Published in final edited form as:

J Clin Psychopharmacol. 2019 ; 39(1): 28–38. doi:10.1097/JCP.0000000000000990.

Does L-Methylfolate Supplement Methylphenidate Pharmacotherapy in Attention-Deficit/Hyperactivity Disorder?:

Evidence of Lack of Benefit From a Double-Blind, Placebo-Controlled, Randomized Clinical Trial

Craig Surman, MD, Atila Ceranoglu, MD, Carrie Vaudreuil, MD, Brittany Albright, MD, Mai Uchida, MD, Amy Yule, MD, Andrea Spencer, MD, Heidi Boland, BA, Rebecca Grossman, BA, Lauren Rhodewalt, BA, Maura Fitzgerald, MPH, Joseph Biederman, MD

Clinical and Research Programs in Pediatric Psychopharmacology and Adult ADHD, Massachusetts General Hospital, Boston, MA.

Abstract

Reprints: Craig Surman, MD, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114 (csurman@partners.org).

AUTHOR DISCLOSURE INFORMATION

Pamlab Inc, supported this research project. C.S. has received, in his lifetime, consulting fees from McNeil, NLS, Nutricia, Pfizer, Rhodes, Shire, Somaxon, Sunovion, and Takeda. He has also received payments for lectures for Alcobra, McNeil, Janssen, Janssen-Ortho, Novartis, Shire, and Reed/MGH Academy, as well as GME CME (both funded by multiple companies). Royalties have been given to C.S. from Berkeley/Penguin for “FASTMINDS How to Thrive if You Have ADHD (or Think You Might)” and from Humana/Springer for “ADHD in Adults: A Practical Guide to Evaluation and Management”. Additionally, C.S. has conducted clinical research at Massachusetts General Hospital (MGH) supported by Abbot, Cephalon, Hilda and Preston Davis Foundation, Eli Lilly, Magceutics/Neurocentria, Johnson & Johnson/McNeil, Lundbeck, Merck, and Nordic Naturals. A.C.: employment/ salary: MGH, private practice; coinvestigator on studies funded by Pamlab Inc, Sunovion Pharmaceuticals Inc, Lundbeck A/S, Pfizer Inc, NIH, Magceutics Inc, Department of Defense, AACAP, and MGH Department of Psychiatry. C. V, B.A., M.U., H.B., M.F, L.R., and A.S. declare no conflicts of interest. A.Y. received grant support from the American Academy of Child and Adolescent Psychiatry Pilot Research Award for Junior Faculty supported by Lilly USA, LLC, in 2012. She received grant support from the MGH Louis V Gerstner III Research Scholar Award from 2014 to 2016. A.Y. is currently receiving funding through the American Academy of Child and Adolescent Psychiatry Physician Scientist Program in Substance Abuse 5K12DA000357–17. She is a consultant to Phoenix House (clinical service). J.B. is receiving research support from the following sources: AACAP, The Department of Defense, Food & Drug Administration, Headspace, Lundbeck, Neurocentria Inc., NIDA, PamLab, Pfizer, Shire Pharmaceuticals Inc, Sunovion, and NIH. J.B. has a financial interest in Avekshan LLC, a company that develops treatments for ADHD. His interests were reviewed and are managed by MGH and Partners Health-Care in accordance with their conflict of interest policies. J.B. ‘s program has received departmental royalties from a copyrighted rating scale used for ADHD diagnoses, paid by Ingenix, Prophase, Shire, Bracket Global, Sunovion, and Theravance; these royalties were paid to the Department of Psychiatry at MGH. In 2017, J.B. is a consultant to Aevi Genomics, Akili, Guidepoint, Ironshore, Medgenics, and Piper Jaffray. He is on the scientific advisory board for Alcobra and Shire. He received honoraria from the MGH Psychiatry Academy for tuition-funded CME courses. Through MGH corporate licensing, he has a US patent (#14/ 027,676) for a nonstimulant treatment for ADHD and a patent pending (#62/33,686) on a method to prevent stimulant abuse. In 2016, J.B. received honoraria from the MGH Psychiatry Academy for tuition-funded CME courses and from Alcobra and APSARD. He was on the scientific advisory board for Arbor Pharmaceuticals. He was a consultant to Akili and Medgenics. He received research support from Merck and SPRITES. In 2015, J.B. received honoraria from the MGH Psychiatry Academy for tuition-funded CME courses and from Avekshan. He received research support from Ironshore, Magceutics Inc, and Vaya Pharma/Enzymotec. In 2014, J.B. received honoraria from the MGH Psychiatry Academy for tuition-funded CME courses. He received research support from AACAP Alcobra, Forest Research Institute, and Shire Pharmaceuticals Inc. In previous years, J.B. received research support, consultation fees, or speaker’s fees for/from the following additional sources: Abbott, Alza, APSARD, AstraZeneca, Boston University, Bristol-Myers Squibb, Cambridge University Press, Celltech, Cephalon, The Children’s Hospital of Southwest Florida/Lee Memorial Health System, Cipher Pharmaceuticals Inc, Eli Lilly and Co, Esai, ElMindA, Fundacion Areces (Spain), Forest, Fundación Dr Manuel Camelo A.C., Glaxo, Gliatech, Hastings Center, Janssen, Juste Pharmaceutical Spain, McNeil, Medice Pharmaceuticals (Germany), Merck, MGH Psychiatry Academy, MMC Pediatric, NARSAD, NIDA, New River, NICHHD, NIMH, Novartis, Noven, Neurosearch, Organon, Otsuka, Pfizer, Pharmacia, Phase V Communications, Physicians Academy, The Prechter Foundation, Quantia Communications, Reed Exhibitions, Shionogi Pharma Inc, Shire, the Spanish Child Psychiatry Association, The Stanley Foundation, UCB Pharma Inc, Veritas, and Wyeth.

Purpose/Background: Interventions for attention-deficit/hyperactivity disorder (ADHD) may be inadequate for some patients. There is evidence that supplementation with L-methylfolate augments antidepressant agent effects and thus might also augment ADHD treatment effects by a common catecholaminergic mechanism.

Methods: Forty-four adults with *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* diagnosis of ADHD participated in a randomized, double-blind, placebo-controlled, 12-week trial of 15 mg of L-methylfolate in combination with osmotic-release oral system methylphenidate. Osmotic-release oral system methylphenidate was dose optimized over the first 6 weeks. We evaluated the effects on ADHD symptoms, self-report on the Behavior Rating Inventory of Executive Function of executive function, methylphenidate dosing, neuropsychological test measures, the Adult ADHD Self-report scale, emotional dysregulation, social adjustment, and work productivity, as well as moderating effects of body mass index, autoantibodies to folate receptors, and select genetic polymorphisms.

Results: L-Methylfolate was well tolerated, with no significant effect over placebo except improvement from abnormal measures on the mean adaptive dimension of the ASR scale ($\chi^2 = 4.36$, $P = 0.04$). Methylphenidate dosing was significantly higher in individuals on L-methylfolate over time ($\chi^2 = 7.35$, $P = 0.007$). Exploratory analyses suggested that variation in a guanosine triphosphate cyclohydrolase gene predicted association with higher doses of methylphenidate ($P < 0.001$).

Conclusions: L-Methylfolate was associated with no change in efficacy on measures relevant to neuropsychiatric function in adults with ADHD, other than suggestion of reduced efficacy of methylphenidate. Further investigation would be required to confirm this effect and its mechanism and the genotype prediction of effects on dosing.

Keywords

attention-deficit/hyperactivity disorder; L-methylfolate; medical food; methylphenidate

Attention-deficit/hyperactivity disorder (ADHD) is a neurobiological disorder associated with high levels of impairment in adulthood^{1–4} and is estimated to affect up to 5% of adults worldwide.^{5–7} Adults who are considered responders to medication clinically often show a 50% or less reduction in the core symptoms of ADHD.⁸ In addition to residual ADHD symptom burden, pharmacologically treated patients may also have residual executive function deficit (EFD) burden.⁹

Conventional therapies for ADHD are thought to work in part through reuptake blockade and/or increased release of the catecholamines dopamine and norepinephrine.^{10,11} Folate is a building block in the synthesis of the monoamine neurotransmitters serotonin, dopamine, and norepinephrine. Folate levels influence the rate of synthesis of tetrahydrobiopterin, which is a cofactor in the hydroxylation of phenylalanine and tryptophan—rate-limiting steps in the formation of the catecholamines dopamine, serotonin, and norepinephrine.^{12,13} Therefore, it is possible that elevating folate levels in the brain could support higher levels of these neurotransmitters. L-Methylfolate is thought to be actively transported across the blood-brain barrier and may bind to presynaptic glutamate receptors, which could modulate release of catecholamines or other neurotransmitters.¹⁴ L-Methylfolate is approved as

Deplin for suboptimal folate levels in depressed individuals or hyperhomocysteinemia in schizophrenia (Deplin [package insert]. Covington, LA: PamLab, LLC; 2011). L-Methylfolate as Deplin is not approved for the treatment of ADHD.

The hypothesis that L-methylfolate supplementation can improve mental health through effect on catecholamines is supported by evidence that L-methylfolate may aid depression symptoms, both in combination with antidepressant treatment¹⁵ and as monotherapy.¹⁶ Improvement in depressive symptoms has been observed in individuals with both normal and low folate levels.^{15,17–19} Furthermore, L-methylfolate may be particularly effective in the presence of body mass index (BMI) greater than 30 kg/m².²⁰

To evaluate the effect of L-methylfolate on attention and executive function, we conducted a randomized, double-blind, placebocontrolled pilot clinical trial of L-methylfolate augmentation of osmotic-release oral system methylphenidate. We hypothesized that methylfolate exposure would be well tolerated and associated with a greater improvement and faster rate of improvement in ADHD symptoms. Secondly, we hypothesized this could result in a lower dose of methylphenidate treatment for subjects on L-methylfolate than those on placebo, at the end of study participation. We further hypothesized that L-methylfolate would be associated with larger improvements in executive function. We also hypothesized that BMI, presence of genotypes associated with folate metabolism and catecholamine activity, and presence of antibodies to the folate receptor would differentially predict response on these outcome variables. Because psychopharmacology currently in use can produce robust improvement in ADHD with titration over several weeks, but timeline of methylfolate effects is not known, we planned primary endpoint evaluations at weeks 6 and 12. Finally, we planned assays to identify low folate, B₁₂ levels, high homocysteine, genotype, and folate receptor autoantibody as possible biomarkers that might be used to predict response to L-methylfolate.

METHODS

We recruited subjects from the pool of existing subjects and new subjects contacting the Pediatric Psychopharmacology and Adult ADHD Program at the Massachusetts General Hospital. All study participants were assessed by board-certified or board-eligible psychiatrists to confirm presence of medical conditions or treatments, mental health conditions, current treatments, and a diagnosis of ADHD meeting criteria according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (Washington, DC: American Psychiatric Association, 2000). Childhood-onset criteria were operationalized, according to established research criteria and consistent with criteria from the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* definition of ADHD, by requiring onset of at least 2 symptoms of either inattentive or of impulsive/hyperactive traits by the age of 12 years. A score of 24 or more on the Adult ADHD Investigator Symptom Report Scale (AISRS) was also required.

We excluded individuals who had a history of intolerance to L-methylfolate supplementation, those who were currently using MAO inhibitors, those who used supplemental folic acid greater than 400 µg/d, those who used L-methylfolate or omega-3

fatty acids greater than 800 mg/d within 2 weeks prior to study start, those who had multiple adverse drug reactions, those on any other concomitant medication considered to be effective for management of ADHD (however, individuals on stable treatment with other agents with central nervous system activity were allowed to participate), those with serious unstable medical illness, and those with clinically unstable psychiatric conditions or lifetime history of conditions exacerbated by a stimulant. We also excluded subjects with significant impairment due to tics or diagnosis of Tourette syndrome or current (within 3 months) *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* criteria for abuse or dependence with any psychoactive substance other than nicotine. We excluded individuals who were related to the investigator or were pregnant or nursing females. Subjects did not need to be methylphenidate naive, and subjects could be removed from an agent for 5 half-lives prior to study participation. However, no individual was removed from an effective and stable treatment regimen.

Participants treated with any psychotropic medication not thought to help ADHD, such as selective serotonin reuptake inhibitors, could participate as long as they had been on a stable dose for at least 2 months prior to study enrollment, would stay on this dose during the study, and have scores of less than 17 on the Hamilton Anxiety (HAM-A) scale and less than 13 on the Hamilton Depression (HAM-D) scale. Any agents with probable efficacy for the treatment of ADHD were not allowed in this study.

The Partners Human Research Committee Institutional Review Board granted approval for this study.

Study Measures

Demographic information was collected. Vital signs (blood pressure, pulse, weight) were measured at every visit. Height, waist circumference, and BMI were measured at baseline and at the end of the study. An electrocardiogram was conducted at the beginning and the end of the study to monitor cardiac safety. A urine drug screen was performed at evaluation, week 6, and week 12.

Subjects were evaluated at weeks 1, 2, 3, 4, 5, 6, 9, and 12. These evaluations occurred in our research clinic by a study physician, except where in-person visits were not feasible, at which time a phone visit was conducted covering all assessments other than vital signs. However, the initial evaluation visit, baseline visit, midpoint visit (week 6), and the final study visit were not conducted over the phone. Additionally, phone visits did not replace scheduled office visits for more than 2 consecutive visits. At each office visit, overall severity and change in severity of ADHD were assessed with the Clinical Global Impression (CGI) scale.²¹ The CGI scale and AISRS were completed at every office visit during study participation. The Global Assessment of Functioning (GAF) scale was rated according to guidelines in *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* at baseline, week 6, and at end of study. Adverse events were elicited by spontaneous reports through open-ended questions at each visit. We utilized an a priori definition of clinical improvement, CGI-Improvement of 2 or greater (much improved), and 30% reduction on the AISRS. Clinicians completed the HAM-D and HAM-A scales to evaluate depression/anxiety symptoms at baseline, week 6, and week 12 visits.²²

Height, weight, and waist circumference were measured before first study agent exposure. Blood samples were also collected from participants before first study agent exposure for biomarker analyses. Participants were given the option of providing an additional blood sample for analysis of genetic polymorphisms.

Subtests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) neuropsychological assessments occurred at baseline and at study endpoint.²³

Subjects also completed: the Adult ADHD Self-report Scale at weeks 1 to 6, 9, and 12; the Behavior Rating Inventory of Executive Function–Adult Form (BRIEF-A) at baseline, week 6, and week 12.²⁴

Subjects also completed the following forms at baseline, week 6, and week 12/dropout: the Adult Self-report (ASR) Form,²⁵ the Social Adjustment Self-report Questionnaire, the Quality of Life Enjoyment & Satisfaction Questionnaire, an 8-item emotional dysregulation scale generated from the Barkley Current Behavior Scale–Self-report (CBS DESR),²⁶ and the Endicott Work Productivity Scale.

OROS Methylphenidate and L-Methylfolate Administration

At the baseline visit, subjects received a prescription for OROS-MPH and were randomized to receive either L-methylfolate or placebo (under double-blind conditions), dispensed from our research unit. Subjects were instructed to self-administer OROS-MPH and L-methylfolate or placebo concurrently. OROS-MPH was prescribed starting with an initial dose of 36 mg/d (n = 41). OROS-MPH was titrated to optimal response by 18 to 36 mg/wk (not exceeding a maximum daily dose of 1.3 mg/kg or 108 mg/d, whichever was lower), according to clinician judgment, during the first 6 weeks of the trial. Dose could be changed at any time based on clinician judgment.

L-Methylfolate dosing remained at 15 mg/d of L-methylfolate for the duration of the study in the form of PAMLAB brand of L-methylfolate or identically encapsulated placebo.

All subjects gave their informed consent before study procedures occurred and after possible adverse effects were fully explained.

Participation Flow

In total, 47 subjects signed consent and were enrolled in the trial. Of these, 44 were randomized to L-methylfolate or placebo; however, 41 were actually exposed. Of these 41 subjects left, 40 made it through week 6, with this 1 individual being dropped from the study because of not adhering to study protocol. A further 4 subjects did not complete the study, because of the subjects' desire to leave the study, due to time burden and/or mild adverse effects. Thirty-six subjects completed all study procedures. Thirty-nine subjects provided serum samples adequate for evaluation of presence of folate receptor antibodies. Thirty-five subjects consented and provided samples for genotyping (Fig. 1).

All enrolled subjects had a blood draw performed before study agent exposure and were also given the choice of providing a genetic sample. Some samples were inadvertently thawed

from -20°C to 0°C , for up to 19 hours. Comparing thawed and unthawed samples, variation in folate receptor antibody assay results was within a range expected for duplicate samples. Tests performed on thawed and unthawed paired aliquots of split samples showed that there were no significant differences in these values due to thawing and storage at 0°C .

Single-nucleotide polymorphisms (SNPs) with possible association to folate metabolism and catecholamine activity were identified in online databases (www.snpedia.com and www.ncbi.nlm.nih.gov/projects/SNP/) for assay (Table 1). Major and minor allele designations were determined through the representation on the dbSNP database or with literature searches where the information was not available (dbSNP reference). Single-nucleotide polymorphism genotyping was performed at the Massachusetts General Hospital Psychiatric Neurogenetics Unit under the supervision of Dr Jordan Smoller using the iPLEX Gold application and MassARRAY system.

Biomarker Assays

Folate receptor autoantibody (*FOLR1*) and ligand interactions were evaluated at the University of Texas, Austin, under the supervision of Dr Robert Cabrera. The assay procedure used was previously described²⁷ with chemiluminescent modification.²⁸

Other biomarkers were evaluated at Brigham and Women's Hospital, Boston, Mass, under the supervision of Dr Raina Fichorova.

Statistical Analysis

Demographic characteristics and baseline efficacy measures were analyzed using Student *t* tests for continuous outcomes, Pearson χ^2 tests for binary outcomes, and Wilcoxon rank-sum tests for ordinal outcomes. We analyzed binary outcomes with Fisher exact tests in the event of cells with expected counts of less than 5. Our primary outcome measure, the AISRS, was analyzed using a mixed-effects Poisson regression model with the drug group, time, and group-by-time interaction as predictors. Secondary outcome measures were analyzed using mixed-effects Poisson, linear, or ordered logistic regression models. Additional analyses for secondary outcomes included stratifying the subjects by whether they had abnormal or normal scores (defined by clinical cutoffs for each outcome) at baseline and comparing the rate of normalization at study endpoint using either Pearson χ^2 or Fisher exact test. We also analyzed the secondary measures in only those with abnormal scores at baseline using mixed-effects regression models. We did not analyze subscales if the placebo or L-methylfolate groups had a sample size of fewer than 5 subjects with abnormal scores. The time course of adverse events was analyzed using a mixed-effects Poisson regression model, and the total number of adverse events during the entire study was analyzed using a Poisson regression model. Vitals were analyzed using mixed-effects linear regression models. Compliance was analyzed using Student *t* tests. Dosing at weeks 6 and 12 was analyzed using Student *t* tests, and dosing over the course of the trial was analyzed using mixed-effects linear regression. To assess the effect of genetic polymorphisms, we added the 3-way interaction between drug group, time, and biomarker to our mixed-effects regression models. All mixed-effects regression models used robust standard errors to account for the repeated measures on each subject. In models where multicollinearity was a

problem, we centered all predictor variables and reran the analysis. All tests were 2-tailed and performed at the 0.05 α level using Stata (version 14; StataCorp LLC, College Station, TX).

RESULTS

Subjects on L-methylfolate and subjects on placebo did not significantly differ in demographic characteristics or baseline measures of efficacy (all $P > 0.05$) (Table 2).

We did not find a significant group-by-time interaction effect for the AISRS ($\chi^2 = 0.14$, $P = 0.71$). Subjects who received L-methylfolate did not show a significantly greater reduction in ADHD symptoms relative to those who received placebo (Fig. 2).

At the study endpoint, we did not find a statistically significant difference in the rate of subjects who had clinically significant response (a CGI-Improvement score ≥ 2 and an AISRS total reduction $\geq 30\%$) between subjects who received L-methylfolate and subjects who received placebo (Fisher exact, $P = 1.00$). Exploring proportion of all participants who experienced a reduction of 30% or greater in AISRS scores, we found that 86% of individuals receiving active methylfolate and 84% of those receiving placebo had an improvement of 30% or greater in AISRS scores. Additionally, we did not find a significant group-by-time interaction effect for the CGI-I ($\chi^2 = 0.30$, $P = 0.59$) or the GAF ($\chi^2 = 1.97$, $P = 0.16$).

Exploring self-reported measures of mental health, we did not find significant group-by-time interaction effects for the HAM-D ($\chi^2 = 0.00$, $P = 0.98$), the HAM-A ($\chi^2 = 1.44$, $P = 0.23$), or the Adult ADHD Self-report Scale ($\chi^2 = 0.04$, $P = 0.85$). We also did not find a significant group-by-time interaction effect for self-reported control over emotional expression as measured by the CBS-DESR scale ($\chi^2 = 2.24$, $P = 0.13$). When we looked at subjects with abnormal scores at baseline (scores ≥ 9)²⁶ (L-methylfolate: $n = 7$, placebo: $n = 8$), we did not find a significant difference in the percentage of subjects who normalized (scores < 9) by the study endpoint (Fisher exact, $P = 1.00$). Furthermore, when we looked at subjects with abnormal scores at baseline, we did not find a significant group-by-time interaction effect for the CBS-DESR ($\chi^2 = 0.18$, $P = 0.67$).

We evaluated self-report of executive function capacities, finding no significant group-by-time interaction effects for any of the BRIEF-A subscales: inhibition ($\chi^2 = 0.95$, $P = 0.33$), shifting ($\chi^2 = 0.00$, $P = 0.98$), emotional control ($\chi^2 = 0.24$, $P = 0.62$), self-control ($\chi^2 = 0.72$, $P = 0.40$), initiate ($\chi^2 = 0.00$, $P = 0.98$), working memory ($\chi^2 = 0.03$, $P = 0.85$), planning/organizing ($\chi^2 = 0.01$, $P = 0.93$), task monitor ($\chi^2 = 3.59$, $P = 0.06$), organization of materials ($\chi^2 = 0.13$, $P = 0.72$), Behavioral Regulation Index ($\chi^2 = 0.00$, $P = 0.96$), Metacognition Index ($\chi^2 = 0.20$, $P = 0.65$), and Global Executive Composite ($\chi^2 = 0.06$, $P = 0.81$). When we looked at subjects with abnormal scores at baseline (T scores ≥ 65) (sample size differed by subscale; L-methylfolate: $n = 7$ – 21 , placebo: $n = 6$ – 16), we did not find a significant difference in the percentage of subjects who normalized (T scores < 65) by the study endpoint for any of the subscales (all $P \geq 0.05$).

We also did not find any significant group-by-time interaction effects when we restricted our sample to only individuals with abnormal BRIEF-A subscale scores at baseline (all P 0.05).

We did not find significant group-by-time interaction effects for the 12 ASR scales in which we were interested: friends ($\chi^2 = 0.00$, $P = 0.95$), spouse/partner ($\chi^2 = 2.97$, $P = 0.08$), family ($\chi^2 = 2.83$, $P = 0.09$), job ($\chi^2 = 0.24$, $P = 0.62$), mean adaptive ($\chi^2 = 1.64$, $P = 0.20$), anxious/depressed ($\chi^2 = 0.27$, $P = 0.60$), attention problems ($\chi^2 = 0.05$, $P = 0.82$), aggressive behavior ($\chi^2 = 0.01$, $P = 0.91$), intrusive ($\chi^2 = 0.71$, $P = 0.40$), depressive problems ($\chi^2 = 0.05$, $P = 0.82$), anxiety problems ($\chi^2 = 0.00$, $P = 0.98$), and ADHD problems ($\chi^2 = 0.16$, $P = 0.69$).

Because we had variable size samples of individuals reporting abnormal scores on ASR scales, we chose for convenience to compare change from baseline in those scales for which there were at least 5 such individuals: job, mean adaptive, anxious/depressed, attention problems, intrusive, depressive problems, and ADHD problems (sample sizes differed by subscale; L-methylfolate: $n = 5$ –20, placebo: $n = 5$ –16). When we looked at subjects with abnormal scores at baseline (T scores ≥ 35 for the job and mean adaptive subscales; T scores ≥ 65 for the anxious/depressed, attention problems, intrusive, depressive problems, and ADHD problems subscales), we did not find a significant difference in the percentage of subjects who normalized (T scores < 35 , T scores < 65) by the study endpoint for any of the subscales (all $P > 0.05$). When we looked for improvement in subjects with abnormal ASR subscale scores at baseline, only the mean adaptive subscale had a significant group-by-time interaction ($\chi^2 = 4.36$, $P = 0.04$).

Evaluating changes in cognitive performance during the study, we did not find significant group-by-time interaction effects for any of the CANTAB subtests (Table 3). We could not analyze Reaction Time (RTI) simple error all or RTI five-choice error, all because there was no variation in responses between any of the subjects.

Only the Spatial Working Memory (SWM) strategy subtest had sufficient numbers to analyze abnormal subjects (L-methylfolate: $n = 7$, placebo: $n = 5$). Abnormal subjects were defined as having a z score of -1 or less at baseline. We did not find a significant difference in the percentage of subjects who normalized (z scores of greater than -1) by the study endpoint on the SWM strategy subtest (Fisher exact, $P = 1.00$).

When we looked at subjects with abnormal scores at baseline, we did not find a significant group-by-time interaction effect for the SWM strategy subtest ($\chi^2 = 0.28$, $P = 0.60$).

We defined EFDs as having 2 or more tests (Intra-Extra Dimensional Set Shifting, Rapid Visual Information Processing, Stockings of Cambridge, SWM) with at least 1 subtest with a z score of -1 or less. There was no significant difference between those on L-methylfolate and those on placebo in the rate of subjects with EFDs at baseline (L-methylfolate: $n = 8$, placebo: $n = 6$) who no longer had EFDs at study endpoint (L-methylfolate: 63% vs placebo: 83%; Fisher exact, $P = 0.58$).

Looking at self-reported quality of life, we did not find a significant group-by-time interaction effect for the Quality of Life Enjoyment & Satisfaction Questionnaire ($\chi^2 = 0.09$, $P = 0.76$) or in measures of work productivity on the Endicott Work Productivity Scale ($\chi^2 = 0.00$, $P = 0.98$).

There were no serious adverse events. The most common adverse events (2 occurrences in the same subject) are reported in Table 4. There was no significant difference between those who received L-methylfolate and those who received placebo in the number of adverse events reported throughout the trial (L-methylfolate: mean $\pm$ SD=9.6 $\pm$ 7.1, placebo: mean $\pm$ SD=9.2 $\pm$ 4.3; $\chi^2 = 0.20$, $P = 0.65$). We did not find a significant group-by-time interaction effect for adverse events ($\chi^2 = 0.33$, $P = 0.56$).

Mean changes in vital signs from baseline to endpoint did not significantly differ between those who received L-methylfolate and those who received placebo (Table 5). There were no significant differences between the 2 groups in the rates of having a heart rate of greater than 100 beats/min at any time during the study (L-methylfolate: 24% vs placebo: 47%; $\chi^2 = 2.43$, $P = 0.12$), having systolic blood pressure of greater than 140 mm Hg or diastolic blood pressure of greater than 90 mm Hg at any time during the study (L-methylfolate: 38% vs placebo: 53%; $\chi^2 = 0.85$, $P = 0.36$), or having systolic blood pressure of greater than 140 mm Hg or diastolic blood pressure of greater than 90 mm Hg at 2 or more consecutive visits between weeks 1 and 12 (L-methylfolate: 14% vs placebo: 21%; Fisher exact, $P = 0.69$).

We evaluated differences in compliance, which we identified as the total number of pills missing from bottles returned by subjects over the whole study divided by the total number of pills expected to be missing over the whole study. We found there was no significant difference in the mean L-methylfolate compliance rate between those on L-methylfolate and those on placebo (L-methylfolate: mean $\pm$ SD = 0.95 $\pm$ 0.08, placebo: mean $\pm$ SD = 0.96 $\pm$ 0.09; $t_{38} = 0.31$, $P = 0.76$).

There was no significant difference in the mean OROS-MPH compliance rate between those on L-methylfolate and those on placebo (L-methylfolate: mean $\pm$ SD = 0.91 $\pm$ 0.08, placebo: mean $\pm$ SD = 0.85 $\pm$ 0.17; $t_{38} = -1.45$, $P = 0.16$). One subject in the placebo group was missing compliance data. Five subjects were on selective serotonin reuptake inhibitors during the course of the study; of the five, 3 were on L-methylfolate and 2 were on placebo. One of the subjects on placebo did not complete the study because of lack of study drug efficacy after completing the week 6 (midpoint) study visit.

Dosing

There was a significant difference in the mean OROS-MPH dose at week 6 ($t_{38} = -2.69$, $P = 0.01$). Subjects on L-methylfolate had a higher mean OROS-MPH dose at week 6 compared with subjects on placebo. There was a significant difference in the mean OROS-MPH dose at week 12 ($t_{38} = -2.19$, $P = 0.04$). Subjects on L-methylfolate had a higher mean OROS-MPH dose at week 12 compared with subjects on placebo.

We found a significant group-by-time interaction effect for dosing ($\chi^2 = 7.35$, $P = 0.007$).

Completers Analyses

In addition to our primary intention-to-treat analysis, we also performed a per-protocol analysis. This analysis excluded 5 subjects who did not complete the study and 1 subject who completed the study but was not compliant. Thus, our final per-protocol sample included 15 subjects on placebo and 20 subjects on L-methylfolate. We found a few results that reached significance below our a priori-defined significance level of 0.05. The group-by-time interaction was significant for the CANTAB Verbal Recognition Memory Recognition Correct–Immediate test when we looked at all subjects ($\chi^2 = 4.37$, $P = 0.04$). The group-by-time interaction was also significant for the ASR spouse/partner subscale when we looked at all subjects ($\chi^2 = 5.39$, $P = 0.02$). Doses at week 6 (placebo: mean $\pm$ SD: 57.50 ± 20.51 ; L-methylfolate: mean $\pm$ SD: 73.64 ± 17.49 ; $t_{38} = -2.69$, $P = 0.01$) and week 12 (placebo: 59.63 ± 20.49 ; L-methylfolate: mean $\pm$ SD: 73.80 ± 18.34 ; $t_{38} = -2.19$, $P = 0.04$) significantly differed between the 2 groups, with higher doses at both time points for subjects on active L-methylfolate than those on placebo (week 6: $t_{33} = -2.65$, $P = 0.01$; week 12: $t_{33} = -2.25$, $P = 0.03$). The group-by-time interaction was also significant when we had dose as an outcome ($\chi^2 = 8.24$, $P = 0.004$).

Moderator Analyses

We found no subjects with low folate before study drug exposure (range, 5.6–29.4 ng/mL; reference range, >3 ng/mL). There were limited numbers of abnormal B₁₂ levels: 2/19 subjects on placebo had low results, and the remaining 17 of 19 subjects had normal results; 1 of 20 active subjects had high results, with the remaining 19/20 subjects having normal results (range, 175.2–1248.9 pg/mL; reference range, 200–835 pg/mL). For homocysteine, 12 of 19 subjects on placebo had low results, and 7 of 19 remaining had normal results; 13 of 20 active subjects had low results, and 7/20 remaining had normal results (range, 3.0–5.7 μ mol/L; reference range, 5–9 μ mol/L). In the absence of a sufficient number of subjects with levels of interest on these values, we could not assess whether these assays were predictors of endpoint analyses.

We assessed how the presence of abnormal genotypes at baseline interacted with drug group and study week to predict dosing, AISRS scores, and BRIEF-GEC scores. Considering BMI (L-methylfolate: 6/22 had BMI ≥ 30 kg/m²; placebo: 6/19 had BMI ≥ 30 kg/m²), we found no prediction for the 3 outcome measures. Considering genotype variants (Table 1), we found significant 3-way interactions with both *MTHFR* (rs 1801131) and *GCH1* (rs8007267) in the models predicting dosing. There were no significant 3-way interactions with any of the genotypes in models predicting BRIEF-GEC scores. Considering immunoglobulin M (IgM) or immunoglobulin G (IgG) antibodies to the folate receptor assay results (Table 5), presence of 1 or both of these antibodies did not significantly predict any 1 of these 3 outcomes.

DISCUSSION

This is the first study, to our knowledge, to evaluate the tolerability and neuropsychiatric effects of methylfolate supplementation to standard-of-care stimulant treatment in individuals with ADHD. We found that L-methylfolate was well tolerated, as there were no

new categories of adverse events not expected from methylphenidate treatment and no elevation in rates of such adverse events. We did not find a difference in improvement in our primary clinical outcome, clinical measure of ADHD symptoms. L-Methylfolate administration was not associated with significant change in several measures of mental health and function, except improvement from abnormal measures prior to study agent exposure on the ASR mean adaptive subscale. Our analysis of moderating effects of elevated BMI, genotypes thought to contribute to folate metabolism and catecholamine activity, and presence of antibodies to the folate receptor overall suggest little clear impact on ADHD symptoms, executive function, or medication dosing, with the exception of 4 genotypes that each predicted scores of either ADHD symptoms or dosing. Of these, as judged by the P value we report, only 1 result is most likely to survive a correction for multiple comparisons with a $P < 0.001$ —the guanosine triphosphate cyclohydrolase polymorphism (rs8007267)—on dosing. On inspecting trends, in the presence of the less frequent allele, individuals on L-methylfolate ended up on higher doses over the study. The product of this gene produces tetrahydrobiopterin, which in turn helps to make serotonin and dopamine. Because of our small sample size, this finding should be viewed as very preliminary (Table 6).

We found 1 study examining folic acid supplementation in children with ADHD that did not support improvement of symptoms,²⁹ and high folic acid may produce negative functional consequences if metabolism is limited by dihydrofolate reductase or methylenetetrahydrofolate reductase.³⁰ However, in this study, the reduced form of folate, methylfolate, does not require reduction by dihydrofolate reductase, which is highly variable,³¹ and is not dependent on methylenetetrahydrofolate reductase for activation. Therefore, we would expect that potential negative consequences of unmetabolized folic acid would have been avoided.³²

Overall, our study suggests that methylfolate does not have beneficial effects on methylphenidate treatment for ADHD. Our study has important limitations. Our ability to evaluate the impact of L-methylfolate on ADHD was limited by the presence of methylphenidate, which has rapid and robust effects that could mask any benefit of this agent.

We also observed that all patients had adequate serum folate and vitamin B₁₂ concentrations. Our study thus does not allow evaluation of effects in individuals with low folate status. Folate and vitamin B₁₂ metabolism are required for homocysteine conversion to methionine in the remethylation pathway. We observed low homocysteine concentrations, consistent with the expected interaction where folate and vitamin B₁₂ concentrations are inversely associated with total homocysteine. It is not surprising that our study population was found to be folate replete, as less than 1% of the population is thought to have low folate.³³ However, this study replicates 1 other study,³⁴ finding that deficiencies in plasma folate or folate metabolism may not be overrepresented in an adult ADHD population.

Our study had limited ability to evaluate the predictive value of biomarkers (BMI, autoantibodies to folate receptors, and select folate metabolism genotypes) on the efficacy of L-methylfolate because of small numbers of individuals with relevant potentially moderating biomarker values of interest and lack of robust effect of L-methylfolate on outcome

measures. Our inability to produce parallel findings to studies demonstrating that BMI and some of the genotypes we studied predicted response to depression treatment could be a product of significant differences in the mental health of our participants, who had low rates of mood or anxiety comorbidity and low rates of antidepressant agent use (5 subjects). Of note, the majority of our subjects were female.

It is of interest that subjects on methylfolate were prescribed higher mean doses of methylphenidate. If replicated, this may mean that methylfolate in some way reduces the effect of methylphenidate. Further study may elaborate on our very preliminary finding that genetic differences could underlie doses that individuals were prescribed and whether this is more a result of efficacy or of tolerability differences. However, subjects had similar adverse effect patterns on active or placebo, consistent with the concept that methylfolate does not have strong catecholaminergic effects, which might be reflected in higher vital sign or sympathomimetic adverse event rates. Our ability to investigate the effect of L-methylfolate on methylphenidate dose-response relationship was significantly limited, however, by lack of fixed, forced doses, which could have been a more useful design to evaluate the biological effect of methylfolate on dose-response relationships in methylphenidate treatment. We believe that a larger project with multicenter design recruiting individuals with abnormal B₁₂, folate, and homocysteine levels would potentially have a higher chance of identifying augmentation benefits of L-methylfolate.

We wish to emphasize that the small sample sizes in our secondary analyses limit the meaningfulness of related findings. Although a larger study would have greater power to detect effects, our study suggests, in conclusion, that methylfolate is well tolerated in the context of methylphenidate treatment with ADHD and that measures previously suggested as predicting response to folate effects on depression treatment (BMI, genetic polymorphisms associated with folate metabolism, and catecholamine effects) may not identify individuals sensitive to ADHD-related benefits of folate supplementation.

ACKNOWLEDGMENTS

The authors thank the Pediatric Psychopharmacology and Adult ADHD Program staff at the Massachusetts General Hospital.

Appendix 1. Addendum: Methods for Genotype, Antibody to the Folate Receptor, Immunoinflammatory and Metabolic Biomarkers, and Thawed Sample Stability Assays

Genetic Polymorphisms

The major steps in identifying genetic polymorphisms included the following: primer and multiplex assay design using MassARRAY Designer software, DNA amplification by polymerase chain reaction, post-polymerase chain reaction nucleotide deactivation using shrimp alkaline phosphatase to remove phosphate groups from unincorporated dNTPs, single-base extension reaction for allele differentiation, salt removal using ion-exchange resin, and mass correlated genotype calling using SpectroCHIP array and matrix-assisted

laser desorption/ionization time-of-flight mass spectrometry. Quality control to determine sample and genotyping quality and to potentially remove poor SNPs and/or samples was performed in PLINK, a whole-genome association analysis tool set.

Antibodies to the Folate Receptor

Assays for antibodies to the folate receptor were conducted as previously described²⁷ using 96-well microtiter plates (Corning COSTAR high-binding 96-well plate; Fischer Scientific, Cambridge, MA). The *FOLR1* was extracted from human placenta by Selhub as previously described³⁵ and printed onto the surface in 2.0- μ L volumes under ambient conditions. Serum folates were extracted under acidic conditions (activated charcoal–dextran, ascorbate 1% wt/vol), filtered to remove charcoal, and neutralized (pH 7–7.5) with 10% NaOH before competitive hybridization with immobilized *FOLR1*. Folic acid–labeled horseradish peroxidase (HRP) (FA-HRP; Ortho-Clinical Diagnostics, Raritan, NJ) binding was developed with SuperSignal enzyme-linked immunosorbent assay (ELISA) Pico Chemiluminescent Substrate (Thermo Scientific, Waltham, MA). Signals were captured on a Q-View Imager (Quansys Biosciences, Logan, Utah). Antibodies were determined in a similar method, briefly; samples were diluted 1:50 in buffer (1 $\times$ tris-buffered saline with 0.1% Tween 20) and incubated with immobilized FOLR1. The autoantibodies were bound with a secondary antibody to human IgG or IgM (IgG HRP, IgM HRP; Sigma, St Louis, MO) and detected with SuperSignal ELISA Pico Chemiluminescent Substrate. All samples were run in triplicate. Average intensities were normalized using standard curves of folic acid or printed human IgG or IgM (Sigma). Sample concentrations were determined relative the standard curve. Raw data were normalized using a grand mean for cases and controls run on the same 96-well plates (intra-assay). The resulting control data were used to establish the positive threshold based on the upper quartile. This upper quartile data were used to categorize case and control data.

Metabolic Biomarkers

Colorimetric ELISAs were performed according to manufacturers' instructions to measure vitamin B₁₂ (AccuDiag vitamin B₁₂ ELISA cat #3125–15; Diagnostic Automation/Cortez Diagnostics Inc, Woodland Hills, Calif), folate (folate ELISA cat #3127–15; Diagnostic Automation/Cortez Diagnostics Inc), homocysteine (cat# CELI-66076h; Nova Lifetech Inc), serotonin (cat #K1894; Thermo Fisher Scientific, Pittsburgh, Pa), and methylmalonic acid (cat #MBS288266_data Competitive; MyBiosource Inc, San Diego, Calif).

Biomarker Stability at 0°C

To establish criteria for acceptance of biomarker data generated from the inadvertently thawed samples, assays were run to ascertain preserved stability of the markers measured in this study under the same thawing conditions. Split aliquots of 10 serum samples that were never thawed before were subjected to 0°C for 19 hours and compared with their frozen counterparts. Assays were performed side-by-side on these split samples to compare measures without prolonged thawing and after thawing to 0°C for 19 hours. Comparing the normally thawed and prolonged thawing samples, there was no significant difference in the

measurements of folate or homocysteine. There was a significant difference in the thawed and nonthawed assays for B₁₂ ($P < 0.001$); however, the mean difference among the 10 samples was very small and less than a typically acceptable assay variation of 10%.

Additional Genotyping Methods Information

All but 2 of the SNPs were in line with Hardy-Weinberg proportions (rs1799732 and rs4818). One SNP was a deletion variant, SNP rs1799732, and no polymorphism was found. Call/pass rates of the SNPs and the minor allele frequency of the SNPs can be found below.

Call/Pass Rates for SNPs

SNP	P	MAF	F_MISS
rs2274976	1	0.1207	0
rs1801131	1	0.3276	0
rs1801133	0.6788	0.3103	0
rs1805087	0.1647	0.1724	0
rs6265	0.3356	0.1207	0
rs61886492	1	0.03448	0
rs202676	0.6714	0.3276	0
rs1800497	0.5578	0.1724	0
rs1079596	1	0.1607	0.03448
rs1799732	0	0	0.1379
rs8007267	0.5578	0.1724	0
rs2297291	1	0.4828	0
rs12659	0.7129	0.4483	0
rs1051266	0.2678	0.4483	0
rs737865	0.6385	0.2759	0
rs4633	0.02319	0.4138	0
rs4818	0.00005871	0.431	0
rs4680	0.059	0.431	0

MAF indicates minor allele frequency.

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FIGURE 1.

Participation flow. Flowchart of participant numbers and status throughout various study time points.

	Coefficient	Standard Error	Test Statistic	P-Value
L-methylfolate	0.08	0.11	$\chi^2=0.48$	0.49
Time			$\chi^2=46.36$	<0.001
L-methylfolate x Time			$\chi^2=0.14$	0.71

FIGURE 2.

Mixed-effects Poisson model predicting total AISRS scores from the group, time, and group $\times$ time interaction (n = 41). Poisson model predicting total AISRS scores from the group, time, and group $\times$ time interaction is shown.

TABLE 1.

Three-Way Interaction Between Drug Group, Week, and Gene*

SNP Name	Gene Name	Alleles	Placebo	Active	AIIRS	BRIEF-GEC	Dosing
rs1805087	<i>MTR</i>	Major: AA Minor: AG/GG	10	11	$\chi^2 = 0.46, P = 0.50$	$\chi^2 = 0.00, P = 0.95$	$\chi^2 = 1.06, P = 0.30$
rs6265	<i>BDNF</i>	Major: GG Minor: AG/AA	3	5	$\chi^2 = 0.60, P = 0.44$	$\chi^2 = 0.02, P = 0.88$	$\chi^2 = 0.57, P = 0.45$
rs1800497	<i>DRD2</i>	Major: CC Minor: CT	8	11	$\chi^2 = 1.76, P = 0.18$	$\chi^2 = 1.01, P = 0.32$	$\chi^2 = 2.76, P = 0.10$
rs1079596	<i>DRD2</i>	Major: GG Minor: AG	5	5	$\chi^2 = 2.08, P = 0.15$	$\chi^2 = 1.46, P = 0.23$	$\chi^2 = 1.98, P = 0.16$
rs8007267	<i>GCHI</i>	Major: CC Minor: CT	4	5	$\chi^2 = 0.54, P = 0.46$	$\chi^2 = 0.42, P = 0.52$	$\chi^2 = 12.23, P < 0.001$
rs4633	<i>COMT</i>	Major: CC Minor: CT/TT	11	8	$\chi^2 = 0.01, P = 0.92$	$\chi^2 = 0.68, P = 0.41$	$\chi^2 = 0.18, P = 0.67$ (centered)
rs4680	<i>COMT</i>	Major: GG Minor: AG/AA	3	5	$\chi^2 = 0.01, P = 0.92$	$\chi^2 = 0.68, P = 0.41$	$\chi^2 = 0.18, P = 0.67$ (centered)
rs202676	<i>GCPII (FOLH1)</i>	Major: TT Minor: TC/CC	10	11	$\chi^2 = 4.84, P = 0.03$	$\chi^2 = 0.14, P = 0.71$	$\chi^2 = 0.14, P = 0.71$ (centered)
rs737865	<i>COMT</i>	Major: TT Minor: TC/CC	9	8	$\chi^2 = 0.32, P = 0.57$	$\chi^2 = 1.06, P = 0.30$	$\chi^2 = 2.60, P = 0.11$
rs2274976	<i>MTHFR</i>	Major: GG Minor: AG	4	9	$\chi^2 = 0.07, P = 0.79$	$\chi^2 = 2.11, P = 0.15$	$\chi^2 = 1.34, P = 0.25$
rs1801131	<i>MTHFR</i>	Major: AA Minor: AC/CC	4	3	$\chi^2 = 0.01, P = 0.94$	$\chi^2 = 2.43, P = 0.12$	$\chi^2 = 5.13, P = 0.02$
rs1801133	<i>MTHFR</i>	Major: CC Minor: CT/TT	7	6	$\chi^2 = 2.32, P = 0.13$	$\chi^2 = 0.03, P = 0.86$	$\chi^2 = 0.54, P = 0.46$ (centered)
			4	9			
			9	7			

Interactions between drug group, week, and gene are listed.

* Allele patterns reflect instances available in the study sample.

TABLE 2.
Demographic Characteristics and Baseline Efficacy Measures of Subjects on Placebo and L-Methylfolate

	Placebo (n = 19) n(%)	L-Methylfolate (n = 22) n(%)	Test Statistic	P
Sex, % male	7(37)	8(36)	$\chi^2 = 0.001$	0.98
Race, % white	15 (79)	19(86)	Fisher exact	0.69
Psychiatric comedication	2(11)	3(14)	Fisher exact	1.00
Clinically significant ASR scores at baseline [*]				
Anxious/depressed	7(39)	5 (23)	$\chi^2 = 1.23$	0.27
Aggressive behaviors	2(11)	3(14)	Fisher exact	1.00
Intrusive	5(28)	7(32)	$\chi^2 = 0.08$	0.78
Depressive problems	5(28)	6 (27)	$\chi^2 = 0.001$	0.97
Anxiety problems	3(17)	4(18)	Fisher exact	1.00
	Mean $\pm$ SD	Mean $\pm$ SD		
Age, y	37.7 $\pm$ 8.9	41.3 $\pm$ 11.4	$t_{39} = 1.12$	0.27
Socioeconomic status [*]	1.5 $\pm$ 0.5	1.6 $\pm$ 0.9	$z = 0.14$	0.89
BMI, kg/m ²	27.3 $\pm$ 4.3	28.1 $\pm$ 5.2	$t_{39} = 0.52$	0.61
Baseline Efficacy Measures				
AISRS	35.0 $\pm$ 10.3	35.4 $\pm$ 8.8	$t_{39} = 0.14$	0.89
CGI-Severity	4.6 $\pm$ 0.7	4.6 $\pm$ 0.6	$z = 0.16$	0.87
GAF [*]	61.3 $\pm$ 5.2	61.0 $\pm$ 4.3	$t_{39} = -0.25$	0.80

Demographic characteristics and baseline efficacy measures of subjects on placebo (n = 19) and L-methylfolate (n = 22).

^{*} Smaller sample sizes. ASR subscales: placebo n = 18; SES: Deplin n = 21; GAF: placebo n = 18.

TABLE 3.

Predicting CANTAB Scores From the Group x Time Interaction (CANTAB Collected at Weeks 0, 6, and 12)

CANTAB Subtest	Result
SWM	Total Errors ($\chi^2 = 0.00, P = 0.99$) Between Errors ($\chi^2 = 0.01, P = 0.90$) Between Errors 8 Boxes ($\chi^2 = 0.02, P = 0.89$) Strategy ($\chi^2 = 1.79, P = 0.18$)
Stockings of Cambridge	Mean Moves 2 Moves ($\chi^2 = 1.07, P = 0.30$) Mean Moves 3 Moves ($\chi^2 = 0.40, P = 0.53$) Mean Moves 4 Moves ($\chi^2 = 0.06, P = 0.81$) Mean Moves 5 Moves ($\chi^2 = 0.21, P = 0.65$) Minimum Moves ($\chi^2 = 3.88, P = 0.05$)
Intra-Extra Dimensional Set Shifting (IED)	Mean Initial Thinking Time ($\chi^2 = 0.51, P = 0.47$) Total Errors ($\chi^2 = 0.15, P = 0.70$), Total Errors Adjusted ($\chi^2 = 0.19, P = 0.66$), Stages Completed ($\chi^2 = 152, P = 0.22$); Simple Reaction Time ($\chi^2 = 0.00, P = 0.96$)
RTI	Five-Choice Reaction Time ($\chi^2 = 0.66, P = 0.42$) Simple Movement Time ($\chi^2 = 0.28, P = 0.60$) Five-Choice Movement Time ($\chi^2 = 2.03, P = 0.15$)
Rapid Visual Information Processing	A' ($\chi^2 = 0.34, P = 0.56$) Response Latency ($\chi^2 = 0.71, P = 0.40$) Probability of Hit ($\chi^2 = 0.55, P = 0.46$) Total False Alarms ($\chi^2 = 1.51, P = 0.22$) Mean Correct Latency ($\chi^2 = 176, P = 0.18$)
Affective Go/No-Go	Mean Correct Latency Positive ($\chi^2 = 0.53, P = 0.47$) Mean Correct Latency Negative ($\chi^2 = 2.20, P = 0.14$) Total Commissions ($\chi^2 = 2.79, P = 0.10$) Total Omissions ($\chi^2 = 0.06, P = 0.80$)

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CANTAB Subtest	Result
Verbal Recognition Memory	Free Recall Immediate ($\chi^2 = 172, P = 0.19$)
	Recognition Correct Immediate ($\chi^2 = 0.66, P = 0.42$)
	Recognition Correct Delayed ($\chi^2 = 1.24, P = 0.26$)

Predicting CANTAB scores from the group $\times$ time interaction was not predictive using a P value of <0.05 .

TABLE 4.

Adverse Events (2 Occurrences)

	Placebo (n = 19)	L-Methylfolate (n = 22)
	n (%)	n (%)
Headache	3 (16)	10 (45)
Musculoskeletal	2 (11)	6 (27)
Mucosal dryness	6 (32)	5 (23)
Nausea/vomit/diarrhea	4 (21)	5 (23)
Insomnia	5 (26)	5 (23)
Cold/infection/allergies	3 (16)	4 (18)
Decreased appetite	8 (42)	3 (14)
Sedation	0 (0)	2 (9)
Tense/jittery	0 (0)	2 (9)
Anxious/worried	2 (11)	1 (5)
Neurological	1 (5)	1 (5)
Agitated/irritable	0 (0)	1 (5)
Sad/down	0 (0)	1 (5)
Cardiovascular	3 (16)	0 (0)
Autonomic: drool/sweat	1 (5)	0 (0)

Instances of adverse events broken down by placebo (n = 19) and L-methylfolate (n = 22).

TABLE 5.
Predicting Study Outcome Measures by Interaction of Drug Group and Presence of IgM and/or IgG Folate Receptor Antibodies

	Placebo (n = 19)	L-Methylfolate (n = 20)	Results		
			AISRS	BRIEF-A GEC	Dosing
Both Negative	8	7	$\chi^2 = 0.04, P = 0.84$	$\chi^2 = 0.04, P = 0.85$	$\chi^2 = 1.45, P = 0.23$
1 Positive	11	13			

The presence of IgM and/or IgG folate receptor antibodies did not have a significant impact on predicting study outcome measures on either placebo and L-methylfolate groups as determined by the AISRS, BRIEF-A GEC, and dosing.
GEC indicates Global Executive Composite.

TABLE 6.

Cardiovascular Changes

	Placebo (n = 19)			L-methylfolate (n = 21) *			Test Statistic [†]	P [‡]
	Baseline	Endpoint	Change	Baseline	Endpoint	Change		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
Heart rate	72.53 ± 10.79	86.63 ± 16.08	14.11 ± 12.66	70.10 ± 9.24	81.81 ± 13.89	11.71 ± 12.28	$\chi^2 = 0.38$	0.54
Systolic blood pressure	118.63 ± 13.40	122.79 ± 9.08	4.16 ± 14.09	118.76 ± 11.93	119.71 ± 11.01	0.95 ± 7.64	$\chi^2 = 0.80$	0.37
Diastolic blood pressure	76.32 ± 9.91	79.58 ± 7.54	3.26 ± 6.89	73.71 ± 9.58	77.76 ± 9.69	4.05 ± 7.00	$\chi^2 = 0.13$	0.72

Blood pressure was measured in millimeters of mercury, and heart rate was measured in beats per minute.

* One subject did not have valid vitals because of white coat hypertension and was excluded.

[†] Mixed effects linear regression was used to predict cardiovascular outcomes from drug group, time (baseline vs endpoint), and the drug group by time interaction. The test statistic and P-value reflect the drug by time interaction.