The Role and Postulated Biochemical Mechanism of L-Methylfolate Augmentation in Major Depression: A Case-Report

Pat Rabjohn, MD, PhD

ABSTRACT
Investigators have been examining the link between folate deficiency and major depression for the past five decades. Folate requires enzymatic conversion to L-methylfolate, which is the biologically active form of folic acid and can be prescribed as a prescription medical food. The enzyme MTHFR, which catalyzes the rate-determining step in L-methylfolate synthesis, is subject to a common polymorphism rendering the enzyme less effective. This can lead to lower levels of L-methylfolate being available to activate tetrahydrobiopterin for serotonin production in the raphe nucleus. Recent data strongly suggest that L-methylfolate is an effective augmentation strategy for major depression at both the initial onset of symptoms and in patients with treatment-resistant depression. Because there is limited evidence-based information available on successful augmentation strategies, physicians often make augmentation decisions on the presence of a particular symptom, avoidance of side effects, or cost. This article summarizes data suggesting that patients with a combination of genetic mutations at MTHFR, early-life adversity, and/or obesity are potentially excellent candidates for L-methylfolate augmentation.

The link between folate deficiency and major depression has been investigated for the past 50 years. Several older studies have reported that low folate levels may be a risk factor for a depressive episode, and recent studies have implied that depressed patients who are folate deficient may respond more poorly to treatment with standard antidepressants. The U.S. Food and Drug Administration (FDA) mandated folate supplementation for many foods in 1996, making clinical folate deficiency a seemingly rare occurrence; however, rates of major depression have remained consistent in U.S. men and women since then.

A variety of trials with differing designs were conducted in the early 1990s with the hypothesis that L-methylfolate (the biologically active form of folate),
was effective for patients with major depression. Godfrey and colleagues reported the first augmentation trial, which investigated 41 patients hospitalized with major depression who exhibited low folate levels in red blood cells. Patients received either a tricyclic antidepressant or monoamine oxidase inhibitor and were augmented with 7.5 mg of L-methylfolate or placebo over a 6-month period. Twenty-four patients receiving augmentation with L-methylfolate had better clinical outcomes than those receiving placebo augmentation at both 3 months and 6 months, and none of the patients receiving augmentation with L-methylfolate experienced a relapse. Meanwhile, a monotherapy, double-blind controlled trial in elderly patients with a diagnosis of both dementia and major depression compared Hamilton Depression Rating Scale (HAMD) scores in depressed patients randomized to either trazodone (n = 47) at 100 mg/d or L-methylfolate (n = 49) at 25 mg/d. Both groups experienced a significant decrease in HAMD scores at weeks 4 and 8 (45% of the patients receiving L-methylfolate saw a decrease), but a much greater proportion of the L-methylfolate group showed improvement in immediate recall compared with those taking trazodone. Other monotherapy trials, limited by sample size and open-label designs, have also demonstrated efficacy. Therefore, the question remains: Is folate (or any of its many metabolites) a safe and effective treatment option, either as monotherapy or as an augmentation agent, for patients with major depression?

CASE PRESENTATION

The patient, Ms. X, is a 36-year-old, married Hispanic woman with a 15-year history of treatment-resistant depression and generalized anxiety. Her medical history includes type 2 diabetes mellitus under partial control and hypertension. She is 5’3” tall with a body weight of 192 lbs, resulting in a body mass index (BMI) of 34.0 kg/m². The following history was obtained at her initial psychiatric evaluation.

Ms. X’s internist placed her on escitalopram, clonazepam, and trazodone 4 months prior to her initial visit at my office, and after 1 month of limited improvement, the internist added aripiprazole at a dosage of 5 mg/d. The internist referred her for a psychiatric evaluation after she developed possible akinesia on aripiprazole and for “non-compliance,” because she had stopped the medication without consulting him. Records from the primary care clinic indicated that all of her recent blood work, including chemistry panels, complete blood count, liver function tests, and thyroid, was within normal limits.

Ms. X’s primary stressors include a husband who travels often for work, two children (ages 9 and 15 years, the older of whom is being treated for depression), and financial stress due to being a one-income family. Her current antidepressive symptoms include poor sleep with racing thoughts at night, low interests and isolation, poor appetite, and increasing sadness and irritability. She finds herself crying frequently and feels it is getting “harder and harder” to hide her depression from friends and family. Her anxiety seems to be evolving toward panic disorder, with recent physical symptoms of anxiety, including minor shortness of breath, chest pressure, and agoraphobia, occurring in both grocery stores and at church. She is not suicidal and there is no history suggestive of mania or psychosis. She uses no illicit drugs and rarely uses alcohol. Her current Quick Inventory of Depressive Symptomology–Self Rated (QIDS-SR) score is 23. Her Hamilton Anxiety Rating Scale (HAMD-A) is 25.

Her current regimen includes escitalopram at 20 mg in the morning, trazodone at 50 mg at bedtime, and clonazepam at 0.5 mg daily as needed for anxiety (which she takes four to five times per week). Ms. X states the clonazepam is very helpful for anxiety but she is afraid of becoming addicted and it makes her somewhat tired. She recently took herself off aripiprazole (5 mg in the morning) after gaining 5 pounds over a 3-month period and feeling restless with increased anxiety/irritability. Past treatments include fluoxetine at 20 mg for 3 months, venlafaxine extended-release for 5 years at 150 mg, sertraline at 150 mg plus bupropion sustained-release 150 mg twice daily for 1 year, and a recent failed trial on duloxetine with minimal benefit and resultant weight gain.

She reports a similar pattern occurring with her medications in that “they work for the first 2 to 3 months then they seem to fizzle out and not work anymore.” She does not feel she has ever reached remission or “felt how I should feel.” Ms. X has tried therapy several times but is unable to maintain the time commitment or financial commitment for sustained psychotherapy. There is no history of inpatient hospitalization and no history of violence or violent thoughts.

The patient reported a very difficult childhood for multiple reasons. Ms. X’s parents separated several times before she was a teenager and they finalized their divorce when she was 15 years old. Her father was physically abusive to her, and she described a pattern of alcohol use by him that is suggestive of alcohol dependence. In addition, her family was very poor and had to rely on neighbors and extended family for financial and emotional support.
Ms. X’s family history is significant for two sisters treated consistently for depression, one of whom was hospitalized after an overdose attempt. Both her mother and maternal grandmother show evidence of depressed mood, but she is unsure if they received treatment. Her father was frequently irritable and “moody” with possible alcohol dependence, but to her knowledge he received no psychiatric treatment. As mentioned previously, her 15-year-old daughter is being treated for depression, with a partial response to vilazodone and hydroxyzine.

**DIAGNOSIS:**
**Major Depressive Disorder; Recurrent, Moderate, and Generalized Anxiety Disorder**

After reviewing Ms. X’s history, the diagnosis of Major Depressive Disorder; Recurrent, Moderate, and Generalized Anxiety Disorder was established. Based on her presenting symptoms and clinical and family history, genetic testing was performed that revealed she had a heterozygous mutation at 5,10-methylenetetrahydrofolate reductase (MTHFR), with a corresponding phenotype of (C/T) at position 667; she was also heterozygous at position 1298, with a phenotype of A/C. Based on these results, she was placed on L-methylfolate at 15 mg in the morning; her escitalopram was increased to 30 mg in the morning and her trazodone was increased to 150 mg at bedtime. No change was made to her clonazepam dosing, and she was told to continue taking it as needed for the onset of physical anxiety symptoms.

At her first follow-up appointment 4 weeks later, Ms. X reported improved sleep, less irritability, more interactions with neighbors and friends, only one crying episode, and no further physical symptoms of anxiety. Her QIDS-SR score was 11, her HAM-A score was 16, and she reported no side effects other than mild nausea the first few days her escitalopram was at 30 mg. Her medications were continued with no changes, and no referral was made for therapy. Ms. X was seen 3 months later and reported feeling “great” with no current symptoms of depression and only mild anticipatory anxiety. She was happy to report that she had not taken any clonazepam in 8 weeks. Her QIDS-SR score at that visit was 3 (indicating remission), her HAM-A score was 11, and she elected to continue her medications for another 6 months. In addition, she reported better compliance with her diabetes and hypertension treatments.

**THE BIOCHEMISTRY OF FOLATE AND ITS METABOLIC CONVERSION TO L-METHYLFOLATE**

As an essential water-soluble B vitamin (B9), folate’s primary metabolic role is to promote the transfer of methyl and formyl groups. These one-carbon transfers are necessary for proper biosynthesis, repair, and methylation of DNA. Other important roles for folate include the catabolism and breakdown of proteins, along with effective red blood cell maturation and cell reproduction. In addition, folate’s role in methylation facilitates several important central nervous system (CNS) reactions that are described in the following text.

Because humans cannot synthesize folate, it must be supplied in dietary form. The synthetic form of folate is referred to as folic acid, and this type is found in over-the-counter vitamins and used to fortify the food supply. The dietary form is referred to as dihydrofolate and exists in various foods such as asparagus, spinach, eggs, and certain vegetables.
whole grains. Neither of these forms have biological activity. Both folic acid and dihydrofolate are inactive substrates that must undergo a series of enzymatic reactions to become the usable, biologically active form of folate, termed L-methylfolate (Figure 1). L-methylfolate is the only metabolite of folate that can cross the blood-brain barrier, and it is this form that can directly impact several important CNS reactions, most notably the synthesis of three important neurotransmitters: serotonin, norepinephrine, and dopamine.

The rate-limiting step in L-methylfolate synthesis is catalyzed by the enzyme MTHFR. The gene encoding MTHFR is highly susceptible to a single nucleotide polymorphism (SNP) at position 667, leading to thymidylate (T) being substituted for the standard nucleotide cytosine (C). This missense mutation results in an altered codon that replaces the amino acid alanine with valine. This single amino acid alteration generates a more thermolabile MTHFR enzyme with reduced enzymatic activity. A person with two normal genes/alleles would have “C” at position 667 and be referred to as C/C. A single gene mutation typically substitutes “T” at that position, and a heterozygous person would be referred to as C/T, whereas a person with homozygous mutations (altered copies on both genes) would be labeled T/T. A C/C carrier has 100% enzyme activity, implying full conversion of folate/dihydrofolate to L-methylfolate. A C/T heterozygous person has approximately 70% enzyme activity, implying that only 70% of inactive folate is converted to L-methylfolate, whereas a T/T homozygous person only has approximately 25% to 35% enzyme activity (Table 1). Population studies have determined that individuals with the homozygous C667T polymorphism have lower levels of red blood cell folate, plasma folate, and vitamin B12.10,11

Another MTHFR site susceptible to polymorphism is located at gene position 1298. This single nucleotide substitution of cytosine for adenosine at 1298 leads to the amino acid change of glutamate to alanine. Although it is mostly agreed within the medical literature that the polymorphism at C667T has a major impact on enzyme function (leading to increased homocysteine concentration and altered levels of folate metabolites), much less can be agreed upon regarding the polymorphism at 1298 and its role in folate metabolism. In vitro studies have reported that the 1298 homozygous mutation (1298 C/C) generates an enzyme with 60% effectiveness of the normal enzyme.12

### Table 1.

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<th>MTHFR Allele Genotype</th>
<th>Enzyme Activity Population Prevalence</th>
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<tr>
<td>Normal</td>
<td>C/C</td>
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<td>Heterozygous</td>
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<td>Homozygous</td>
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### THE ROLE OF L-METHYLFOLATE IN THE CNS

L-methylfolate has been shown to serve two important roles within the CNS. First, L-methylfolate is a required substrate for the activation of tetrahydrobiopterin (BH₄), which, once activated, serves as an essential co-factor for both tryptophan hydroxylase (the rate-limiting enzyme for serotonin synthesis) and tyrosine hydroxylase (the rate-limiting enzyme for norepinephrine and dopamine synthesis).13 Secondly, L-methylfolate is required by methionine synthase to recycle homocysteine to methionine. Methionine is then converted to S-adenyl-methionine (SAM), and it is SAM that serves a critical role as a methyl donor for the formation of all three previously mentioned monoamines (serotonin, norepinephrine, and dopamine).11 Theoretically, low CNS levels of L-methylfolate would lead to both a decreased activation of BH₄ (causing decreased activation of the rate-limiting enzyme for serotonin synthesis) while also reducing the capacity of methionine synthase to remethylate homocysteine (leading to decreased SAM production). Therefore, neither SAM (the primary methyl donor for serotonin production) nor tryptophan hydroxylase (the rate-limiting enzyme for serotonin production in the CNS) would be working at optimal capacity, which means the amount of serotonin being produced is restricted.

### L-METHYLFOLATE DEFICIENCY: CAUSATION AND REPLACEMENT

Multiple reasons exist for why certain patients or groups may be deficient in L-methylfolate. In addition to the possibility of a gene polymorphism in MTHFR as described previously, low L-methylfolate levels can result from smoking, excessive alcohol intake, gastrointestinal/absorptive disorders, and medications.1 For example, superoxide generated from the metabolism of ethanol to acetaldehyde can lead to cleavage of folic acid before it can be absorbed and converted to L-methylfolate.14 Two anticonvulsants used to treat bipolar disorder, valproic acid and lamotrigine, have been shown to decrease folate levels,1 as has the use of oral contraceptives.15

Although vitamins such as folic acid can be bought at health-food stores and...
other retailers, L-methylfolate is classified as a “medical food” and requires a prescription. Medical foods were established by the Orphan Drug Act Amendment of 1988 and must be formulated for oral or enteral administration, be prescribed by a physician, and be intended for specific dietary management of a disease. Current thinking is that prescribing L-methylfolate to patients with major depression would be beneficial in three situations: 1) as a replacement or supplement for individuals with major depression affected by an MTHFR enzyme deficiency; 2) for individuals prescribed medications that lower levels of L-methylfolate; or 3) for patients impacted with chronic diseases, such as alcoholism, that can impair L-methylfolate synthesis.

ASSOCIATION OF MTHFR POLYMORPHISMS WITH PSYCHIATRIC DISORDERS

A fair estimate for an MTHFR polymorphism in the general population would be about 50%. Although some studies reported no link between MTHFR 667 polymorphisms and depression or cognitive decline, a recent meta-analysis of 10 studies indicated higher rates of depression in individuals with the C667T homozygous mutation for MTHFR. In addition, a recent study found that the presence of the 1298 CC genotype correlated with a higher risk of depression in the Slovak population. One report showed an association between the C667T polymorphism and inadequate treatment response to citalopram in patients with major depression due to a traumatic brain injury. A recent case-control study found no association between the MTHFR C667T mutation and Alzheimer’s disease, but did find a strong correlation between development of Alzheimer’s disease and the MTHFR A1298C mutation. A meta-analysis of eight case-control studies showed that the C667T polymorphism, but not the A1298C mutation correlated with an increased risk for autism in children from countries without folate fortification of the food supply.

TWO RECENT STUDIES EXAMINING L-METHYLFOLATE AS AN AUGMENTING AGENT FOR MAJOR DEPRESSION

Two recent studies strengthen the argument that L-methylfolate should be considered a first-line augmentation strategy for patients with major depression. Ginsberg et al. used a retrospective chart review to evaluate the efficacy of L-methylfolate-plus-SSRI/SNRI group versus 3.6% of the SSRI/SNRI-monotherapy group.

Major improvement was observed in 8.9% of the L-methylfolate-plus-SSRI/SNRI group versus 3.6% of the SSRI/SNRI-monotherapy group.
they demonstrated a greater response rate than the placebo group. This led to the design of the second trial with 75 patients, in which only the higher dosage of 15 mg/day of L-methylfolate was used for augmentation. In this second trial, L-methylfolate at 15 mg/day plus an SSRI was superior in both outcome measures to SSRI monotherapy. Response rates for the L-methylfolate-plus-SSRI group (32.3%) were higher than the SSRI-plus-placebo group (14.6%), and the corresponding differences in the degree of improvement on the HAM-D were higher (-5.58 versus -3.04). In addition, L-methylfolate augmentation in both the Ginsberg and the Papakastos studies did not lead to any significant difference with regard to gastrointestinal side effects, sedation, weight gain, or sexual dysfunction.

RELATIONSHIP BETWEEN EARLY-LIFE EVENTS, BMI, AND DEPRESSION: APPLICATION TO THIS CASE

It has long been assumed that abuse and mistreatment in childhood would predispose individuals to an increased risk for future major depressive episodes. A recent meta-analysis inspecting the relationship between the course of depressive illness and treatment outcome in studies concerning childhood maltreatment came to two significant conclusions. First, individuals with childhood maltreatment were much more likely to develop recurrent and persistent depressive episodes, and secondly, those with childhood maltreatment were less likely to achieve remission during treatment. In addition, a 32-year prospective, longitudinal study followed a Dutch birth cohort of 1037 people at 2- to 3-year intervals with final data collection on 972 people at age 32 years. The purpose of this study was to examine the impact of early-life adversity/childhood maltreatment on the development of major depression, increased inflammation as measured by elevated C-reactive protein, and metabolic risk factors such as obesity, high cholesterol, and elevated A1c levels. They concluded that children who suffered from early-life psychosocial adversity were more likely to be depressed, have obesity and increased metabolic risk factors, and have higher rates of inflammation.

Severity of depression also correlated with the number and severity of childhood traumatic events.

Multiple reports have concluded that obese individuals are more susceptible to depression than non-obese individuals, and that the prevalence of depression increases with BMI. Reports have also demonstrated a strong relationship between elevated BMI and poor antidepressant response.

If we accept that early-life adversity is a central risk factor for the development of depression and obesity, and obese individuals are less likely to respond to traditional antidepressant treatment, what hypothesis exists to explain this cause-and-effect relationship?

Obviously, no one specific candidate gene has been identified or found to be predictive for depression, but data are emerging about complex gene-environment interactions that may provide a molecular link between early-life adversity, obesity, and depression. Lok et al examined the gene-environment relationship between early-life adversity and MTHFR genotype for major depressive disorder recurrence and concluded that MTHFR polymorphism combined with traumatic childhood events was predictive of depression. Patients with a history of recurrent depression currently in remission were followed for 5.5 years for depression recurrence. The researchers concluded that presence of the MTHFR T allele coupled with early-life adversity was most predictive of depression recurrence. Severity of depression was highest in the T/T population and lowest in the C/C population. Severity of depression also correlated with the number and severity of childhood traumatic events. How the MTHFR polymorphism disrupts one carbon metabolism or promotes oxidative stress along with how early-life adversity may trigger or “unlock” certain genes that alter methylation patterns and expression of inflammatory markers will be a primary focus of future research.

Ms. X had a history of early-life adversity and childhood trauma. She became obese (with an elevated BMI) and developed type 2 diabetes mellitus. She suffered from recurrent depression and responded poorly to traditional treatment. Genetic testing revealed her to possess the MTHFR C/T genotype, which produces an enzyme with limited ability to produce biologically active L-methylfolate. Augmentation of her antidepressant regimen with L-methylfolate improved her symptoms and she reached remission. This highlights the importance of understanding complex gene-environment interactions, because they may lead to more personalized psychiatry (ie, the ability to choose treatment strategies that account for individual variability, which can achieve a greater response).

CONCLUSIONS

Prior studies and two recent studies strongly suggest that L-methylfolate is an effective augmentation treatment to standard antidepressant therapy for major depression. It has been well established in multiple studies, including the Sequenced Treatment Alternatives for Depression study (STAR*D), that monotherapy with an SSRI or SNRI is not likely to achieve remission in pa-
tients with major depression. Three second-generation antipsychotics have FDA approval as augmentation agents for major depression: olanzapine, quetiapine extended-release (XR), and aripiprazole. The number needed to treat (NNT) can be calculated as a measure of the number of patients needed to treat to obtain one desirable event. Based on the three aripiprazole registration trials for depression augmentation, the NNT to generate a response to treatment was calculated as 5, 7, and 11, with a pooled NNT of 7.34 Citrome34 also calculated the NNT for olanzapine plus fluoxetine from five registration trials and computed a pooled NNT of 8. When a similar approach was performed for quetiapine XR as an augmenting agent, the pooled NNT for the 150-mg dose was 14 and for the 300-mg dose was 9. Although L-methylfolate has not been directly compared in a trial to augmentation with atypical antipsychotics, the L-methylfo- late NNT extrapolated from the Papakostas trial26 is 6, which is comparable to the aforementioned augmentation strategies. Unfortunately, there is a paucity of evidence-based augmentation data for the treatment of major depression. Excellent reviews regarding depression augmentation (although limited by the amount of data) have been done by Nelson and colleagues.35,36

If major depression is hypothesized as a low serotonin state, then we can imagine a serotonergic neuron producing a less-than-desired amount of neurotransmitter. When an SSRI/SNRI is prescribed, less serotonin returns to the neuron via reuptake inhibition and it begins to accumulate in the post-synaptic cleft, where it can serve its role in neurotransmission. Therefore, consistent synthesis of serotonin would be required to provide adequate amounts of neurotransmitter for release into the synaptic cleft.37 All too often, patients such as Ms. X will make the following comment: “My antidepressant worked great the first 2 to 3 months, then it just fizzled out and stopped working.” Although it is possible that additional stressors arose to worsen their clinical state or that certain symptoms returned for unknown reasons, it is unlikely that the prescribed antidepressant is no longer binding effectively to the serotonin reuptake site, nor is it likely that tolerance developed. What may be occurring is that the prescribed antidepressant worked very effectively and that over several weeks of reuptake inhibition, it effectively “drained” the neuron of serotonin. Something similar was shown in prior research involving partial responders to fluoxetine who experienced depressive symptom relapse after tryptophan-depletion challenges.38 Augmentation of SSRI/SNRI with L-methylfolate may, because of its biological actions, help maintain serotonin levels in some patients and also contribute to a more sustained treatment effect. This may increase the chance of achieving remission, and in those who have reached remission, it may reduce the risk of symptom relapse.

As psychiatry advances at the molecular level, we will gain more understanding of how changes to gene expression relate to environmental stressors and how this influences disease progression. People with certain genotypes may be more vulnerable to particular stressors than people with other genotypes. The possibility exists that treatment could be tailored even further to specific individuals. Currently, certain traumatic events or stressors may lead to a specific psychotherapy intervention, such as cognitive-behavioral therapy, eye movement desensitization and reprocessing, or even exercise. In the future, biological interventions aimed at oxidative stress, one carbon metabolism, or blockage of certain DNA-protein interactions could be the norm. Understanding the role of MTHFR and its relationship to depression may be one of the first steps in this process.

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