentation in adults with MDD using standardized tools and consensus criteria. Whereas previous reviews and meta-analyses have focused on both folate and L-methylfolate [14], we looked exclusively at L-methylfolate augmentation and added an additional study that was not included in previous studies [20].

Methods

Search Strategy

This systematic review and meta-analysis were conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) recommendations and registered with PROSPERO (CRD42020218394) [21]. Two reviewers (AAM and LCB) independently searched PubMed for published articles written in



▶ **Fig. 1** Folate and methylation cycles.

▶ **Table 1** A summary of the characteristics of the studies (n = 10) included in the systematic review on L-methylfolate augmentation in depressive disorders.

Study (First Author, Year)	Sample Size	Subject Characteristics (Age in years, Women %)	Study Design	Treatment	Treatment Duration	Comparator	Primary Outcome Measurement	Result
Ginsberg, 2011	242	43, 66.9%	Retrospective	L-methylfolate 7.5 or 15 mg + SSRI/SNRI	Minimum of 60 days	SSRI/SNRI	CGI-S	The treated group had greater improvement of symptoms versus the comparator
* Godfrey, 1990	24 (patients with depression)	45.5, 54%	Double-blinded, randomized controlled trial	L-methylfolate 15 mg + psychotropic medication	6 months	Placebo + psychotropic medication	HAM-D17	The treated group trended towards having a greater reduction in HAM-D versus the comparator
* Kakar, 2017	260	36.9, 53.5%	Double-blinded, randomized controlled trial	L-methylfolate 15 mg + escitalopram	30 days	Placebo + escitalopram	HAM-D17	Response in the treated was better than the comparator
Shelton, 2013	554	49.6, 76.5%	Prospective, naturalistic study	L-methylfolate 7.5 or 15 mg monotherapy or as an adjuvant with SSRI, SNRI, or other psychotropic medications	90 days	None	PHQ-9	68.1% (n = 342) responded and 45% (n = 226) achieved remission
Shelton, 2015	69 patients (same cohort as Papakostas trial 2)	*Unable to calculate*	Post-hoc analysis of double-blinded, randomized controlled trial	L-methylfolate 15 mg + SSRI	60 days	Placebo + SSRI	HAM-D17/28	The treated group had a greater response than the comparator. Inflammatory and obesity-related factors were associated with improved response in the treated group
* Papakostas (Trial 1), 2012	148	47.9, 69.5%	Double-blinded, randomized controlled trial	L-methylfolate 7.5/15 mg + SSRI	60 days	Placebo + SSRI	HAM-D17	No difference in response between treated and comparator groups
* Papakostas (Trial 2), 2012	75	48.4, 70.6%	Double-blinded, randomized controlled trial	L-methylfolate 15 mg + SSRI	60 days	Placebo + SSRI	HAM-D17	The treated group had a greater response versus the comparator group
Papakostas, 2014	74 provided data & 61 completed the study (same cohort as Papakostas trial 2)	*Unable to calculate*	Post-hoc analysis of double-blinded, randomized controlled trial	L-methylfolate 15 mg + SSRI	60 days	Placebo + SSRI	HAM-D28	The treated group had improved response versus the comparator group when stratified by biological and genetic biomarkers

▲ **Table 1** Continued

Study (First Author, Year)	Sample Size	Subject Characteristics (Age in years, Women %)	Study Design	Treatment	Treatment Duration	Comparator	Primary Outcome Measurement	Result
Wade, 2014	5,404	45.4, 74.5 %	Retrospective	7.5 (96.5 % of cohort) or 15 mg L-methylfolate + SSRI/SNRI	> 231 days of continuous augmentation	SSRI/SNRI + second-generation antipsychotic	NCQA mHEDIS AMM	The treated group had better adherence scores and lower total and depression-related costs and utilization than the comparator group
Zajecka, 2016	68 (same cohort as Papakostas trial 2)	48.3, 52.3 %	Open-label follow-up	L-methylfolate 15 mg + SSRI	12 months	None	HAM-D17	38 % (n = 26) fully recovered with no recurrence of MDD

* Included in the meta-analysis (n = 4). Participants from all studies were from European ancestry with the exception of Kakar et al. (East Asian).[22] Abbreviations: HAM-D17/28, Hamilton Depression Rating Scale; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin-norepinephrine reuptake inhibitor; NCQA, National Committee for Quality Assurance; mHEDIS, modified Healthcare Effectiveness Data and Information Set; AMM, Antidepressant Medication Management; MADRS, Montgomery-Asberg Depression Rating Scale; MDD, major depressive disorder; PHQ-9, 9-item Patient Health Questionnaire; CGI-S, Clinical Global Impression – Severity.

English up to December 31, 2020. The search strategy was "((L-methylfolate OR Deplin OR methylenetetrahydrofolate reductase OR MTHFR OR folate) AND (therapy OR augmentation OR treatment OR adjuvant OR adjunctive OR supplementation) AND (depression OR depressive * OR MDD OR antidepressant))." Two reviewers (AAM and LCB) independently searched the titles and abstracts of all articles identified by the search strategies for eligibility. The full-text copies of articles that met the inclusion criteria were assessed to ensure consistency with the eligibility criteria. Bibliographies of all full-text research articles and review articles were searched manually for additional references not identified in the primary searches (AAM). Articles for which a consensus between the two reviewers was not obtained were assessed by a third reviewer (EAP). The articles that met the inclusion criteria were then selected for data extraction. Extracted information included database ID, authors, publication year, study title, subject characteristics, study design and sample size, diagnosis studied, L-methylfolate dose and duration, comparator, phenotypes investigated, how and what outcomes were measured, main findings, safety/side effects, and conflicts of interest information.

Study Selection Criteria

Article inclusion criteria were: (1) examined L-methylfolate adjunctive therapy in depressive disorders or its effect on antidepressant response in humans, (2) published in a peer-reviewed, English-language journal, and (3) availability of the full-text. Review articles, commentaries, books, book chapters, editorial pieces, or any published material not deemed original research were excluded in the evaluation. Case and single-arm studies of clinical relevance identified from the systematic search are reviewed in the discussion section but were not included as eligible studies in the systematic review and meta-analysis.

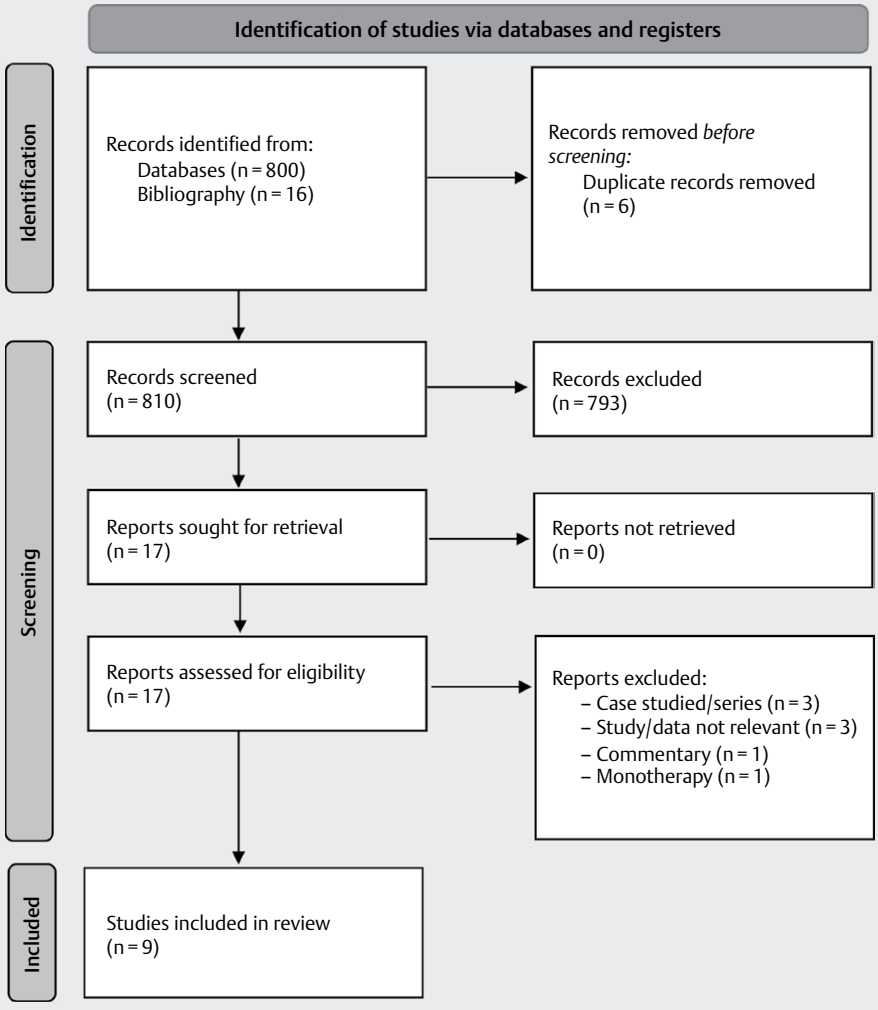
Meta-Analysis and Risk of Bias Assessment

Studies were reviewed to identify randomized controlled trials evaluating the use of adjunctive L-methylfolate in the treatment of depressive disorders by two independent reviewers (LCB and EAP). For meta-analyses, we only included studies reporting Hamilton Rating Scale for Depression (HAM-D17) response or scores at the trial endpoint. Random-effects models were utilized to determine the relative risk (RR) of response for adjunctive L-methylfolate versus antidepressant monotherapy. Using the Mantel-Haenszel meta-analytic method, effect sizes were pooled with 95 % confidence intervals (CI) and the alpha threshold was set at 0.05. Fixed-effects models were performed as a sensitivity analysis. A second meta-analysis was performed to determine the standardized mean difference (SMD) of HAM-D17 scores at the trial endpoint. Missing standard deviation values at the endpoint were estimated using baseline scores. Using Hedge's g as the meta-analytic method, effect sizes were pooled with 95 % CI and the alpha threshold was set at 0.05. For both outcomes, a sensitivity meta-analysis was performed after removing one study (that used 7.5 mg L-methylfolate) to mitigate variability between studies regarding different L-methylfolate doses [22]. Response rates were pooled from the studies included in the meta-analysis to calculate the number needed to treat with L-methylfolate augmentation. Due to the lack of reporting of adverse events, the number needed to harm was not calcu-

▶ **Table 2** Cochrane risk of bias assessment [26] of included studies in the meta-analysis (n = 4).

	Godfrey et al., 1990 [27]	Papakostas et al., 2012 (Trial 1, 7.5 mg) [22]	Papakostas et al., 2012 (Trial 2, 15 mg) [22]	Kaker et al., 2017 [20]
Random sequence generation	+	+	+	+
Allocation concealment	+	+	+	+
Blinding of participants and personnel	+	+	+	+
Blinding of outcome assessment	?	+	+	?
Incomplete outcome data	+	+	+	+
Selective reporting	+	+	+	?
Other sources of bias	+	-	-	+

(+) Low risk of bias; (-) High risk of bias, (?) Unknown risk of bias.



▶ **Fig. 2** Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart detailing the article selection process.

lated. Funnel plots and statistical testing for publication bias were not performed as these techniques are discouraged when the number of studies is less than 10 [23]. Random-effects models were performed as a sensitivity analysis. All analyses were performed using the "meta" and "metafor" packages in *R* version 4.0.3 (Vienna, Austria) [24, 25]. The risk of bias of these trials was assessed using the Cochrane risk of bias tool [26].

Results

Systematic Review

The article selection process is summarized as a PRISMA flowchart in ▶ **Fig. 2**. In total, 800 articles were identified through PubMed database searches and an additional 16 articles were identified

through manual searches of review article bibliographies, resulting in a total of 810 articles after duplicates were removed. Seventeen articles were included for full-text screening. Bibliographies of all full-text research articles were searched manually for additional references; however, no additional articles were identified. After the full-text screening, 9 articles were included in the systematic review, and three articles were identified for meta-analyses. One article provided data from two independent randomized controlled trials and both trials met inclusion criteria for the systematic review and meta-analysis [22]. Therefore, 10 studies were included for the systematic review and four studies for the meta-analyses. Four studies were double-blind, randomized controlled trials, with three additional studies reporting results from a *post-hoc* analysis of one of the trials [22, 27–30]. All studies involving clinical trial data used HAM-D17 as the primary outcome measurement. One additional study used a prospective, naturalistic study design and did not have a comparator group [31]. Lastly, two studies were retrospective case-controlled studies, one of which evaluated the efficacy of adjunctive L-methylfolate in combination with SSRIs or serotonin-norepinephrine reuptake inhibitor (SNRI) whereas the other study evaluated adherence and cost-utilization [32, 33]. All studies used L-methylfolate doses of either 7.5 or 15 mg/day.

Summary of Included Studies

The largest studies of L-methylfolate augmentation included two randomized, double-blind, parallel-sequential trials of augmentation with L-methylfolate (7.5 mg and 15 mg) in adults with partial SSRI response [28]. In the first trial, 7.5 mg L-methylfolate augmentation did not differ from placebo in terms of treatment efficacy as measured by HAM-D17 in 148 MDD patients. In the second trial, 15 mg L-methylfolate augmentation to SSRI treatment in adults with MDD (N = 75) produced greater symptomatic improvement (–5.58 vs. –3.04, $p = 0.05$) and response rates (32.3 % vs. 14.6 %, $p = 0.04$) on the HAM-D17 compared to SSRI therapy plus placebo. Taken together, these studies suggest that augmentation of 15 mg L-methylfolate with SSRIs may improve outcomes for individuals with MDD and inadequate response to SSRIs. Two other studies also investigated the efficacy of L-methylfolate augmentation in patients with MDD. Kakar and colleagues reported that augmentation with escitalopram and L-methylfolate improved outcomes compared to placebo in patients with MDD (N = 260) [20]. Godfrey and colleagues found that L-methylfolate augmentation over six months improved clinical symptoms compared to placebo in depressed adults (N = 24), but the improvement in HAM-D17 score was not significant despite lower scores in the L-methylfolate arm [27].

In an open-label, naturalistic trial, patients with depression who were treated with L-methylfolate (7.5 mg or 15 mg) monotherapy or as augmentation had improved Patient Health Questionnaire-9 scores and improved functioning [31]. However, without a comparison group, it is difficult to determine if L-methylfolate would outperform placebo in these patients. Interestingly, one study sought to understand the economic effects of adjunctive L-methylfolate compared to antipsychotics. In a large registry of SSRI- and SNRI-treated patients who received adjunctive L-methylfolate or antipsychotics, patients who received L-methylfolate had improved adherence scores, as well as lower medical utilization and depression-related costs compared to those who receive augmentation with second generations antipsychotics [33]. This indicates that L-methylfolate augmentation may have greater economic savings than adjunctive antipsychotic therapy in patients with MDD who are treated with SSRIs and SNRIs.

While MTHFR is crucial to converting folic acid to L-methylfolate (▶ **Fig. 1**) and may play a role in depression and antidepressant response, only one of the included studies sought to determine if genetic variations in *MTHFR* play a role in treatment response with L-methylfolate [28]. The authors reported that carriers of the 677 T variant trended towards more improvement; however, the result was not significant, and neither was the response rate. The A1298C variant also did not correlate with improvement or response. Therefore, whether MTHFR variants play a role in response to L-methylfolate augmentation is not known and additional studies are needed.

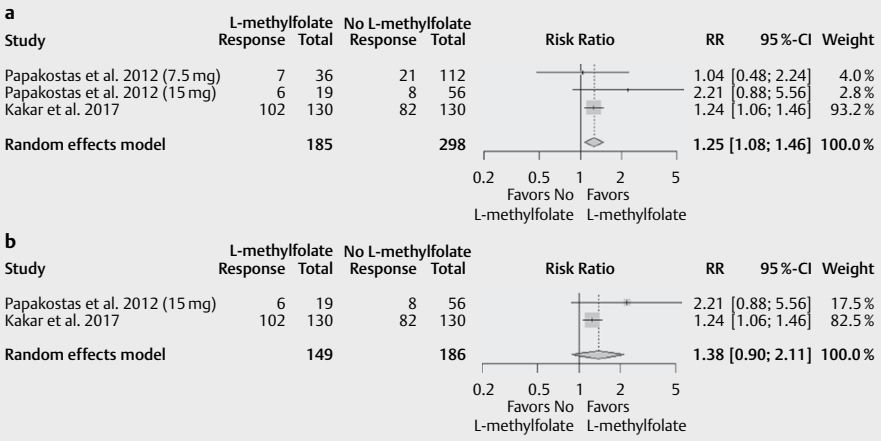
Meta-Analysis

For the meta-analysis focusing on HAM-D17 response, two articles reporting three independent trials were included [20, 22]. No heterogeneity between the included studies was detected ($I^2 = 0\,\%$, $p = 0.43$). However, heterogeneity across the studies is likely present despite the lack of statistical proof of such heterogeneity. The random-effects model revealed a 25 % greater likelihood of response among those receiving L-methylfolate augmentation compared to those that received SSRI or SNRI monotherapy (▶ **Fig. 3a**, RR = 1.25, 95 % CI = 1.08 to 1.46, $p = 0.004$). Further, a fixed-effects model maintained this significant association (RR = 1.26, 95 % CI: [1.07 to 1.48], $p = 0.005$). A sensitivity analysis after removing the one trial that evaluated adjunctive L-methylfolate at a dose of 7.5 mg, thereby only including the two trials assessing a 15 mg dose of L-methylfolate doses, did not result in a significant effect (▶ **Fig. 3b**, RR = 1.38, 95 % CI = 0.90 to 2.11, $p = 0.14$) [18].

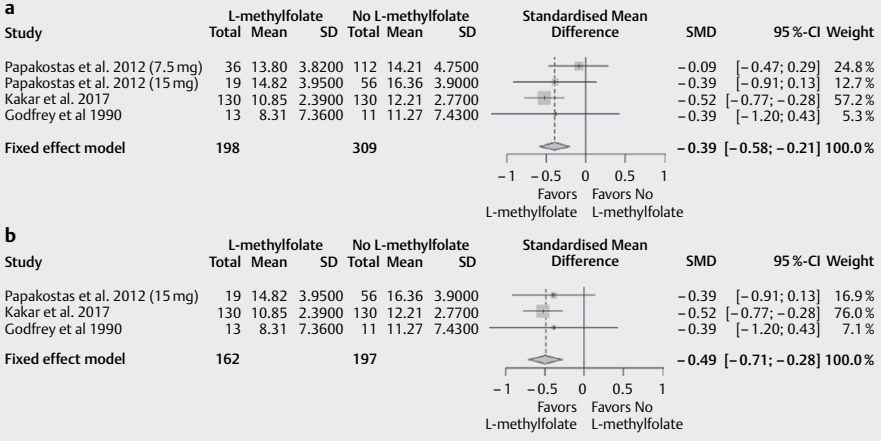
For the meta-analysis focusing on HAM-D17 scores at trial endpoint, three articles reporting four independent trials were included [20, 22, 27]. There was minimal heterogeneity between the included studies ($I^2 = 16\,\%$, $p = 0.31$). The random-effects model showed an effect size of SMD = − 0.38 (▶ **Fig. 4a**, 95 % CI = − 0.59 to − 0.17, $p = 0.0003$). A fixed-effects model maintained this significant association (SMD = − 0.39, 95 % CI = − 0.58 to − 0.21, $p < 0.0001$). A sensitivity analysis after removing the one trial that evaluated adjunctive L-methylfolate at a dose of 7.5 mg, thereby only including the two trials assessing a 15 mg dose of L-methylfolate doses, resulted in a similar significant effect (▶ **Fig. 4b**, SMD = − 0.49, 95 % CI = − 0.71 to − 0.28, $p < 0.0001$).

Risk of Bias

The risk of bias was analyzed based on the Cochrane risk of bias assessment tool [26] and a consensus was reached by all authors. The included studies have a moderate to high risk of bias. It is not known whether the studies by Godfrey et al. [27] and Kakar et al. [20] were blinded. No dropouts were reported in the study by Kakar et al. [20]. An author in the studies by Papakostas also held a patent for the trial design which may present some risk of bias as well [22, 30].



▶ **Fig. 3** Hamilton Rating Scale for Depression-17 (HAM-D17) response meta-analysis of L-methylfolate augmentation studies. (**a**) All controlled studies (N = 3), (**b**) Only 15 mg of L-methylfolate augmentation (N = 2).



▶ **Fig. 4** Hamilton Rating Scale for Depression-17 (HAM-D17) scores at trial endpoint meta-analysis of L-methylfolate augmentation studies. (**a**) All controlled studies (N = 4), (**b**) Only 15 mg of L-methylfolate augmentation (N = 3).

Discussion

The findings from our systematic review and meta-analysis favor the use of L-methylfolate as an adjunct to antidepressant (i. e., SSRI, SNRI) therapy for the treatment of MDD. However, the effect sizes were notably small and were derived from a modest number of patients. Our findings concur with a previous meta-analysis of folate and its derivatives and the British Association for Psychopharmacology guidelines for the treatment of depression, which recommended to (1) not prescribe L-methylfolate as monotherapy in patients with MDD and (2) consider 15 mg as an adjunct to an SSRI for the treatment of MDD [14, 19].

The decision to utilize L-methylfolate as an augmentation strategy, however, should be considered in light of several caveats of the current evidence. First, the patients included in published studies to date may not be representative of all MDD patients. The majority were women of European background in their 40's, who had experienced an inadequate response to multiple antidepressant medication trials, potentially owing to treatment-resistant depression. Whether L-methylfolate augmentation varies by age, sex, ethnicity, disease severity, or stage of treatment has yet to be determined and warrants further study. Second, it is unclear whether specific SSRIs or SNRIs are superior for L-methylfolate augmentation. Among the RCTs we identified, SSRIs were the most common antidepressants examined but head-to-head trials will be required to determine superiority. There is also no data on the efficacy of L-methylfolate augmentation for other antidepressant classes (e. g., tricyclics). Third, we did not meta-analytically examine adverse drug reactions or side effects due to the lack of available data in the included trials. However, adjunctive L-methylfolate appears to be well-tolerated, with gastrointestinal and somatic adverse

events being the most common [20, 22, 30]. Previous work has also suggested tolerability does not vary by *MTHFR* genotypes [22]. Fourth, the impact of *MTHFR* genetic variation on adjunctive L-methylfolate efficacy is unclear. Despite strong biological plausibility, the evidence to date is inconclusive. We only identified one small study (N = 75) that met our inclusion criteria, which failed to find an association between the C677T polymorphism and response to L-methylfolate augmentation. Likewise, a recent large study of 426 children and adolescents with mood disorders, reported that neither improvement nor response to adjunctive L-methylfolate was influenced by C677T *MTHFR* genotype [34]. Fifth, we did not perform a meta-regression to adjust for methodological differences across included studies, given the minimal number of studies included. Sixth, our systematic review and meta-analysis focused exclusively on adults with MDD. While most of the studies with L-methylfolate augmentation have been studied in adult patients with MDD, one small open-label registry study of L-methylfolate in ten patients with bipolar depression found improvement in outcomes [35]. Likewise, in a case series of 10 adolescents with treatment-resistant depression, 80 % showed improvement with adjunctive L-methylfolate and another study of 190 children and adolescents with anxiety and mood disorders found fewer adverse events in the L-methylfolate group compared to the comparator arm [36, 37]. However, in 426 children and adolescents with mood disorders, neither improvement nor response was influenced by adjunctive L-methylfolate use [34]. These initial findings suggest the need for additional research to understand the effect of L-methylfolate in other disorders and examine developmental factors that may contribute to differences in response in pediatric patients. Finally, the study by Kakar et al. [20] had several methodological issues, e. g., they reported the study as an augmentation study although, they introduced L-methylfolate as a combination with SSRIs. Moreover, the study did not have a detailed statistical analysis plan and did not report any dropouts.

Given the limited evidence and modest effect size, this study precludes a thorough discussion of the clinical considerations for L-methylfolate. While L-methylfolate appears to play a role in the pathophysiology and treatment of MDD, establishing its specific place within the psychopharmacologic armamentarium is an ongoing challenge. Further understanding this heterogeneity of treatment response, especially in patients with treatment-resistant depression, is of critical importance.

We meta-analytically identified the effects of L-methylfolate on endpoint HAM-D17 response and HAM-D17 score at trial endpoints in patients with MDD. While endpoint differences are important, faster improvement is also very important for clinicians, particularly as patients with treatment-resistant depression who improve earlier have triple the likelihood of achieving remission over longer-term follow-up [38]. The time course of response to adjunctive L-methylfolate remains unclear. Relatedly, the role of L-methylfolate within sequential or staged approaches warrants further study. Should L-methylfolate be added early in treatment or after less than predicted improvement over the first 4 weeks and should this differ based on genotype or other factors? Answering these questions by understanding the trajectory and heterogeneity of L-methylfolate response will be critical to determining the specific role of L-methylfolate in MDD.

Acknowledgments

The study is registered with PROSPERO (CRD42020218394) and did not receive any funding.

Conflict of Interest

LCB was an employee of Tempus Labs which provides MTHFR genetic testing. AAM, EAP, CAB, and LCB are members of the Pharmacogenomics Research Network (PGRN) and Clinical Pharmacogenetics Implementation Consortium (CPIC). AAM, LCB, and CAB are members of the International Society for Psychiatric Genetics (ISPG).

References

[1] Malhi GS, Mann JJ. Depression. The Lancet 2018; 392: 2299–2312

[2] Kennedy SH, Lam RW, McIntyre RS et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: Section 3. Pharmacological treatments. Can J Psychiatry 2016; 61: 540–560

[3] Warden D, Rush AJ, Trivedi MH et al. The STAR * D Project results: a comprehensive review of findings. Curr Psychiatry Rep 2007; 9: 449–459

[4] Rush AJ, Trivedi MH, Stewart JW et al. Combining medications to enhance depression outcomes (CO-MED): acute and long-term outcomes of a single-blind randomized study. Am J Psychiatry 2011; 168: 689–701

[5] Nelson JC. The evolving story of folate in depression and the therapeutic potential of l-methylfolate. Am J Psychiatry 2012; 169: 1223–1225

[6] Frankenburg FR. The role of one-carbon metabolism in schizophrenia and depression. Harv Rev Psychiatry 2007; 15: 146–160

[7] Bottiglieri T, Laundy M, Crellin R et al. Homocysteine, folate, methylation, and monoamine metabolism in depression. J Neurol Neurosurg Psychiatry 2000; 69: 228–232

[8] Frosst P, Blom HJ, Milos R et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111–113

[9] Weisberg I, Tran P, Christensen B et al. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998; 64: 169–172

[10] Auton A, Brooks LD, Durbin RM et al. A global reference for human genetic variation. Nature 2015; 526: 68–74

[11] Wu YL, Ding XX, Sun YH et al. Association between MTHFR C677T polymorphism and depression: An updated meta-analysis of 26 studies. Prog Neuropsychopharmacol Biol Psychiatry 2013; 46: 78–85

[12] Stahl SM. Novel therapeutics for depression: L-methylfolate as a trimonoamine modulator and antidepressant-augmenting agent. CNS Spectr 2007; 12: 739–744

[13] Hoepner CT, McIntyre RS, Papakostas GI. Impact of supplementation and nutritional interventions on pathogenic processes of mood disorders: A review of the evidence. Nutrients 2021; 13(3): 767

[14] Roberts E, Carter B, Young AH. Caveat emptor: Folate in unipolar depressive illness, a systematic review and meta-analysis. J Psychopharmacol 2018; 32: 377–384

[15] Ravindran AV, Balneaves LG, Faulkner G et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: Section 5. Complementary and alternative medicine treatments. Can J Psychiatry 2016; 61: 576–587

[16] American Psychiatric Association Workgroup on Major Depressive Disorder. Practice Guideline for the Treatment of Patients With Major Depressive Disorder. Am Psych Publishing 2010; http://www.psychiatryonline.com/pracGuide/pracGuideTopic_7.aspx

[17] National Institute for Health and Care Excellence (NICE). Depression in Adults: Recognition and Management Clinical Guideline [CG90], 2009; https://www.nice.org.uk/guidance/cg90

[18] Bauer M, Pfennig A, Severus E. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of unipolar depressive disorders, part 1: Update 2013 on the acute and continuation treatment of unipolar depressive disorders. World J Biol Psychiatry 2013; 14: 334–385

[19] Cleare A, Pariante CM, Young AH et al. Evidence-based guidelines for treating depressive disorders with antidepressants: A revision of the 2008 British Association for Psychopharmacology guidelines. J Psychopharmacol 2015; 29: 459–525

[20] Kakar SM, Jehangir S, Mustafa M et al. Therapeutic efficacy of combination therapy of L-methylfolate and escitalopram in depression. PAFMJ 2017; 67: 976–981

[21] Page MJ, McKenzie JE, Bossuyt PM et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 2021; 372: n71

[22] Papakostas GI, Shelton RC, Zajecka JM et al. L-methylfolate as adjunctive therapy for SSRI-resistant major depression: results of two randomized, double-blind, parallel-sequential trials. Am J Psychiatry 2012; 169: 1267–1274

[23] Lau J, Ioannidis JP, Terrin N et al. The case of the misleading funnel plot. BMJ 2006; 333: 597–600

[24] Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: A practical tutorial. Evid Based Ment Health 2019; 22: 153–160

[25] Viechtbauer W. Conducting ceta-analyses in R with the metafor package. J Stat Softw 2010; 36: 1–48

[26] Sterne JAC, Savović J, Page MJ et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. BMJ 2019; 366: l4898

[27] Godfrey PS, Toone BK, Carney MW et al. Enhancement of recovery from psychiatric illness by methylfolate. Lancet 1990; 336: 392–395

[28] Papakostas GI, Shelton RC, Zajecka JM et al. Effect of adjunctive L-methylfolate 15 mg among inadequate responders to SSRIs in depressed patients who were stratified by biomarker levels and genotype: Results from a randomized clinical trial. J Clin Psychiatry 2014; 75: 855–863

[29] Zajecka JM, Fava M, Shelton RC et al. Long-term efficacy, safety, and tolerability of L-methylfolate calcium 15 mg as adjunctive therapy with selective serotonin reuptake inhibitors: A 12-month, open-label study following a placebo-controlled acute study. J Clin Psychiatry 2016; 77: 654–660

[30] Shelton RC, Pencina MJ, Barrentine LW et al. Association of obesity and inflammatory marker levels on treatment outcome: results from a double-blind, randomized study of adjunctive L-methylfolate calcium in patients with MDD who are inadequate responders to SSRIs. J Clin Psychiatry 2015; 76: 1635–1641

[31] Shelton RC, Sloan Manning J, Barrentine LW et al. Assessing effects of l-methylfolate in depression management: Results of a real-world patient experience trial. Prim Care Companion CNS Disord 2013; 15: PCC.13m01520

[32] Ginsberg LD, Oubre AY, Daoud YA. L-methylfolate plus SSRI or SNRI from treatment initiation compared to SSRI or SNRI monotherapy in a major depressive episode. Innov Clin Neurosci 2011; 8: 19–28

[33] Wade RL, Kindermann SL, Hou Q et al. Comparative assessment of adherence measures and resource use in SSRI/SNRI-treated patients with depression using second-generation antipsychotics or L-methylfolate as adjunctive therapy. J Manag Care Pharm 2014; 20: 76–85

[34] Poweleit EA, Bopp EA, Coz Mm et al. Ineffectiveness of adjunctive L-methylfolate in pediatric mood disorders. American Society for Clinical Pharmacology & Therapeutics Annual Meeting 2021

[35] Nierenberg AA, Montana R, Kinrys G et al. L-methylfolate for bipolar I depressive episodes: An open trial proof-of-concept registry. J Affect Disord 2017; 207: 429–433

[36] Dartois LL, Stutzman DL, Morrow M. L-methylfolate augmentation to antidepressants for adolescents with treatment-resistant depression: A case series. J Child Adolesc Psychopharmacol 2019; 29: 386–391

[37] Rainka M, Aladeen T, Westphal E et al. L-methylfolate calcium supplementation in adolescents and children: A retrospective analysis. J Psychiatr Pract 2019; 25: 258–267

[38] Emslie GJ, Mayes T, Porta G et al. Treatment of resistant depression in adolescents (TORDIA): Week 24 outcomes. Am J Psychiatry 2010; 167: 782–791Discussion