Folate-Based Interventions in Major Depressive Disorder: From Folic Acid and L-Methylfolate to MTHFR and Precision Psychiatry

Jozélio Freire de Carvalho
Núcleo de Pesquisa em Doenças Crônicas não Transmissíveis (NUPEn-DC), School of Nutrition, Federal University of Bahia, Salvador, Bahia, Brazil

* Corresponding Author: **Jozélio Freire de Carvalho**

Article Info

E-ISSN: 3107-7137
Volume: 02
Issue: 05
Received: 09-05-2026
Accepted: 07-07-2026
Published: 05-09-2026
Page No: 39-50

Abstract

Background: Folate metabolism has long been implicated in major depressive disorder (MDD), through its involvement in one-carbon metabolism, methylation reactions, homocysteine regulation, and monoamine synthesis. However, folic acid, folinic acid, and L-methylfolate (5-MTHF) are pharmacologically distinct compounds, and their clinical evidence in depression differs substantially. Interest has also increased in methylenetetrahydrofolate reductase (MTHFR) polymorphisms and metabolic and inflammatory biomarkers as potential predictors of treatment response.

Objective: To critically review the evidence linking folate metabolism to MDD and to evaluate the efficacy, dose-response relationships, biological rationale, and potential predictors of response to folic acid, folinic acid, and L-methylfolate, with particular emphasis on antidepressant augmentation and the clinical relevance of MTHFR polymorphisms.

Methods: A structured narrative review was conducted using MEDLINE/PubMed, Embase, Scopus, Web of Science, PsycINFO, Cochrane Library, Google Scholar, ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform from inception through September 2026. Evidence was synthesized hierarchically, prioritizing umbrella reviews, meta-analyses, systematic reviews, and randomized controlled trials, followed by prospective, observational, biomarker, genetic, and mechanistic studies. Folate formulations were analyzed separately because of their pharmacological differences.

Results: Higher-level evidence supports a modest overall benefit of folate-based interventions in depression, but substantial heterogeneity exists across formulations and doses. The most consistent formulation-specific evidence was identified for adjunctive L-methylfolate 15 mg/day in patients with inadequate antidepressant response. In contrast, L-methylfolate 7.5 mg/day has not demonstrated consistent efficacy. Evidence for folic acid is mixed; although smaller studies reported beneficial effects, the large FolATED randomized trial failed to demonstrate clinically meaningful augmentation with folic acid 5 mg/day. Evidence for folinic acid remains limited and predominantly uncontrolled. MTHFR C677T and, less consistently, A1298C have been associated with depression susceptibility and possibly treatment resistance; however, current evidence does not validate routine MTHFR genotyping for selecting L-methylfolate treatment. Exploratory analyses suggest that obesity, inflammatory biomarkers, oxidative stress, and abnormalities in one-carbon metabolism may identify patients with greater L-methylfolate response, raising the possibility of a broader folate–metabolic–inflammatory phenotype.

Conclusions: Folate-based interventions in MDD should not be considered a homogeneous therapeutic class. Current evidence most strongly supports L-methylfolate 15 mg/day as an adjunctive strategy in patients with inadequate response to antidepressants, whereas high-dose folic acid cannot presently be considered an evidence-based substitute and data for folinic acid remain insufficient. Although MTHFR is biologically relevant, isolated MTHFR genotyping has not been prospectively validated as a treatment-selection tool. Future biomarker-stratified trials should determine whether integrated genetic, nutritional, metabolic, and inflammatory profiles can identify patients most likely to benefit from L-methylfolate augmentation.

DOI: https://doi.org/10.54660/IJBBR.2026.2.5.39-50

Keywords: major depressive disorder, L-methylfolate, 5-methyltetrahydrofolate, folic acid, folinic acid, MTHFR, treatment-resistant depression, antidepressant augmentation, one-carbon metabolism, homocysteine

Introduction

Major depressive disorder (MDD) is a heterogeneous psychiatric disorder characterized by substantial variability in clinical presentation, biological substrates, treatment response, and long-term outcome. Although multiple effective pharmacological treatments are available, a considerable proportion of patients fail to achieve remission after an initial antidepressant trial, and

incomplete response remains one of the major challenges in contemporary psychiatric practice. This therapeutic gap has stimulated increasing interest in adjunctive strategies directed not only at neurotransmitter receptors and transporters but also at metabolic pathways potentially involved in antidepressant response.

Among these pathways, folate-dependent one-carbon metabolism has attracted particular attention. Folate participates in the transfer of one-carbon units required for nucleotide synthesis, methylation reactions, homocysteine remethylation, and the production of S-adenosylmethionine (SAM), the principal methyl donor in numerous biochemical reactions. Through interactions with tetrahydrobiopterin-dependent pathways, folate metabolism may also influence the synthesis of serotonin, dopamine, and norepinephrine. These mechanisms provide biological plausibility for an association between disturbances of folate metabolism and mood disorders.

Observational studies have repeatedly reported associations between low folate status and depression, while low serum folate has also been associated with poorer antidepressant response and an increased risk of relapse [1–3]. Elevated homocysteine, another potential indicator of disturbed one-carbon metabolism, has likewise been associated with depression in epidemiological studies, although its predictive value for antidepressant response has been inconsistent.

Interest in this pathway has expanded further with the recognition of common functional variants in the gene encoding methylenetetrahydrofolate reductase (MTHFR), particularly C677T (rs1801133) and A1298C (rs1801131). MTHFR catalyzes the conversion of 5,10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF), an essential methyl donor for the remethylation of homocysteine to methionine. The C677T variant reduces MTHFR activity, particularly in TT homozygotes, and meta-analytic evidence suggests a modest association between this variant and depression susceptibility in some populations [4]. More recently, MTHFR variants have been investigated in relation to treatment-resistant depression (TRD), although their clinical predictive value remains uncertain [5].

Importantly, an association between MTHFR variants and depression does not establish the clinical utility of MTHFR testing. Genetic susceptibility, biochemical function, and prediction of therapeutic response represent distinct questions. This distinction is particularly relevant because commercial pharmacogenetic testing has increased the visibility of MTHFR polymorphisms, sometimes exceeding the strength of the evidence supporting genotype-guided treatment.

A further source of confusion is the tendency to consider all forms of folate therapeutically equivalent. Folic acid, folinic acid, and L-methylfolate are pharmacologically and metabolically distinct. Folic acid is a synthetic oxidized precursor requiring several metabolic conversion steps before entering active folate pathways. Folinic acid (5-formyl-tetrahydrofolate; leucovorin) is already a reduced folate and bypasses some of these steps. L-methylfolate is the biologically active 5-MTHF form and bypasses the MTHFR-dependent conversion step. These distinctions may be clinically relevant and argue against assuming that increasing the dose of folic acid necessarily reproduces the pharmacological effects of L-methylfolate.

Clinical studies have produced apparently conflicting findings. Early trials suggested that folic acid or methylfolate could enhance antidepressant response [6,7]. However, the large FolATED trial subsequently failed to demonstrate clinically meaningful augmentation with folic acid 5 mg/day [8]. In contrast, randomized trials of L-methylfolate in patients with inadequate response to selective serotonin reuptake inhibitors (SSRIs) demonstrated a dose-dependent pattern, with 15 mg/day showing significant efficacy while a strategy beginning at 7.5 mg/day did not [9].

More recent systematic reviews and meta-analyses have strengthened the evidence supporting adjunctive folate-based interventions while simultaneously highlighting important heterogeneity among formulations [10–12]. A meta-analysis specifically examining L-methylfolate demonstrated modest but significant improvement in antidepressant response, whereas an umbrella meta-analysis pooling broader folate interventions found an overall reduction in depressive symptoms [10,12]. These findings emphasize the need to distinguish a general biological relationship between folate and depression from the efficacy of specific pharmacological formulations.

The possibility of identifying patients particularly likely to benefit from L-methylfolate has generated further interest. Post-hoc analyses of randomized trials suggest that obesity, inflammation, oxidative stress, abnormalities in methylation capacity, and selected genetic markers may be associated with greater response to L-methylfolate [13,14]. These findings raise the possibility that treatment response may reflect a multidimensional metabolic-inflammatory phenotype rather than a single MTHFR polymorphism.

The present narrative review therefore critically examines the role of folate-based interventions in MDD, with particular emphasis on the distinction among folic acid, folinic acid, and L-methylfolate; dose-response relationships; antidepressant augmentation; folate status and homocysteine; MTHFR C677T and A1298C; metabolic and inflammatory predictors of response; safety; and the potential future role of biomarker-guided treatment.

Methods

This narrative review was conducted using a structured and comprehensive literature search. MEDLINE/PubMed, Embase, Scopus, Web of Science, PsycINFO, the Cochrane Library, and Google Scholar were searched from database inception through September 2026. ClinicalTrials.gov and the World Health Organization International Clinical Trials Registry Platform were additionally examined to identify relevant registered or ongoing studies.

Search terms were used individually and in combination and included “major depressive disorder,” “depression,” “treatment-resistant depression,” “folate,” “folic acid,” “folinic acid,” “leucovorin,” “5-methyltetrahydrofolate,” “5-MTHF,” “L-methylfolate,” “one-carbon metabolism,” “homocysteine,” “S-adenosylmethionine,” “MTHFR,” “C677T,” “A1298C,” “pharmacogenetics,” “biomarker,” “augmentation,” “antidepressant response,” and “remission.” Priority was given to human studies directly examining depressive disorders and folate-related interventions or biomarkers. Evidence was considered hierarchically, with umbrella reviews and meta-analyses evaluated first, followed by systematic reviews, randomized controlled trials, prospective controlled studies, open-label studies, observational cohorts, genetic association studies, biomarker studies, and mechanistic literature.

Folic acid, folinic acid, and L-methylfolate were analyzed

separately because these compounds differ in metabolic activation and cannot be assumed to be therapeutically interchangeable. Studies evaluating MTHFR variants as risk factors for depression or TRD were distinguished from studies examining genetic variants as predictors of treatment response.

The principal clinical outcomes considered were changes in validated depression rating scales, treatment response, remission, recovery, relapse or recurrence, treatment discontinuation, tolerability, and adverse events. Biochemical outcomes included folate concentrations, vitamin B12, homocysteine, SAM/SAH indices, inflammatory biomarkers, oxidative stress markers, and metabolic variables.

Because the purpose of the present work was critical narrative synthesis rather than generation of a new pooled effect estimate, no de novo meta-analysis was performed.

Results

Overview of The Evidence

The literature search identified a heterogeneous body of evidence examining the relationship between folate metabolism and depressive disorders, ranging from observational and genetic association studies to randomized controlled trials (RCTs), systematic reviews, meta-analyses, and more recent umbrella-level evidence. Therapeutic studies evaluated three principal folate formulations—folic acid, folinic acid (leucovorin), and L-methylfolate (5-MTHF)—across different doses, populations, and clinical settings. The strongest intervention evidence concerned adjunctive treatment in patients with MDD who had experienced an inadequate response to conventional antidepressants.

Overall, higher-level evidence suggests a beneficial effect of folate-based interventions on depressive symptoms, although the magnitude and consistency of benefit vary substantially according to folate formulation and dose (Table 1). This distinction is clinically relevant because studies combining different forms of folate under a single intervention category may obscure formulation-specific effects.

A recent umbrella meta-analysis integrating 11 previous meta-analyses reported an overall reduction in depressive symptoms associated with folate supplementation, with a standardized mean difference (SMD) of −0.42 (95% CI −0.57 to −0.27). The same analysis also supported an association between folate insufficiency and depression (OR 1.35, 95% CI 1.27–1.42) [12]. However, heterogeneity remained across the underlying literature with respect to folate formulation, patient population, treatment duration, and concomitant antidepressant therapy.

A meta-analysis focusing on adjunctive folate treatment identified six RCTs and reported significant improvements in Hamilton Depression Rating Scale scores (mean difference −2.16, 95% CI −3.62 to −0.69), treatment response (RR 1.36, 95% CI 1.16–1.59), and a borderline improvement in remission (RR 1.39, 95% CI 1.00–1.92) [11]. Because this analysis combined folic acid and L-methylfolate studies, formulation-specific conclusions remain limited.

In contrast, a systematic review and meta-analysis specifically evaluating adjunctive L-methylfolate included nine studies comprising 6,707 participants in the qualitative synthesis. Meta-analysis of categorical response from three studies (n=483) demonstrated a modest but significant benefit (RR 1.25, 95% CI 1.08–1.46), while four studies reporting continuous symptom outcomes (n=507) yielded an SMD of −0.38 (95% CI −0.59 to −0.17) [10]. Collectively, these analyses indicate a small-to-moderate augmentation effect while emphasizing the relatively limited number of controlled trials underlying the pooled estimates.

L-Methylfolate: Randomized Evidence and Dose-Response Relationship

The most consistent formulation-specific clinical evidence was identified for L-methylfolate as an adjunct to antidepressant therapy (Table 1). Early evidence was provided by Godfrey *et al*., who evaluated methylfolate 15 mg/day for six months in psychiatric patients with low or borderline red-cell folate. Clinical and social recovery favored methylfolate, including among patients with depression; however, the depression subgroup was small, limiting the precision and generalizability of the findings [7].

The pivotal modern evidence derives from two randomized, double-blind, sequential-parallel trials conducted by Papakostas *et al*. in patients with MDD who had demonstrated an inadequate response to SSRIs [9]. In the first trial (n=148), an initial dose of L-methylfolate 7.5 mg/day, with subsequent escalation to 15 mg/day in one treatment sequence, did not produce a significant overall advantage over placebo.

The second trial (n=75) used L-methylfolate 15 mg/day throughout the active treatment period and demonstrated significantly greater improvement in depressive symptoms and response compared with continued SSRI plus placebo. The reported number needed to treat for an additional response was approximately six. Importantly, adverse-event rates did not differ meaningfully between L-methylfolate and placebo [9].

Taken together, these trials suggest a clinically relevant dose-response pattern. Evidence supporting 7.5 mg/day is weak or inconsistent, whereas 15 mg/day represents the dose with the strongest randomized evidence. This pattern has also been observed in subsequent evidence syntheses in which favorable effects were concentrated in trials employing the 15-mg/day regimen [10–12].

Longer-term data are less robust. In an open-label extension evaluating L-methylfolate 15 mg/day in combination with an SSRI for up to 12 months, 38% of the 68 patients included in the efficacy analysis achieved full recovery. Among patients entering the extension in remission, 10 of 11 achieved full recovery, with no reported relapse or recurrence [15]. However, the absence of a concurrent placebo comparator prevents firm conclusions regarding maintenance efficacy or prevention of recurrence.

Naturalistic studies have similarly reported reductions in depressive symptom scores during L-methylfolate treatment. These findings support real-world feasibility and tolerability but cannot establish causality because of uncontrolled design, concomitant treatments, and potential selection effects.

Folic Acid

Clinical evidence for folic acid was considerably more heterogeneous than that for L-methylfolate (Table 1). An early randomized placebo-controlled study of 127 patients with MDD evaluated folic acid 0.5 mg/day added to fluoxetine. Adjunctive folic acid was associated with improved antidepressant response, with the strongest effect observed among women. In women, response rates were 93.9% with folic acid versus 61.1% with placebo [6]. The pronounced sex-specific finding and absence of consistent

replication, however, limit broad generalization.

A small randomized double-blind study involving 42 women subsequently compared folic acid 1.5 mg/day with 5 mg/day during fluoxetine treatment. Greater reductions in depressive symptoms were reported with 5 mg/day; HDRS remission occurred in 36.8% versus 8.7% of patients, respectively [16]. The small sample size, restriction to women, and absence of a placebo arm substantially limit interpretation.

Another small randomized study of 27 patients compared fluoxetine plus folic acid 10 mg/day with fluoxetine plus placebo and reported greater reduction in depressive symptoms together with a reduction in homocysteine [17]. Again, the limited sample size restricts the certainty of this result.

The larger FolATED trial provided a more rigorous test of high-dose folic acid augmentation. In this pragmatic randomized, double-blind, placebo-controlled study, approximately 475 participants were randomized to receive folic acid 5 mg/day or placebo in addition to antidepressant therapy. High-dose folic acid did not provide clinically significant improvement in depressive outcomes compared with placebo [8]. These findings provide comparatively strong evidence against the assumption that increasing the dose of folic acid to 5 mg/day necessarily enhances antidepressant efficacy.

Thus, the available data do not demonstrate a simple dose-response relationship for folic acid. Small earlier studies produced positive signals at low and higher doses, whereas the substantially larger controlled FolATED trial failed to confirm clinically meaningful augmentation with 5 mg/day.

Folinic Acid

Evidence concerning folinic acid is limited. A small open-label study evaluated leucovorin at doses of 15–30 mg/day as adjunctive treatment in 22 patients with SSRI-resistant MDD [18]. Among intention-to-treat participants, approximately 27% achieved response and 18% achieved remission. Although depressive symptom scores declined during treatment, the absence of a placebo group and the small sample prevent differentiation between a specific folinic acid effect and improvement related to ongoing antidepressant treatment, expectancy, or natural symptom fluctuation.

Consequently, folinic acid remains biologically relevant as a reduced folate but has substantially weaker clinical evidence for antidepressant augmentation than L-methylfolate.

MTHFR Polymorphisms And Depression

Genetic studies have focused predominantly on two common functional variants of the methylenetetrahydrofolate reductase gene, C677T (rs1801133) and A1298C (rs1801131) (Table 2). The C677T variant reduces MTHFR enzymatic activity, with the greatest functional reduction generally observed in individuals homozygous for the T allele.

Meta-analyses of genetic association studies have reported a modest relationship between C677T and susceptibility to depression, although the magnitude and statistical consistency of this association vary according to ethnicity, population characteristics, and genetic model [4].

More recently, attention has shifted from depression susceptibility toward treatment-resistant depression. A 2026 systematic review specifically addressing MTHFR C677T and A1298C in TRD identified seven eligible studies. The available evidence suggested possible associations of the 677TT and 1298CC genotypes with treatment resistance, but conflicting findings, heterogeneous definitions, limited sample sizes, dietary folate exposure, and comorbidities prevented definitive conclusions [5].

Importantly, evidence associating MTHFR variants with depression or treatment resistance is substantially stronger than evidence demonstrating that MTHFR genotyping can prospectively select patients who will respond to L-methylfolate. At present, the latter remains unconfirmed.

Folate Status, Homocysteine, and One-Carbon Metabolism

Biochemical studies provide additional evidence linking altered one-carbon metabolism with depressive disorders (Table 2). Lower serum or red-cell folate concentrations have been associated with depression and, in clinical cohorts, with poorer antidepressant response. In a cohort of 213 patients treated with fluoxetine, low baseline folate was associated with a significantly lower probability of response [1]. In patients who had already failed fluoxetine, low serum folate was again associated with subsequent treatment resistance [2]. Low folate was also associated with a higher risk of relapse during continuation treatment [3].

Elevated homocysteine has been associated with depression in observational literature, although individual treatment cohorts have not consistently demonstrated an association between homocysteine and antidepressant response [1–3].

These biomarkers are nevertheless nonspecific. Homocysteine concentrations are influenced by age, renal function, nutritional status, vitamin B12 and B6 availability, lifestyle factors, and medication use. Likewise, peripheral folate concentrations do not necessarily provide a direct measure of central nervous system folate availability.

The methionine cycle provides a mechanistic bridge between these observations and neuronal function. 5-MTHF participates in the vitamin B12-dependent remethylation of homocysteine to methionine, which subsequently contributes to S-adenosylmethionine (SAM) formation. SAM serves as a major methyl donor, whereas S-adenosylhomocysteine (SAH) inhibits methylation reactions. Consequently, the SAM/SAH ratio has been investigated as an indicator of cellular methylation potential.

Metabolic and Inflammatory Predictors Of L-Methylfolate Response

Post-hoc analyses of randomized L-methylfolate data identified a potentially important interaction between treatment response and metabolic-inflammatory phenotype (Table 2). Patients with BMI ≥30 kg/m² demonstrated a larger L-methylfolate-placebo difference in depressive symptoms than the overall trial population. In pooled analyses, the HDRS-17 treatment effect was −2.74 points overall and −4.66 points (95% CI −7.22 to −1.98) among patients with BMI ≥30 kg/m² [14].

Inflammatory and metabolic biomarkers were also associated with differential response. Higher baseline concentrations of hsCRP, TNF-α, IL-8, and leptin were associated with larger L-methylfolate-placebo differences, while combinations of BMI ≥30 kg/m² with elevated TNF-α, IL-6, IL-8, hsCRP, or leptin yielded pooled treatment effects ranging approximately from −6.31 to −3.98 points [14].

A related exploratory pharmacogenetic analysis found greater L-methylfolate effects among patients with BMI ≥30 kg/m², elevated hsCRP or 4-hydroxy-2-nonenal, a low

SAM/SAH ratio, and selected genetic markers related to L-methylfolate synthesis and metabolism [13].

These findings suggest that response to L-methylfolate may not depend exclusively on folate deficiency or MTHFR genotype. Instead, a broader metabolic-inflammatory phenotype may influence therapeutic response. However, these analyses were post-hoc, involved relatively small samples, and have not yet generated prospectively validated biomarker thresholds or a clinically established treatment-selection algorithm.

MTHFR Genotype Versus Biomarker-Guided Treatment

The available evidence therefore distinguishes three related but separate concepts. First, MTHFR variants may influence folate metabolism and have modest associations with depression susceptibility. Second, abnormalities in folate, homocysteine, methylation capacity, inflammation, or metabolic status may characterize biologically distinct depressive phenotypes. Third, whether any individual marker—or combination of markers—can reliably predict therapeutic response to L-methylfolate remains uncertain.

Post-hoc pharmacogenetic analyses of L-methylfolate trials have identified genetic markers related to folate metabolism that may influence response, but these findings have not undergone prospective replication sufficient to establish MTHFR genotyping as a prerequisite for treatment [13]. Similarly, evidence from broader combinatorial pharmacogenomic platforms cannot be directly extrapolated to the clinical utility of isolated MTHFR testing.

Mechanistic Differentiation of Folate Formulations

The pharmacological differences among folic acid, folinic acid, and L-methylfolate provide a plausible explanation for at least part of the heterogeneity observed in clinical studies (Table 3). Folic acid is an oxidized synthetic precursor requiring sequential metabolic conversion before entering active intracellular folate pathways. Folinic acid is a reduced folate that bypasses some of these metabolic steps. L-methylfolate represents the biologically active 5-MTHF form and bypasses the MTHFR-dependent conversion of 5,10-methylene-THF to 5-MTHF.

5-MTHF serves as the methyl donor for the vitamin B12-dependent remethylation of homocysteine to methionine, thereby supporting SAM production and multiple methylation reactions relevant to neuronal function. Folate metabolism is also linked to tetrahydrobiopterin (BH4)-dependent pathways. BH4 is an essential cofactor for tryptophan hydroxylase and tyrosine hydroxylase, rate-limiting enzymes involved in the synthesis of serotonin and catecholamines, respectively. These pathways provide a mechanistic rationale through which folate metabolism could influence serotonergic, dopaminergic, and noradrenergic neurotransmission [19,20].

Importantly, these biochemical differences demonstrate that folic acid and L-methylfolate are not pharmacologically interchangeable on a milligram-for-milligram basis. They do not, however, independently establish that one formulation is clinically superior to another. Direct head-to-head randomized evidence comparing adequately dosed folic acid with L-methylfolate in MDD remains lacking.

Overall Synthesis of Clinical Evidence

Across the available evidence, the most reproducible therapeutic signal was observed with adjunctive L-methylfolate at 15 mg/day in patients with inadequate antidepressant response. The 7.5-mg/day regimen did not demonstrate consistent benefit, whereas 15 mg/day was associated with improved response in randomized evidence and favorable pooled effects in subsequent meta-analyses [9–12].

Evidence for folic acid was mixed. Positive findings from smaller studies were not confirmed by the larger FolATED trial using 5 mg/day, arguing against the assumption that high-dose folic acid can simply substitute for pharmacological L-methylfolate [6,8,16,17]. Evidence for folinic acid remained preliminary and was based predominantly on a small uncontrolled study [18].

Finally, MTHFR polymorphisms, low folate status, elevated homocysteine, impaired methylation indices, obesity, and inflammatory biomarkers provide convergent evidence for biological heterogeneity within MDD. However, none currently constitutes a prospectively validated standalone biomarker for selecting L-methylfolate treatment. The emerging evidence instead raises the possibility that a composite folate–metabolic–inflammatory phenotype, rather than an isolated MTHFR genotype, may ultimately prove more informative for precision use of folate-based augmentation.

Discussion

The present review highlights an important evolution in the understanding of folate-based interventions in major depressive disorder (MDD). The available literature no longer supports treating “folate supplementation” as a single therapeutic concept. Folic acid, folinic acid, and L-methylfolate differ substantially in their metabolic requirements, pharmacological characteristics, clinical evidence, and potentially their effects within the central nervous system. When these formulations are considered separately and the evidence is organized hierarchically, a more coherent pattern emerges: among currently studied folate-based strategies, the most consistent evidence supports L-methylfolate 15 mg/day as an adjunctive intervention in patients with an inadequate response to antidepressant therapy, whereas evidence for folic acid is inconsistent and that for folinic acid remains preliminary.

This distinction is clinically relevant because several systematic reviews and meta-analyses have pooled different folate formulations [11,12]. Although such analyses generally suggest an overall benefit of folate-based interventions on depressive symptoms, response, or remission, they may obscure important formulation- and dose-specific effects. An overall positive effect for “folate” should therefore not be interpreted as evidence that all forms of vitamin B9 are therapeutically interchangeable.

Why Does L-Methylfolate 15 Mg/Day Appear Different?

One of the most striking findings in the clinical literature is the apparent dose-dependent effect of L-methylfolate. In the pivotal randomized trials conducted in patients with SSRI-resistant MDD, a strategy beginning with 7.5 mg/day did not demonstrate consistent efficacy, whereas treatment with 15 mg/day throughout the active phase produced significantly greater improvement than placebo, with a number needed to treat of approximately six [9]. Subsequent meta-analytic evidence has supported a modest but significant augmentation effect for L-methylfolate [10].

The biological explanation for this apparent threshold

remains uncertain. One possibility is that the doses used for antidepressant augmentation should be viewed as pharmacological rather than nutritional. Fifteen milligrams of L-methylfolate greatly exceeds normal dietary folate requirements and may therefore exert effects on one-carbon metabolism and central methylation pathways that are qualitatively different from simple correction of nutritional folate deficiency.

This distinction may also explain why baseline folate deficiency does not appear to be an absolute prerequisite for therapeutic response. Historically, folate-based treatment was largely conceptualized as replacement therapy for patients with low folate status. Contemporary L-methylfolate augmentation, however, represents a different therapeutic model: modification of metabolic pathways relevant to neurotransmitter synthesis and methylation in patients who may have normal conventional serum folate concentrations.

Nevertheless, caution is required in interpreting the apparent superiority of 15 mg/day over 7.5 mg/day. The evidence does not derive from a large conventional head-to-head dose-ranging trial specifically powered to compare these doses. Rather, the dose-response inference emerges from differences between treatment sequences and trials. Thus, although 15 mg/day is the best-supported dose, the precise dose-response curve has not been definitively established.

L-Methylfolate Versus Folic Acid: Not Simply A Question Of Dose

The contrasting clinical results obtained with L-methylfolate and folic acid are particularly informative. Earlier controlled studies suggested that relatively low-dose folic acid could improve antidepressant response, with some of the strongest findings occurring among women receiving fluoxetine [6]. Small subsequent studies also reported beneficial effects with higher doses [16,17]. However, these encouraging findings were not confirmed in the substantially larger FolATED trial, in which folic acid 5 mg/day failed to produce a clinically meaningful augmentation effect [8].

The negative FolATED findings are important because they challenge the intuitive assumption that higher doses of folic acid should approximate the therapeutic effects of pharmacological L-methylfolate. The two compounds cannot be regarded as milligram-equivalent interventions. Folic acid requires several metabolic steps before contributing to the biologically active intracellular folate pool, whereas L-methylfolate bypasses the MTHFR-dependent conversion of 5,10-methylene-THF to 5-MTHF.

This provides a biologically plausible explanation for differences in clinical efficacy, particularly in individuals with reduced folate-cycle efficiency. However, biological plausibility should not be confused with direct comparative evidence. No adequately powered head-to-head randomized trial has established that L-methylfolate 15 mg/day is clinically superior to folic acid in MDD. The evidence instead consists of positive placebo-controlled L-methylfolate trials contrasted with mixed and, in the largest trial, negative evidence for folic acid. A direct comparison remains an important research priority.

The available findings also argue against the indiscriminate use of high-dose folic acid as a low-cost pharmacological substitute for L-methylfolate. Although folic acid remains essential for prevention and correction of nutritional deficiency, its role as a pharmacological antidepressant augmentation strategy appears considerably less certain.

Folinic Acid: Biologically Attractive But Clinically Underexplored

Folinic acid occupies an intermediate metabolic position between folic acid and L-methylfolate. Because it is already a reduced folate, it bypasses some metabolic steps required by folic acid, providing a theoretical rationale for psychiatric applications. Nevertheless, clinical evidence in depression remains remarkably limited.

The available open-label study of adjunctive folinic acid in SSRI-resistant depression demonstrated modest response and remission rates, but its small sample size and uncontrolled design preclude firm efficacy conclusions [18]. Folinic acid therefore represents an interesting but insufficiently investigated alternative. Direct comparative studies among folic acid, folinic acid, and L-methylfolate would be particularly informative for determining whether metabolic proximity to 5-MTHF translates into clinically meaningful differences.

MTHFR and Depression: Biological Relevance Does Not Equal Clinical Utility

Few aspects of folate-related psychiatry have attracted as much attention as MTHFR polymorphisms, particularly C677T and A1298C. The biological rationale is compelling. MTHFR catalyzes the conversion of 5,10-methylene-THF to 5-MTHF, and the C677T variant can substantially reduce enzyme activity, particularly in TT homozygotes. Meta-analytic evidence suggests a modest association between C677T and susceptibility to depression, although results vary according to ethnicity, nutritional environment, and study population [4].

More recent evidence suggests that MTHFR variants may also be associated with treatment-resistant depression [5]. However, the evidence base remains small and heterogeneous. Importantly, three distinct questions must not be conflated: whether MTHFR variants are associated with depression susceptibility; whether they are associated with treatment resistance; and whether they predict response to L-methylfolate. Evidence supporting the first question does not automatically establish the second, and neither establishes the third.

This distinction has direct clinical consequences. The fact that L-methylfolate bypasses the MTHFR-dependent step provides a strong biochemical rationale for its use in individuals with reduced MTHFR activity. However, routine MTHFR genotyping as a prerequisite for prescribing L-methylfolate is not supported by current evidence. Prospective trials have not demonstrated that selecting patients according to C677T or A1298C genotype produces superior outcomes compared with treatment decisions made without genotyping.

This is especially important given the growing availability of commercial pharmacogenetic testing. Evidence supporting multigene pharmacogenomic strategies cannot be extrapolated to isolated MTHFR testing. MTHFR is biologically relevant to folate metabolism, but its clinical predictive value must be demonstrated independently.

Beyond MTHFR: A Folate–Metabolic–Inflammatory Phenotype?

Perhaps the most intriguing finding emerging from the L-methylfolate literature is that treatment response may be related less to an isolated MTHFR genotype than to a broader biological phenotype. Post-hoc analyses of randomized trial

data suggested greater treatment effects among patients with obesity, elevated inflammatory biomarkers, markers of oxidative stress, and abnormalities related to one-carbon metabolism [13,14].

The observation involving obesity is particularly notable. Patients with BMI ≥30 kg/m² showed a substantially greater L-methylfolate-placebo difference in depressive symptom scores than the overall study population [14]. When obesity was combined with elevated inflammatory biomarkers—including hsCRP, TNF-α, IL-6, IL-8, or leptin—the treatment differences became even larger in some analyses.

These findings are biologically plausible. Obesity is frequently accompanied by low-grade systemic inflammation, oxidative stress, and metabolic abnormalities. Inflammatory processes can influence tetrahydrobiopterin availability, monoamine synthesis, oxidative pathways, and potentially one-carbon metabolism. A metabolically compromised patient might therefore require greater availability of active folate to maintain pathways involved in methylation and neurotransmitter synthesis.

This possibility leads to a concept that may ultimately prove more clinically useful than isolated genetic testing: a folate–metabolic–inflammatory phenotype. Such a phenotype could potentially incorporate folate and vitamin B12 status, homocysteine, BMI, inflammatory markers, metabolic characteristics, and selected genetic variants.

At present, however, this remains a hypothesis rather than a validated clinical algorithm. Most of the supporting biomarker findings are derived from post-hoc analyses of relatively small trials. Prospective biomarker-stratified RCTs are required before specific laboratory thresholds can be recommended for selecting patients for L-methylfolate treatment.

Folate Deficiency and Pharmacological Augmentation Are Different Clinical Questions

Another important distinction emerging from this review is the difference between correcting folate deficiency and using L-methylfolate pharmacologically to augment antidepressant treatment.

Patients with nutritional folate deficiency should receive appropriate replacement irrespective of whether they have depression. Low folate status may also identify patients at increased risk of depressive symptoms or poorer antidepressant response [1–3]. However, L-methylfolate 15 mg/day augmentation should not simply be conceptualized as treatment of folate deficiency.

The doses employed in antidepressant trials are considerably higher than physiological nutritional requirements. Moreover, therapeutic effects have been reported in populations not selected solely on the basis of folate deficiency. Thus, nutritional replacement and pharmacological augmentation represent overlapping but conceptually distinct therapeutic strategies.

This distinction may explain apparently contradictory observations in the literature. Serum folate concentrations may be normal while intracellular one-carbon metabolism, methylation capacity, inflammatory regulation, or central folate availability remains suboptimal. Conversely, low serum folate does not necessarily establish that pharmacological-dose L-methylfolate is required.

Homocysteine and Vitamin B12 Should Not Be Overlooked

Although MTHFR has received disproportionate attention, the folate cycle cannot be considered in isolation from vitamin B12 and homocysteine metabolism. 5-MTHF participates in the vitamin B12-dependent remethylation of homocysteine to methionine. Consequently, vitamin B12 deficiency can impair this pathway even when folate availability is adequate.

Elevated homocysteine may provide a functional indication of impaired one-carbon metabolism, but it is nonspecific and influenced by renal function, age, nutritional factors, vitamin B6 and B12 status, medications, and lifestyle factors. Nevertheless, from a biological perspective, simultaneous assessment of folate, vitamin B12, and homocysteine may provide more information about one-carbon metabolism than isolated MTHFR genotyping.

The clinical relevance of vitamin B12 assessment is also reinforced by the potential for high-dose folate exposure to obscure hematological manifestations of vitamin B12 deficiency while neurological consequences progress. Evaluation of B12 status is therefore particularly important when prolonged pharmacological folate therapy is considered.

From One-Carbon Metabolism to Monoamine Synthesis

The mechanistic rationale for L-methylfolate extends beyond homocysteine reduction. Through the methionine cycle, folate availability influences the generation of S-adenosylmethionine (SAM), the principal methyl donor for numerous reactions involving DNA, RNA, proteins, phospholipids, and neurotransmitter-related pathways [19,20]. Alterations in the SAM/SAH ratio may therefore reflect impaired methylation capacity.

Folate metabolism also intersects with tetrahydrobiopterin (BH4)-dependent pathways. BH4 is an essential cofactor for tryptophan hydroxylase and tyrosine hydroxylase, which participate in the synthesis of serotonin, dopamine, and norepinephrine. This provides a plausible mechanism through which L-methylfolate could augment antidepressant effects upstream of conventional monoamine reuptake mechanisms.

This hypothesis is attractive in patients with incomplete response to SSRIs. Rather than further inhibiting serotonin reuptake, L-methylfolate could theoretically enhance the metabolic availability required for monoamine synthesis. Nevertheless, the relative contribution of methylation, BH4 availability, monoamine synthesis, inflammatory modulation, and other mechanisms to the observed clinical effect remains uncertain.

Clinical Positioning of L-Methylfolate

Based on current evidence, L-methylfolate should be considered primarily as an augmentation strategy rather than antidepressant monotherapy. The best-supported clinical scenario is an adult with MDD who has achieved an inadequate or partial response to an adequately administered antidepressant, particularly an SSRI [9,10].

The evidence does not establish that every patient with treatment-resistant depression should receive L-methylfolate. The average effect observed in meta-analysis is modest, and

the randomized evidence base is much smaller than that supporting several established pharmacological augmentation strategies. Nevertheless, the favorable tolerability profile observed in controlled trials makes L-methylfolate a potentially useful option in selected patients [9].

The available data support 15 mg/day as the evidence-based pharmacological dose. Evidence for 7.5 mg/day is substantially weaker. Whether lower doses are effective in selected folate-deficient individuals remains a separate question and should not be extrapolated from augmentation trials.

Safety and Tolerability

L-methylfolate was generally well tolerated in controlled trials, with overall adverse-event rates similar to placebo [9]. This favorable safety profile represents a potentially important advantage over some conventional augmentation strategies.

Nevertheless, pharmacological doses should not be regarded as equivalent to ordinary nutritional supplementation. Long-term controlled safety data remain less extensive than short-term efficacy data. The available 12-month open-label extension is reassuring but cannot substitute for large controlled long-term studies [15]. Future research should therefore examine not only acute response but also sustained remission, relapse prevention, long-term tolerability, and treatment discontinuation.

Limitations of The Current Evidence

Several limitations should temper interpretation of the literature. First, despite considerable interest in folate and depression, the number of high-quality randomized trials specifically evaluating L-methylfolate remains relatively small. Meta-analytic estimates are therefore influenced heavily by a limited number of pivotal studies.

Second, considerable heterogeneity exists across studies regarding folate formulation, dose, baseline nutritional status, concomitant antidepressant treatment, duration, depression severity, and definitions of treatment resistance. Meta-analyses that combine different folate formulations may consequently produce estimates that are difficult to translate into formulation-specific clinical recommendations.

Third, several of the most interesting precision-treatment findings—including obesity, inflammatory biomarkers, oxidative stress markers, and genetic variants—derive from exploratory or post-hoc analyses [13,14]. These observations require prospective replication before they can be incorporated into treatment algorithms.

Fourth, direct comparative trials are notably absent. In particular, adequately powered studies directly comparing L-methylfolate 15 mg/day, folic acid, and folinic acid would help determine whether the apparent differences across the literature reflect genuine pharmacological superiority or differences in study populations and trial design.

Finally, evidence regarding MTHFR testing remains insufficient for routine treatment selection. Future studies should move beyond single polymorphisms toward integrated biological models incorporating genetic, nutritional, metabolic, and inflammatory information.

Future Directions

Future research should prioritize adequately powered, biomarker-stratified randomized trials. Rather than asking only whether L-methylfolate is superior to placebo, trials should determine which patients derive clinically meaningful benefit.

A particularly informative design would prospectively stratify patients according to MTHFR genotype, folate and vitamin B12 status, homocysteine, BMI, hsCRP, and selected inflammatory or metabolic biomarkers before randomization to L-methylfolate 15 mg/day or placebo. Such a design could test whether the proposed folate–metabolic–inflammatory phenotype predicts treatment response.

Head-to-head trials comparing L-methylfolate with folic acid and folinic acid are also needed. These studies would directly address whether bypassing specific metabolic steps translates into superior antidepressant efficacy.

Finally, studies should distinguish nutritional deficiency correction from pharmacological augmentation and include longer follow-up periods assessing sustained remission, relapse, functional recovery, quality of life, safety, and cost-effectiveness.

Conclusion

Folate metabolism represents a biologically plausible and clinically relevant pathway in major depressive disorder, but the therapeutic evidence is strongly dependent on folate formulation and dose. Folic acid, folinic acid, and L-methylfolate should not be considered therapeutically interchangeable.

Among currently available folate-based interventions, L-methylfolate 15 mg/day has the most consistent evidence as an adjunctive treatment for patients with MDD and inadequate response to antidepressant therapy. Evidence for 7.5 mg/day is less convincing, while high-dose folic acid has produced inconsistent findings and failed to demonstrate clinically meaningful augmentation in the large FolATED trial. Evidence for folinic acid remains preliminary.

MTHFR polymorphisms are biologically relevant and have been associated with depression and potentially treatment resistance, but current evidence does not support routine MTHFR genotyping as a prerequisite for L-methylfolate treatment or as a validated standalone predictor of response. Emerging data instead suggest that therapeutic response may reflect a broader interaction among one-carbon metabolism, nutritional status, obesity, inflammation, oxidative stress, and genetic background.

Thus, the future of folate-based treatment in depression may lie not in a simple “MTHFR-positive versus MTHFR-negative” approach, but in defining a multidimensional folate–metabolic–inflammatory phenotype capable of identifying patients most likely to benefit from targeted metabolic augmentation. Prospective biomarker-guided trials are required to determine whether this promising precision-psychiatry model can be translated into routine clinical practice.

Declarations

Ethics Approval and Consent to Participate

Not applicable. This article is a narrative review of previously published literature and did not involve the recruitment of human participants, collection of individual patient data, or animal experimentation.

Consent for Publication

Not applicable.

Availability of Data and Materials

No new datasets were generated or analyzed specifically for this narrative review. All information discussed in this article was obtained from previously published studies and publicly available sources cited in the manuscript.

Competing Interests

The authors declare that they have no competing interests.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' Contributions

All authors contributed to the conception and design of the review, literature evaluation, interpretation of the evidence, critical revision of the manuscript, and approval of the final version. All authors agree to be accountable for the accuracy and integrity of the work.

Acknowledgments

The authors have no additional acknowledgments to declare.

Use of Artificial Intelligence

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to translate portions of the manuscript originally written in Portuguese into English and to assist with English-language editing, grammar correction, and improvement of clarity and readability. The authors subsequently reviewed, revised, and approved the entire manuscript and take full responsibility for the accuracy, integrity, and scientific content of the final version.

Table 1: Hierarchy of clinical evidence for folate-based interventions in depressive disorders

Study	Design/population	Intervention	Comparator	Main findings	Interpretation/evidence level
Gao *et al*., 2024	Umbrella meta-analysis; 11 meta-analyses, 8 quantitative	Folate supplementation	Control/placebo across meta-analyses	Depressive symptoms: SMD −0.42 (95% CI −0.57 to −0.27); WMD −3.20 (−4.00 to −2.41). Folate insufficiency associated with depression: OR 1.35 (1.27–1.42)	Highest-level synthesis supports adjunctive benefit, but formulations were heterogeneous
Altaf *et al*., 2021	Systematic review/meta-analysis; 6 RCTs	Folic acid or L-methylfolate + SSRI/SNRI	Antidepressant alone/placebo augmentation	HAM-D MD −2.16 (−3.62 to −0.69); response RR 1.36 (1.16–1.59); remission RR 1.39 (1.00–1.92)	Supports folate augmentation overall; formulation pooling limits specific conclusions
Al Maruf *et al*., 2022	Systematic review/meta-analysis; 9 studies, N=6,707 qualitative synthesis	Adjunctive L-methylfolate	Antidepressant monotherapy/control	Response: RR 1.25 (1.08–1.46), 3 studies, n=483; continuous symptoms: SMD −0.38 (−0.59 to −0.17), 4 studies, n=507	Most relevant formulation-specific meta-analysis; modest benefit
Papakostas *et al*., 2012, Trial 1	Randomized, double-blind sequential-parallel RCT; n=148, SSRI-resistant MDD	L-methylfolate 7.5 mg/day initially, with escalation to 15 mg in one sequence	SSRI + placebo	No significant overall difference	Does not support consistent efficacy of the 7.5-mg strategy
Papakostas *et al*., 2012, Trial 2	Randomized, double-blind sequential-parallel RCT; n=75	L-methylfolate 15 mg/day + SSRI	SSRI + placebo	Significant benefit in response and symptom change; NNT ≈6; tolerability comparable to placebo	Strongest RCT evidence supporting 15 mg/day
Papakostas *et al*., 2014	Post-hoc biomarker/genotype analysis of RCT; n=75	L-methylfolate 15 mg/day	Placebo augmentation	Greater effects in selected metabolic, inflammatory and genetic subgroups	Hypothesis-generating; not prospective biomarker validation
Godfrey *et al*., 1990	Double-blind placebo-controlled trial; 41 psychiatric patients with low/borderline RBC folate	Methylfolate 15 mg/day + standard therapy	Placebo + standard therapy	Improved clinical and social recovery in depression and schizophrenia	Early positive evidence; small and selected population
Coppen & Bailey, 2000	Randomized double-blind placebo-controlled trial; n=127 MDD	Folic acid 0.5 mg/day + fluoxetine 20 mg	Fluoxetine + placebo	Overall improvement; strongest in women; female response 93.9% vs 61.1%	Positive signal but prominent sex interaction
Venkatasubramanian *et al*., 2013	Randomized double-blind dose comparison; n=42 women	Folic acid 5 mg/day + fluoxetine	Folic acid 1.5 mg/day + fluoxetine	Greater HDRS/BDI improvement with 5 mg; HDRS remission 36.8% vs 8.7%	Small study; no placebo arm
Resler *et al*., 2008	Small randomized controlled study; n=27	Folic acid 10 mg/day + fluoxetine	Fluoxetine + placebo	Greater symptom improvement and reduction in homocysteine	Positive but underpowered evidence
FolATED, 2014	Large pragmatic randomized double-blind placebo-controlled trial; ~475 randomized	Folic acid 5 mg/day + antidepressant	Placebo + antidepressant	No clinically meaningful augmentation effect	Strong evidence against assuming high-dose folic acid is effective augmentation
Alpert *et al*., 2002	Prospective open-label study; n=22 SSRI-refractory MDD	Folinic acid 15–30 mg/day + SSRI	None	ITT response 27%; remission 18%	Preliminary evidence only; uncontrolled

Abbreviations: BDI, Beck Depression Inventory; CI, confidence interval; HAM-D/HDRS, Hamilton Depression Rating Scale; ITT, intention-to-treat; MD, mean difference; MDD, major depressive disorder; NNT, number needed to treat; OR, odds ratio; RBC, red blood cell; RCT, randomized controlled trial; RR, risk ratio; SMD, standardized mean difference; SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; WMD, weighted mean difference.

Note: Folic acid, folinic acid, and L-methylfolate differ pharmacologically and should not be regarded as milligram-equivalent interventions.

Table 2: MTHFR variants, folate-related biomarkers, and potential predictors of treatment response in major depressive disorder

Biomarker/factor	Main evidence	Association with depression/treatment response	Potential clinical implication	Current strength/limitation
MTHFR C677T (rs1801133)	Genetic association studies/meta-analyses	T allele, particularly 677TT, associated with reduced MTHFR activity and modestly increased depression susceptibility in some populations	Biologically plausible marker of altered one-carbon metabolism	Association supported in some populations; predictive treatment utility uncertain
MTHFR A1298C (rs1801131)	Candidate-gene studies; TRD systematic review	Possible association of 1298CC with TRD; findings inconsistent	May contribute to broader metabolic/genetic profile	Limited and inconsistent
MTHFR variants and TRD	Moraes *et al*., 2026 systematic review; 7 studies	Potential association of 677TT and 1298CC with TRD	Supports further investigation in precision psychiatry	Heterogeneous and insufficient for routine clinical use
MTHFR-related genetic markers and L-methylfolate response	Papakostas *et al*., post-hoc RCT analysis	Selected genomic markers associated with greater L-methylfolate response	Potential enrichment strategy	Exploratory/post-hoc; requires prospective replication
Low serum/RBC folate	Clinical cohorts/observational studies	Associated with depression and poorer antidepressant response; low folate also associated with relapse	Folate assessment may be informative in nonresponse or suspected deficiency	Association does not prove benefit from pharmacological L-methylfolate
Elevated homocysteine	Epidemiological and biochemical studies	Associated with depression at population level; inconsistent predictor of antidepressant response	Functional marker of one-carbon metabolism	Nonspecific; influenced by B12/B6, renal function, age, diet
Low SAM/SAH ratio	Exploratory L-methylfolate RCT analysis	Associated with greater treatment benefit	Potential marker of impaired methylation capacity	Not prospectively validated
BMI ≥30 kg/m²	Post-hoc randomized trial analyses	HDRS-17 pooled treatment effect −4.66 (95% CI −7.22 to −1.98)	Possible metabolic phenotype associated with response	Strong exploratory signal; not validated selection criterion
Elevated hsCRP	Post-hoc RCT analysis	Associated with greater L-methylfolate effect	Potential inflammatory phenotype	Exploratory
TNF-α	Post-hoc RCT analysis	Elevated baseline level associated with larger treatment effect	Potential inflammatory predictor	Exploratory
IL-6	Post-hoc RCT analysis	Combination with BMI ≥30 associated with enhanced treatment effect	Component of composite inflammatory/metabolic phenotype	Not validated independently
IL-8	Post-hoc RCT analysis	Elevated IL-8 associated with greater treatment effect	Potential inflammatory predictor	Exploratory
Leptin	Post-hoc RCT analysis	Elevated leptin, particularly with obesity, associated with greater treatment effect	Links adipose/metabolic inflammation to possible responsiveness	Requires prospective confirmation
4-hydroxy-2-nonenal (4-HNE)	Papakostas biomarker analysis	Elevated oxidative-stress marker associated with greater response	Possible oxidative-stress phenotype	Limited exploratory evidence
Composite metabolic/inflammatory profile	Post-hoc analyses	Obesity combined with inflammatory abnormalities produced larger treatment effects than several isolated markers	Supports concept of a folate–metabolic–inflammatory phenotype	Promising hypothesis; no validated clinical algorithm

Abbreviations: 4-HNE, 4-hydroxy-2-nonenal; BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; MDD, major depressive disorder; MTHFR, methylenetetrahydrofolate reductase; RBC, red blood cell; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; TNF-α, tumor necrosis factor-alpha; TRD, treatment-resistant depression.

Note: Associations between MTHFR polymorphisms and depression or treatment resistance should not be interpreted as evidence that MTHFR genotyping alone can predict clinical response to L-methylfolate. Biomarker and genotype findings related to L-methylfolate response remain predominantly exploratory or post-hoc.

Table 3: Folate metabolism and proposed mechanisms linking folate-based interventions to major depressive disorder

Pathway/component	Biochemical role	Relevance to depression	Effect of folate formulation/clinical implication
Folic acid	Synthetic oxidized form of vitamin B9 requiring enzymatic reduction and subsequent metabolic conversion	Provides substrate for one-carbon metabolism	Indirect precursor of active folates; higher doses do not necessarily reproduce L-methylfolate effects
Folinic acid (5-formyl-THF; leucovorin)	Reduced folate entering the folate pool while bypassing some steps required by folic acid	Supports one-carbon metabolism	Metabolically more proximal than folic acid, but antidepressant evidence is limited
5,10-methylene-THF	Substrate for MTHFR and key intracellular folate intermediate	Links folate metabolism to methylation pathways	Availability does not guarantee adequate 5-MTHF production when MTHFR activity is reduced
MTHFR	Converts 5,10-methylene-THF to 5-MTHF	Rate-regulating step in methyl donor generation	Functional variants may reduce activity; genotype-guided treatment remains unvalidated
MTHFR C677T	T variant produces thermolabile enzyme with reduced activity	Associated with altered folate metabolism and modest depression risk in some populations	L-methylfolate bypasses this conversion step, but genotype is not required for treatment
MTHFR A1298C	Functional MTHFR variant with generally less pronounced biochemical effect than C677T	Possible relationship with depression/TRD	Potential component of multigenic phenotype
L-methylfolate (5-MTHF)	Biologically active methylated folate participating directly in homocysteine remethylation	Provides active folate substrate relevant to CNS one-carbon metabolism	Bypasses MTHFR-dependent conversion; strongest formulation-specific augmentation evidence
5-MTHF + vitamin B12	Supports B12-dependent methionine synthase reaction	Links folate availability to methionine cycle	Adequate vitamin B12 is essential
Homocysteine → methionine	Remethylation mediated by methionine synthase using 5-MTHF and B12	Folate/B12 dysfunction can increase homocysteine	Homocysteine is a functional but nonspecific marker
Methionine → SAM	Methionine converted to S-adenosylmethionine	SAM is a major CNS methyl donor	Provides mechanistic link between folate and methylation
SAM → SAH	SAM donates methyl groups and becomes SAH	SAM/SAH ratio reflects methylation potential	Low ratio has been explored as predictor of L-methylfolate response
DNA/RNA/protein/phospholipid methylation	SAM-dependent regulation of gene expression, membrane and protein biology	Potential broader mechanism beyond monoamines	Mechanistically plausible but incompletely established as mediator of clinical response
BH2 → BH4 regeneration	Supports tetrahydrobiopterin availability	BH4 is required for monoamine synthesis	Proposed pathway connecting active folate with neurotransmitter synthesis
Tryptophan hydroxylase + BH4	Rate-limiting pathway in serotonin biosynthesis	Influences serotonin production	Potential mechanism for facilitating serotonergic neurotransmission
Tyrosine hydroxylase + BH4	Rate-limiting pathway in catecholamine synthesis	Required for dopamine and norepinephrine production	Provides mechanism extending beyond serotonin
Serotonin, dopamine, norepinephrine	Major neurotransmitter systems involved in mood, motivation, reward and cognition	Implicated in depressive symptomatology	L-methylfolate may influence monoamine synthesis upstream rather than inhibit reuptake
Inflammation/oxidative stress	Can alter BH4 availability, oxidative pathways and one-carbon metabolism	Characterizes biologically distinct MDD phenotypes	Exploratory data suggest greater L-methylfolate response in inflammatory/metabolic phenotypes
Final proposed therapeutic effect	Improved methyl-donor availability, methylation, BH4-dependent monoamine synthesis and possible metabolic-inflammatory modulation	Potential enhancement of antidepressant response	Clinical evidence strongest for L-methylfolate 15 mg/day as adjunctive therapy

Abbreviations: 5-HT, serotonin; 5-MTHF, 5-methyltetrahydrofolate; BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; CNS, central nervous system; MDD, major depressive disorder; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate; TRD, treatment-resistant depression.

Note: These biochemical pathways provide biological plausibility for folate-based interventions but do not establish that individual metabolic or genetic abnormalities predict antidepressant response. In particular, bypassing the MTHFR-dependent conversion step with L-methylfolate is pharmacologically plausible, whereas routine MTHFR genotyping to select patients for L-methylfolate has not been prospectively validated.

References

1. Fava M, Borus JS, Alpert JE, Nierenberg AA, Rosenbaum JF, Bottiglieri T. Folate, vitamin B12, and homocysteine in major depressive disorder. Am J Psychiatry. 1997;154(3):426-428. doi:10.1176/ajp.154.3.426.
2. Papakostas GI, Petersen T, Mischoulon D, Ryan JL, Nierenberg AA, Bottiglieri T, *et al*. Serum folate, vitamin B12, and homocysteine in major depressive disorder, part 1: predictors of clinical response in fluoxetine-resistant depression. J Clin Psychiatry. 2004;65(8):1090-1095. doi:10.4088/jcp.v65n0810.
3. Papakostas GI, Petersen T, Mischoulon D, Green CH, Nierenberg AA, Bottiglieri T, *et al*. Serum folate, vitamin B12, and homocysteine in major depressive disorder, part 2: predictors of relapse during the continuation phase of pharmacotherapy. J Clin Psychiatry. 2004;65(8):1096-1098. doi:10.4088/jcp.v65n0811.
4. Rai V. Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene and susceptibility to depression in Asian population: a

systematic meta-analysis. Cell Mol Biol (Noisy-le-grand). 2014;60(3):29-36.
5. Brum Moraes J, Oliveira CEC, Alfieri DF, Vila LM, Euzébio NJ, Reiche EMV, *et al*. Systematic review of methylenetetrahydrofolate reductase (MTHFR) 677C>T and 1298A>C variants and treatment-resistant depression: insights for precision psychiatry. Complex Psychiatry. 2026;12(1-4):9-26. doi:10.1159/000548757.
6. Coppen A, Bailey J. Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial. J Affect Disord. 2000;60(2):121-130. doi:10.1016/S0165-0327(00)00153-1.
7. Godfrey PS, Toone BK, Carney MW, Flynn TG, Bottiglieri T, Laundy M, *et al*. Enhancement of recovery from psychiatric illness by methylfolate. Lancet. 1990;336(8712):392-395. doi:10.1016/0140-6736(90)91942-4.
8. Bedson E, Bell D, Carr D, Carter B, Hughes D, Jorgensen A, *et al*. Folate Augmentation of Treatment--Evaluation for Depression (FolATED): randomised trial and economic evaluation. Health Technol Assess. 2014;18(48):vii-viii, 1-159. doi:10.3310/hta18480.
9. Papakostas GI, Shelton RC, Zajecka JM, Etemad B, Rickels K, Clain A, *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(12):1267-1274. doi:10.1176/appi.ajp.2012.11071114.
10. Maruf AA, Poweleit EA, Brown LC, Strawn JR, Bousman CA. Systematic review and meta-analysis of L-methylfolate augmentation in depressive disorders. Pharmacopsychiatry. 2022;55(3):139-147. doi:10.1055/a-1681-2047.
11. Altaf R, Gonzalez I, Rubino K, Nemec EC 2nd. Folate as adjunct therapy to SSRI/SNRI for major depressive disorder: systematic review and meta-analysis. Complement Ther Med. 2021;61:102770. doi:10.1016/j.ctim.2021.102770.
12. Gao S, Khalid A, Amini-Salehi E, Radkhah N, Jamilian P, Badpeyma M, *et al*. Folate supplementation as a beneficial add-on treatment in relieving depressive symptoms: a meta-analysis of meta-analyses. Food Sci Nutr. 2024;12(6):3806-3818. doi:10.1002/fsn3.4073.
13. Papakostas GI, Shelton RC, Zajecka JM, Bottiglieri T, Roffman J, Cassiello C, *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(8):855-863. doi:10.4088/JCP.13m08947.
14. Shelton RC, Pencina MJ, Barrentine LW, Ruiz JA, Fava M, Zajecka JM, *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(12):1635-1641. doi:10.4088/JCP.14m09587.
15. Zajecka JM, Fava M, Shelton RC, Barrentine LW, Young P, Papakostas GI. 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(5):654-660. doi:10.4088/JCP.15m10181.
16. Venkatasubramanian R, Kumar CN, Pandey RS. A randomized double-blind comparison of fluoxetine augmentation by high and low dosage folic acid in patients with depressive episodes. J Affect Disord. 2013;150(2):644-648. doi:10.1016/j.jad.2013.02.029.
17. Resler G, Lavie R, Campos J, Mata S, Urbina M, García A, *et al*. Effect of folic acid combined with fluoxetine in patients with major depression on plasma homocysteine and vitamin B12, and serotonin levels in lymphocytes. Neuroimmunomodulation. 2008;15(3):145-152. doi:10.1159/000151527.
18. Alpert JE, Mischoulon D, Rubenstein GE, Bottonari K, Nierenberg AA, Fava M. Folinic acid (Leucovorin) as an adjunctive treatment for SSRI-refractory depression. Ann Clin Psychiatry. 2002;14(1):33-38. doi:10.1023/A:1015271927517.
19. Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MW, Reynolds EH. Homocysteine, folate, methylation, and monoamine metabolism in depression. J Neurol Neurosurg Psychiatry. 2000;69(2):228-232. doi:10.1136/jnnp.69.2.228.
20. Stahl SM. L-methylfolate: a vitamin for your monoamines. J Clin Psychiatry. 2008;69(9):1352-1353. doi:10.4088/jcp.v69n0901.

How to Cite This

Carvalho JF. Folate-based interventions in major depressive disorder: from folic acid and L-methylfolate to MTHFR and precision psychiatry. Int J Biol Biomed Res. 2026;2(5):39-50. doi:10.54660/IJBBR.2026.2.5.39-50.

Creative Commons (CC) License