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

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ABSTRACT

Objective: Specific genetic or biological markers may predict inadequate response to therapy for major depressive disorder (MDD). The objective of the current post hoc analysis was to evaluate the effect of specific biological and genetic markers on the antidepressant efficacy of adjunctive L-methylfolate 15 mg versus placebo from a trial of inadequate responders to selective serotonin reuptake inhibitors (SSRIs).

Method: The double-blind, randomized, placebo-controlled trial used the sequential parallel comparison design. Outpatients with SSRI-resistant MDD (*DSM-IV* criteria) received L-methylfolate 15 mg/d for 60 days, placebo for 30 days followed by L-methylfolate 15 mg/d for 30 days, or placebo for 60 days. The effects of baseline levels of select biological and genetic markers individually and combined on treatment response to L-methylfolate versus placebo were evaluated; the primary response measure was the 28-Item Hamilton Depression Rating Scale (HDRS-28). The first patient was enrolled July 14, 2009, and the last patient completed April 28, 2011.

Results: Seventy-five patients were enrolled. Patients with specific biological (body mass index ≥ 30 kg/m^2, elevated plasma levels of high-sensitivity C-reactive protein or 4-hydroxy-2-nonenal, low *S*-adenosylmethionine/*S*-adenosylhomocysteine ratio) and genetic markers at baseline had significantly ($P \leq .05$) greater pooled mean change from baseline on the HDRS-28 with L-methylfolate versus placebo. Pooled mean change from baseline on the Clinical Global Impressions-Severity of Illness scale was significantly ($P < .05$) greater with L-methylfolate versus placebo for most genetic markers. Most combinations of baseline biological and genetic markers predicted significantly ($P \leq .05$) greater reductions in pooled mean change from baseline in HDRS-28 scores with L-methylfolate versus placebo.

Conclusions: Biomarkers associated with inflammation or metabolism and genomic markers associated with L-methylfolate synthesis and metabolism may identify patients with SSRI-resistant depression who are responsive to adjunctive therapy with L-methylfolate 15 mg. Confirmatory studies are needed.

Trial Registration: ClinicalTrials.gov identifier: NCT00955955

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Despite the availability of numerous antidepressant drugs, over 60% of patients with major depressive disorder (MDD) fail to experience a complete remission of symptoms following their first antidepressant treatment, and the majority of those who do remit experience relapse or recurrence.[1] An opportunity exists to develop more efficacious treatment strategies with improved success rates for specific patients identified with the use of clinical or biological markers.[2]

Disturbances in metabolic systems have been implicated in the pathophysiology and course of MDD.[3–5] For instance, a prospective cohort study found that participants with depression and comorbid metabolic syndrome had a higher risk of developing chronic, recurrent depression.[4] In parallel, in recent years, an association has also been recognized between MDD and altered cellular immunity and inflammation, characterized by elevated interleukin-6, tumor necrosis factor α, and high-sensitivity C-reactive protein (hsCRP) levels.[5–8] Furthermore, activation of inflammatory pathways within the brain also may contribute to oxidative stress leading to the neuropathologic characteristics of MDD.[7,9,10] In a prospective study of patients hospitalized for cardiac intervention, use of statins, which have anti-inflammatory and antioxidative properties, was associated with a significant reduction in the risk of MDD at 9 months.[9,10]

An association has been observed between folate deficiency, metabolic dysregulation, and inflammation.[4,6–8] The benefits of folic acid and its biologically active form, L-methylfolate, for treating MDD have been recognized; also recently recognized are links between folate deficiency and an increased risk for MDD, reduced antidepressant effectiveness, and a more chronic course of illness.[4,11–14] More recently, our group published the results from a randomized, placebo-controlled trial in MDD patients not achieving an adequate response to selective serotonin reuptake inhibitors (SSRIs), which demonstrated greater efficacy for adjunctive treatment with 15 mg daily of L-methylfolate versus placebo using the sequential parallel comparison design (SPCD).[15]

In light of these findings, we surmised that it would be interesting to examine the treatment effect of 15 mg of L-methylfolate versus placebo as a function of baseline biomarker levels or genotype, focusing on markers of metabolic or inflammatory status. Specifically, in light of the relationship between hypofolatemia and metabolic disturbances as well as inflammation, our hypothesis was that there would be a potential interaction between metabolic or inflammatory status at baseline as defined using specific markers from these domains and treatment outcome with 15 mg daily of L-methylfolate versus placebo augmentation. In addition, a potential interaction is hypothesized in light of the role of L-methylfolate in enhancing tetrahydrobiopterin (BH_4)-dependent monoamine synthesis.[16] Significant correlations have been observed between MDD and levels of red cell folate, monoamine neurotransmitters, and cerebrospinal fluid BH_4. Furthermore, BH_4 regulates the presynaptic release of neurotransmitters from nerve terminals.[12,13,16] Finally, given that L-methylfolate is an intermediary in the one-carbon cycle, we expanded our analyses to test for the influence of markers associated with one-carbon cycle metabolism and treatment outcome.

METHOD

This report presents results from exploratory, post hoc analyses from a multicenter, 60-day, randomized, double-blind trial of L-methylfolate 15 mg as adjunctive therapy for patients with SSRI-resistant MDD.[15] The study was divided into two 30-day phases (phases 1 and 2) according to the SPCD of Fava et al[17] in which patients are randomized to drug or placebo during phase 1, and nonresponders to placebo are re-randomized to drug or placebo in phase 2. The study design and results were described previously[15] but are summarized briefly here. The study protocol was reviewed and approved by the following institutional review boards (IRBs): Partners Human Research Office, Massachusetts General Hospital; Research and Clinical Trials Administration Office, Rush University Medical Center; Goodwyn IRB; Human Research Protections Program, University of California, San Diego; Institutional Review Board Office, University of Cincinnati Medical Center; Institutional Review Board, Vanderbilt University; and Office of Regulatory Affairs, University of Pennsylvania. Written informed consent was obtained from all study patients before any study procedures were conducted. This study was registered at ClinicalTrials.gov (identifier: NCT00955955). The first patient was enrolled July 14, 2009, and the last patient completed April 28, 2011.

Patient Selection

Adults aged 18–65 years and meeting *DSM-IV* criteria for a current episode of MDD were eligible if they had a Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR)[18] score ≥ 12 at screening and baseline visits. Patients must have been treated with an SSRI during the current episode of MDD for ≥ 8 weeks at adequate doses (defined as 20 mg/d or more of fluoxetine, citalopram, or paroxetine; 10 mg/d or more of escitalopram; or 50 mg/d or more of sertraline) as assessed using the Massachusetts General Hospital Antidepressant Treatment Response Questionnaire.[19] Patients also must have been on a stable SSRI dose for the past 4 weeks. Patients were excluded if they had failed more than 2 adequate antidepressant trials during the current episode. Patients who demonstrated ≥ 25% decrease in depressive symptoms on the QIDS-SR total score from screening to baseline were excluded.

Clinical Points

- L-Methylfolate may provide an option for adjunctive treatment of patients with major depressive disorder not adequately responding to antidepressants.
- Patients identified by the presence of certain biological or genetic markers may experience a more robust response to L-methylfolate.

Study Procedures

Eligibility was assessed during the screening and baseline visits, which occurred within 14 days of each other. Patients eligible during the baseline visit were enrolled in the study using the SPCD previously described.[17] Patients were randomized to 1 of 3 treatment groups receiving placebo-placebo, placebo–L-methylfolate 15 mg/d, or L-methylfolate–L-methylfolate 15 mg/d during phases 1 and 2 using a randomization code generated by the primary study center. Each phase was 30 days in duration. Study visits occurred every 10 days, during which the concomitant SSRI doses remained constant, and patients unable to tolerate the study medications were withdrawn from the study. Patients and investigators were blinded to study assignment.

Patients were assessed at each study visit with the 28-Item Hamilton Depression Rating Scale (HDRS-28).[20] In addition, symptom response was evaluated with the HDRS-7,[21] the Cognitive and Physical Functioning Questionnaire (CPFQ),[22] and the Clinical Global Impressions-Severity of Illness scale (CGI-S).[23] Height and weight were measured, and body mass index (BMI) was calculated in kg/m^2. Baseline blood samples were collected to assess baseline levels of plasma hsCRP (within-/between-day coefficient of variation [CV]%: 5.7, 6.9), 4-hydroxy-2-nonenal [4-HNE] (within-/between-day CV%: 4.0, 7.5), and low *S*-adenosylmethionine (SAM)/*S*-adenosylhomocysteine (SAH) ratio (within-/between-day CV%: 6.6, 9.2 for SAM; 7.9, 10.4 for SAH). Also assessed were genetic polymorphisms for (1) the C677T, 1298C, and G1793A genotypes for methylenetetrahydrofolate reductase (*MTHFR*); (2) the A66G genotype for methionine synthase reductase (*MTRR*); and (3) the A2756G genotype for methionine synthase (*MTR*). For additional analyses, baseline samples were assessed for genetic polymorphisms for calcium channel, voltage-dependent, L type, α 1C subunit (*CACNA1C*); catechol-*O*-methyltransferase (*COMT*); DNA (cytosine-5-)-methyltransferase 3 β (*DNMT3B*); dopamine receptor D_2 (*DRD2*); folate hydrolase 1 (*FOLH1*); GTP cyclohydrolase 1 (*GCH1*); GTP cyclohydrolase 1 feedback regulatory protein (*GCHFR*); and solute carrier family 19 (folate transporter), member 1 (also known as *SLC19A1* or reduced folate carrier [*RFC1*]) (Table 1).

Table 1. List of Genetic Markers Examined in Exploratory Analyses

Genetic Variant	RS Number	Genotype Comparisons
CACNA1C	rs1006737	GG vs AG/AA
COMT Val158	rs4633	TT vs CC
COMT Val158Met	rs4680	AA vs GG
DNMT3B	rs1883729	GG vs AG/AA and GG vs AA
DRD2	rs1079596	CC vs TC/TT
DRD2 129	rs6275	CC vs TT
FOLH1	rs202676	AA vs AG/GG
GCH1	rs8007267	CC vs TC/TT
GCHFR	rs7163862	AA vs TA/TT
MTHFR 677	rs1801133	CC vs CT/TT
MTHFR 1298	rs1801131	AA vs AC/CC
MTHFR 1793	rs2274976	AA vs GA
MTR 2756	rs1805087	AA vs AG/GG
MTRR 66	rs1801394	AA vs AG/GG
RFC1 80	rs1051266	GG vs AA
RFC1	rs2297291	GG vs AA
RFC1 815	rs12659	CC vs TT

Table 2. Effect of L-Methylfolate 15 mg/d vs Placebo on Pooled Mean Change From Baseline for HDRS-28 Stratified by Baseline Level of Plasma Marker

Variable	n	Pooled[a] Mean Change vs Placebo	95% Confidence Interval	*P* Value	Pooled[a] Effect Size[b]
SAM/SAH ratio					
≥ 2.71	36	0.07	−3.33 to 3.48	.966	0.01
< 2.71	37	−4.57	−7.73 to −1.41	.005	−0.75
hsCRP					
≥ 2.25 mg/L	37	−3.61	−7.23 to 0.002	.050	−0.50
< 2.25 mg/L	36	−2.29	−5.47 to 0.89	.158	−0.36
4-HNE					
≥ 3.28 μg/mL	37	−4.55	−7.61 to −1.50	.003	−0.74
< 3.28 μg/mL	36	−0.11	−3.67 to 3.46	.953	0.01

[a]Pooled across study phases with equal weights.
[b]A negative pooled effect size indicates that the treatment effect favored the L-methylfolate group.
Abbreviations: 4-HNE = 4-hydroxy-2-nonenal, HDRS-28 = 28-Item Hamilton Depression Rating Scale, hsCRP = high-sensitivity C-reactive protein, SAH = *S*-adenosylhomocysteine, SAM = *S*-adenosylmethionine.

Assay Methods

Serum hsCRP was measured by a commercially available kit latex particle–enhanced immunoturbidimetric assay (Pointe Scientific, Inc; Canton, Michigan). The turbidity (absorbance) was read on an ACE Alera clinical chemistry analyzer (Alfa Wassermann, West Caldwell, New Jersey). Plasma 4-HNE was measured by analysis of the amount of HNE-His protein adducts present in the sample using an enzyme immunoassay (OxiSelect HNE-His adduct ELISA kit; Cell Biolabs, Inc; San Diego, California). Plasma SAM and SAH were determined by stable-isotope dilution liquid chromatography–electrospray ionization tandem mass spectrometry as previously described.[24] Determination of the presence of genetic polymorphisms was performed on DNA purified from whole blood using a DNeasy blood and tissue kit (Qiagen Inc, Valencia, California). Genotyping was conducted using the MassArray platform (Sequenom, Inc; San Diego, California).

Statistical Analyses

For exploratory analyses, the pooled treatment effect was assessed by average differences in mean changes from baseline to endpoint for L-methylfolate and placebo groups, pooled across the 2 phases of the study, consistent with the SPCD of Fava et al.[17] The primary outcome measure was the effect of biomarkers on the response on the HDRS-28 with L-methylfolate compared to placebo, which was stratified by BMI (≥ 30 or < 30 kg/m^2), hsCRP level (median baseline value ≥ 2.25 or < 2.25 mg/L), SAM/SAH ratio (median baseline value ≥ 2.71 or < 2.71), and 4-HNE level (median baseline level ≥ 3.28 or < 3.28 μg/mL). Further, the presence of molecular polymorphisms of genotypes was measured. Elevated BMI, low ratio of SAM/SAH, elevated plasma levels of hsCRP and 4-HNE, and molecular polymorphisms were evaluated as predictors of a greater pooled (phases 1 and 2 according to SPCD) drug/placebo difference.

A standard SPCD analysis approach was employed to analyze the study efficacy data. Specifically, an intent-to-treat/last-observation-carried-forward (ITT/LOCF) approach was employed for patients treated with L-methylfolate during phase 1. The phase 2 dataset of interest was limited to patients treated with placebo during phase 1 who completed phase 1, did not experience a clinical response on the HDRS during phase 1, and entered phase 2. The LOCF approach was applied to the dataset for phase 2, with the final visit of phase 1/first visit of phase 2 serving as the new baseline visit. The ITT/LOCF data comparing L-methylfolate and placebo during phase 1 were combined with the data comparing L-methylfolate and placebo in phase 2 according to the model for SPCD and were analyzed using the general approach outlined in Fava et al[17] using a weight (w = 0.50) and a randomization fraction (a = 0.333).

Dichotomous measures were analyzed according to the method for dichotomous outcomes,[17] while seemingly unrelated regression analysis, controlling for baseline scores, was employed for the comparison of continuous outcomes.[25] All tests were conducted as 2-tailed, with α set at .05. Pooled mean changes from baseline to endpoint for L-methylfolate versus placebo on the HDRS-28 were stratified for each biomarker and genetic marker. Treatment effect, effect size (difference between means divided by a standard deviation), and 95% confidence intervals (CIs) were calculated for each biomarker. In addition, within-group analyses, HDRS-28 response rate (at least 50% reduction from baseline), odds ratio, and number needed to treat were determined. Within-group analyses were conducted separately for individuals who received L-methylfolate (in phase 1 or as placebo nonresponders in phase 2) or placebo (in phase 1 or as placebo nonresponders in phase 2) with the biomarker or genetic marker status as exposure. Because individuals were not randomized on the basis of their biomarker status, the within-group analyses adjusted for potential confounders including age, sex, race, and BMI as well as baseline level of HDRS-28. Adjustment was made using linear regression for continuous HDRS-28 scores and through propensity score–stratified analysis for binary outcomes (to decrease the number of predictors in the final model).

RESULTS

Overall, 74 patients provided data, and 61 (81.3%) completed the study. Detailed results from the primary analysis of the study (efficacy, safety, tolerability of L-methylfolate 15 mg versus placebo) have been published elsewhere.[15] For all analyses, results from both phase 1 and phase 2 of the study were pooled according to the SPCD method.[17] Pooled (phases 1 and 2) mean change from baseline was significantly greater with adjunctive L-methylfolate 15 mg/d than placebo for HDRS-28 (−6.8 ± 7.2 vs −3.7 ± 6.5, $P = .017$).

Pooled mean changes on the HDRS-28 with L-methylfolate versus placebo were examined among subgroups of patients identified by the presence or absence of various biomarkers or their combinations. Pooled mean changes from baseline on the HDRS-28 for L-methylfolate versus placebo were significantly ($P \leq .05$) greater among subgroups of patients with a plasma SAM/SAH ratio below the study median value, hsCRP or 4-HNE blood levels above the study median value, or a BMI ≥ 30 kg/m^2 (consistent with obesity) (Table 2).

Exploratory analyses demonstrated significant ($P \leq .05$) differences for pooled mean change from baseline on the HDRS-28 for L-methylfolate versus placebo based on the presence of most genetic markers at baseline (Table 3). Pooled mean change from baseline on the HDRS-28 with L-methylfolate versus placebo was significantly ($P < .05$) greater among subgroups of patients with the *MTR* 2756 AG/GG or *MTRR* 66 AG/GG genotype but not significantly greater for the *MTHFR* 677 CT/TT or *MTHFR* 1298 AC/CC genotypes compared to the respective homozygous dominant genotypes (Table 3). For the HDRS-28, the pooled effect size ranged from −0.05 to −1.57 for significant mean changes from baseline across all genotypes. Similarly, HDRS-28 response rate (treatment minus placebo) was significantly ($P < .05$) improved with L-methylfolate versus placebo when stratified for baseline presence of most genetic markers. A comparison of the presence of normal and putative positive markers at baseline demonstrated marked differences in the HDRS-28 response rate, with significant ($P < .05$) differences noted for most markers except *MTHFR* 677CT/TT, *FOLH1* AG/GG, and *GCHFR* TA/TT (Figure 1).

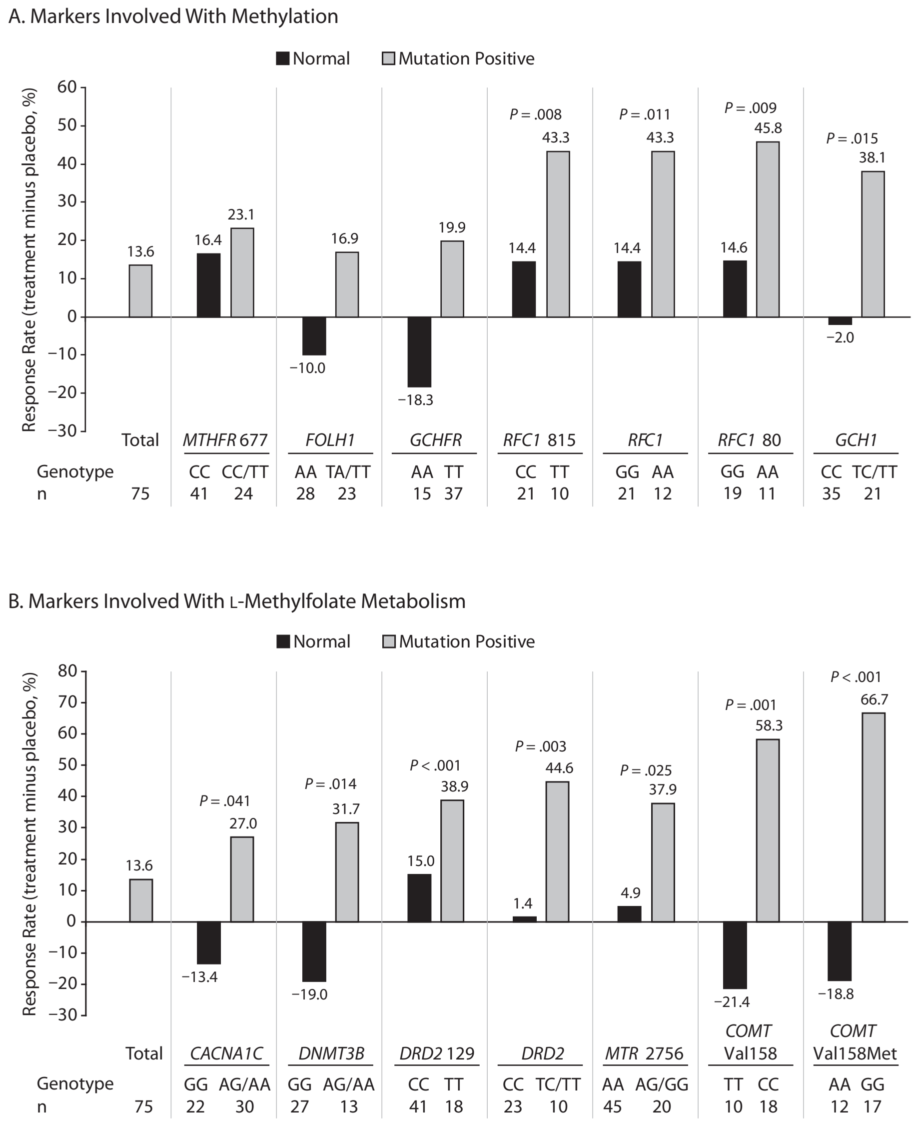
Further exploratory analyses were conducted to determine the effect of L-methylfolate versus

Table 3. Analysis of the Effect of L-Methylfolate Stratified by Baseline Levels of Individual Markers (n = 59)

Variable	n (%)	Pooled[a] Mean Change	Pooled[a] Effect Size	Within–Treatment Group Mean Change[b]	Within–Placebo Group Mean Change[b]	Response Rate, Treatment Minus Placebo (%)[a]	Within–Treatment Group Difference[c]	Pooled Difference Treatment Group[d]	Odds Ratio	Number Needed to Treat
COMT GG (rs4680)	17 (29)	−10.9 $P < .001$	−1.57	−5.70 $P = .079$	6.52 $P = .007$	66.7 $P < .001$	11.219 $P = .084$	0.32 $P = .045$	NA	1
COMT CC (rs4633)	18 (31)	−9.2 $P < .001$	−1.40	−4.45 $P = .162$	6.52 $P = .007$	58.3 $P = .001$	11.219 $P = .084$	0.248 $P = .168$	NA	2
MTR 2756 AG/GG[e] (rs1805087)	20 (31)	−8.2 $P < .001$	−1.15	−6.95 $P = .008$	1.96 $P = .397$	37.9 $P = .025$	30.97 $P = .025$	0.298 $P = .037$	6.24	3
RFC1 (80) AA (rs1051266)	11 (19)	−7.7 $P = .003$	−1.38	−3.58 $P = .29$	−1.13 $P = .73$	45.8 $P = .009$	6.462 $P = .241$	0.146 $P = .314$	NA	2
DRD2 129 TT[f] (rs6275)	10 (17)	−7.6 $P = .028$	−0.70	−0.65 $P = .916$	1.44 $P = .652$	38.9 $P < .001$	0.000 $P = 1.000$	0.09 $P = .693$	8.00	3
DRD2 TC/TT (rs1079596)	18 (31)	−6.9 $P = .002$	−0.98	0.28 $P = .923$	4.63 $P = .047$	44.6 $P = .003$	2.005 $P = .52$	0.126 $P = .413$	13.96	2
RFC1 AA (rs2297291)	12 (20)	−5.5 $P = .02$	−0.96	−2.80 $P = .385$	−0.91 $P = .77$	43.3 $P = .01$	3.106 $P = .416$	0.058 $P = .708$	NA	2
DNMT3B AG/AA (rs1883729)	32 (54)	−5.4 $P < .001$	−0.84	−1.70 $P = .573$	7.93 $P < .001$	31.7 $P = .014$	5.977 $P = .156$	0.113 $P = .481$	13.33	3
GCH1 TC/TT (rs8007267)	24 (41)	−5.1 $P = .01$	−0.78	−4.37 $P = .157$	1.54 $P = .550$	38.1 $P = .015$	119.10 $P = .035$	0.268 $P = .075$	9.53	3
RFC1 815 TT (rs12659)	10 (17)	−4.9 $P = .036$	−0.83	−2.80 $P = .385$	−0.91 $P = .77$	43.3 $P = .008$	3.106 $P = .416$	NA	NA	2
BMI ≥ 30 (kg/m^2)[g]	40 (56)	−4.7 $P = .001$	−0.75	−3.53 $P = .166$	6.31 $P = .003$	30.2 $P = .009$	10.76 $P = .067$	0.22 $P = .117$	7.11	3
CACNA1C AG/AA (rs1006737)	37 (63)	−4.2 $P = .005$	−0.64	−4.73 $P = .095$	1.52 $P = .510$	27.0 $P = .041$	> 1,000 $P = .997$	0.193 $P = .156$	4.08	4
MTHFR 677 CT/TT[e] (rs1801133)	24 (37)	−3.8 $P = .087$	−0.53	−0.67 $P = .822$	5.29 $P = .035$	23.1 $P = .114$	0.36 $P = .402$	−0.06 $P = .728$	3.99	4
FOLH1 AG/GG (rs202676)	30 (51)	−3.2 $P = .076$	−0.40	−2.99 $P = .497$	0.83 $P = .850$	16.9 $P = .119$	> 1,000 $P = .997$	−0.042 $P = .828$	2.46	6
GCHFR TA/TT (rs7163862)	43 (73)	−3.1 $P = .037$	−0.48	−5.62 $P = .225$	0.54 $P = .849$	19.9 $P = .078$	> 1,000 $P = .997$	0 $P = 1.000$	2.92	5
MTRR 66 AG/GG[e] (rs1801394)	49 (75)	−2.9 $P = .041$	−0.45	1.27 $P = .694$	-4.12 $P = .117$	22.6 $P = .041$	1.47 $P = .782$	0.001 $P = .994$	3.38	4
MTHFR 1298 AC/CC[e] (rs1801131)	30 (46)	−0.5 $P = .807$	−0.05	−0.08 $P = .981$	−5.58 $P = .009$	19.1 $P = .19$	2.069 $P = .561$	0.123 $P = .457$	2.47	5

[a]Change from baseline on 28-Item Hamilton Depression Rating Scale; treatment minus placebo. [b]Adjusted for baseline HDRS-28 score, race, age, and BMI. [c]Adjusted (age, sex, race, baseline HDRS-28 score, and BMI) odds ratio of responder status (within those treated) between those with the presence of the genetic variant listed versus those who were "wild-type." [d]Adjusted (age, sex, race, baseline HDRS-28 score, and BMI) difference in responder status (within those treated) between those with the presence of the genetic variant listed versus those who were "wild-type." [e]n = 65 (10 patients did not consent to genetic testing). [f]n = 58 (single-nucleotide polymorphism results were unreadable in 1 patient); for n = 59, samples for genetic testing were depleted. [g]n = 72 (3 patients were missing height value for calculating BMI).
Abbreviations: BMI = body mass index, HDRS-28 = 28-Item Hamilton Depression Rating Scale, NA = not available.

Figure 1. HDRS-28 Response Rate (treatment minus placebo) With L-Methylfolate Stratified by Markers Involved With Methylation (A) and Markers Involved With L-Methylfolate Metabolism (B) That Were Normal and Mutation Positive



Abbreviation: HDRS-28 = 28-Item Hamilton Depression Rating Scale.

placebo stratified by baseline levels of individual markers when response was assessed using HDRS-7, CGI-S, and CPFQ (Table 4). Significant ($P < .05$) improvements were noted for pooled mean change from baseline with L-methylfolate versus placebo for all genetic markers except *MTHFR* 1298 AC/CC on the CGI-S and for many markers on the HDRS-7 and CPFQ.

The effect on the pooled mean change from baseline on the HDRS-28 with L-methylfolate versus placebo in patients with combinations of biological and genetic markers present at baseline was also examined. Combinations of markers demonstrated pooled mean change from baseline for L-methylfolate versus placebo that ranged from −3.6 to −23.3 and pooled effect sizes that ranged from −0.56 to −4.50 (Table 5). Combinations of *MTHFR* 677 CT/TT + *MTR* 2756 AG/GG, *GCH1* TC/TT + *COMT* GG, and *GCH1* TC/TT + *COMT* CC demonstrated the largest pooled mean change (−23.3, −20.7, and −18.2, respectively) for L-methylfolate versus placebo; these values were highly significant ($P < .001$).

DISCUSSION

Results from the primary analyses demonstrated significant differential efficacy with L-methylfolate 15 mg versus placebo as adjunctive therapy among patients with an inadequate response to SSRIs.[15] The overall effect size on the HDRS with L-methylfolate (0.41) was similar to the effect sizes (0.35 to 0.37) observed in other studies of adjunctive therapy in MDD.[26,27] The results from these exploratory analyses revealed a greater differential treatment effect with L-methylfolate versus placebo among patients stratified by the presence of baseline level biological and genetic biomarkers (moderators of outcome) that were associated with metabolic

Table 4. Analysis of the Effect of L-Methylfolate on HDRS-7, CGI-S, and CPFQ Stratified by Baseline Levels of Individual Genetic Markers (n = 59)

Variable	n (%)	Pooled[a] Mean Change HDRS-7	Pooled[a] Mean Change CGI-S	Pooled[a] Mean Change CPFQ
Total	75 (100)	−1.49 $P = .018$	−0.52 $P = .002$	−1.51 $P = .068$
BMI ≥ 30 (kg/m²)[b]	40 (56)	−2.52 $P = .002$	0.23 $P < .001$	−3.04 $P = .032$
CACNA1C AG/AA	37 (63)	−2.43 $P = .009$	−0.83 $P = .001$	−1.92 $P = .177$
COMT (Val158Met) GG	17 (29)	−5.17 $P = .01$	−1.71 $P < .001$	−7.99 $P = .004$
COMT (Val158) CC	18 (31)	−5.06 $P = .009$	−1.53 $P < .001$	−7.36 $P = .008$
DNMT3B AG/AA	32 (54)	−2.64 $P = .003$	−0.82 $P = .001$	−2.53 $P = .079$
DRD2 TC/TT	18 (31)	−3.60 $P = .007$	−0.90 $P = .013$	0.43 $P = .825$
DRD2 129 TT[c]	10 (17)	−1.31 $P = .489$	NA	−5.35 $P = .387$
FOLH1 AG/GG	30 (51)	−2.26 $P = .017$	−0.70 $P = .013$	−2.96 $P = .019$
GCH1 TC/TT	24 (41)	−2.96 $P = .004$	−0.74 $P = .021$	−3.22 $P = .007$
GCHFR TA/TT	43 (73)	−1.75 $P = .038$	−0.61 $P = .003$	−0.56 $P = .631$
MTHFR 677 CT/TT[d]	24 (37)	−1.46 $P = .176$	−0.77 $P = .024$	−2.21 $P = .02$
MTHFR 1298 AC/CC[d]	30 (46)	−0.24 $P = .819$	−0.49 $P = .055$	−0.08 $P = .960$
MTR 2756 AG/GG[d]	20 (31)	−4.56 $P < .001$	−0.99 $P = .003$	−3.01 $P = .05$
MTRR 66 AG/GG[d]	49 (75)	−1.62 $P = .052$	−0.62 $P = .004$	−2.85 $P = .026$
RFC1 AA	12 (21)	−2.91 $P = .036$	−1.07 $P = .021$	−3.82 $P = .096$
RFC1 80 AA	11 (19)	−3.65 $P = .031$	−1.31 $P = .006$	−3.79 $P = .147$
RFC1 815 TT	10 (17)	−2.62 $P = .053$	−1.05 $P = .015$	−1.18 $P = .522$

[a]Treatment minus placebo. [b]n = 72 (3 patients were missing height value for calculating BMI). [c]n = 58 (SNP results were unreadable in 1 patient). [d]n = 65 (10 patients did not consent to genetic testing).
Abbreviations: BMI = body mass index, CGI-S=Clinical Global Impressions-Severity of Illness scale, CPFQ = Cognitive and Physical Functioning Questionnaire, HDRS-7 = 7-Item Hamilton Depression Rating Scale, NA = not available.

dysfunction, inflammation, or variants of L-methylfolate metabolism (eg, BMI, hsCRP, *MTRR, MTR*).

The exploratory analyses reported in this article reveal interesting associations between the presence of select biomarkers at baseline and the response to L-methylfolate. It is encouraging that the treatment effect and effect size with L-methylfolate 15 mg versus placebo when stratified by the presence of specific biological plasma or genetic markers appear larger than those reported from conventional antidepressant-placebo trials.[28,29] Combinations of markers demonstrated an even greater treatment effect with L-methylfolate 15 mg, with effect sizes exceeding 1.0 in most comparisons.

Several biomarkers have been identified that are associated with an increased risk or severity of MDD. Increased body weight and obesity are positively associated with an increased risk of MDD and a poorer response to antidepressant treatment.[30–33] Genetic markers related to folate metabolism have been investigated for their association with MDD.[34–37] The results from these analyses provide further support for the benefits of L-methylfolate as adjunctive treatment for patients not responding adequately to SSRIs and suggest additional avenues for identifying those individuals most likely to respond to this treatment. These results could lead to an opportunity for individualizing treatment approaches for depressed patients unresponsive to initial antidepressant therapy.

The HDRS is widely used as the standard for assessing drug response in clinical trials of MDD. However, the HDRS has been criticized because it is multidimensional, lacks sensitivity to detect clinical change, and lacks discriminative power to define remission.[38] For these analyses, the HDRS-28 rather than the HDRS-17 item score was used to compare symptom improvement with L-methylfolate versus placebo because the longer version is more sensitive to changes in patients with symptoms of atypical or melancholic depression.[39,40] Additionally, the HDRS-7 and CPFQ were used in the exploratory analyses because the HDRS-7 may be more sensitive to change in clinical trials of depression,[21] and the CPFQ has been found to measure cognitive and physical symptoms of depression, which are predictive of residual symptoms.[22]

The limitations of this study include a relatively small sample size, particularly when the study population was segmented by biomarker level or genotype. Further, the biomarker analysis was conducted across mean values and was not associated with a specific baseline value. Lastly, this was a short-term study of only a 30-day duration, and the effects of biomarkers on long-term treatment response remain unknown. Nevertheless, the results demonstrate a robust association of biomarkers with antidepressant response with L-methylfolate despite the short treatment period.

In conclusion, greater efficacy was observed with L-methylfolate when used as an adjunct to SSRI treatment in inadequate responders. Our present analyses suggest that the relative superiority of L-methylfolate versus placebo with respect to efficacy may be further enhanced among subsets of patients stratified by the presence of metabolic and genetic markers related to inflammation and disturbance of

Table 5. Analysis of the Effect of L-Methylfolate 15 mg/d on Pooled Mean Change From Baseline vs Placebo for HDRS-28 Stratified by Combinations of Biomarker Level Status and Genotype (n = 59)

Variable	n (%)	Pooled[a] Mean Change vs Placebo	95% CI	Pooled[a] Effect Size	Response Rate, Treatment Minus Placebo	Number Needed to Treat
MTHFR 677 CT/TT + *MTR* 2756 AG/GG	8 (14)	−23.3 *P* < .001	−32.09 to −14.50	−2.51	66.7% *P* = .002	1
GCH1 TC/TT + *COMT* (rs4680) GG	11 (19)	−20.7 *P* < .001	−29.99 to −11.33	NA	75.0% *P* < .001	1
GCH1 TC/TT + *COMT* (rs4633) CC	12 (20)	−18.2 *P* < .001	−24.70 to −11.78	NA	66.7% *P* < .001	1
CACNA1C AG/AA + *COMT* (rs4680) GG	13 (22)	−16.2 *P* < .001	−24.70 to −7.77	−2.93	83.3% *P* < .001	1
MTR 2756 AG/GG + *COMT* (rs4633) CC	7 (12)	−15.1 *P* = .001	−24.17 to −6.04	−4.50	100% *P* < .001	1
BMI ≥ 30 kg/m² + *MTR* 2756 AG/GG	10 (17)	−14.4 *P* < .001	−19.45 to −9.41	−2.83	92.9% *P* < .001	1
CACNA1C AG/AA + *MTR* 2756 AG/GG	13 (22)	−13.5 *P* < .001	−17.13 to −9.91	−2.90	83.8% *P* < .001	1
DNMT3B AG/AA + *COMT* (rs4680) GG	15 (25)	−13.1 *P* < .001	−18.49 to −7.67	−2.55	100% *P* < .001	1
CACNA1C AG/AA + *COMT* (rs4633) CC	14 (24)	−13.0 *P* < .001	−19.27 to −6.65	−2.67	75.0% *P* < .001	1
BMI ≥ 30 kg/m² + *GCH1* TC/TT	16 (27)	−12.4 *P* < .001	−16.95 to −7.78	−2.62	70.0% *P* < .001	1
DNMT3B AG/AA + *MTR* 2756 AG/GG	13 (22)	−12.0 *P* < .001	−14.93 to −9.08	−2.38	70.8% *P* < .001	1
GCHFR TA/TT + *MTR* 2756 AG/GG	12 (20)	−12.0 *P* < .001	−16.61 to −7.48	−2.25	65.0% *P* < .001	2
FOLH1 AG/GG + *COMT* (rs4680) GG	15 (25)	−11.8 *P* = .001	−18.33 to −5.23	−1.63	66.7% *P* < .001	1
BMI ≥ 30 kg/m² + *COMT* (rs4680) GG	14 (24)	−11.8 *P* = .011	−20.94 to −2.65	−1.39	58.3% *P* = .010	2
GCH1 TC/TT + *RFC1* 80 AA	7 (12)	−11.4 *P* = .012	−20.20 to −2.51	−2.02	75.0% *P* < .001	1
DNMT3B AG/AA + *COMT* (rs4633) CC	16 (27)	−10.9 *P* < .001	−15.55 to −6.24	−2.24	83.3% *P* < .001	1
RFC1 AA + *GCH1* TC/TT	7 (12)	−10.5 *P* = .01	−18.51 to −2.56	−2.66	75.0% *P* = .001	1
GCH1 TC/TT + *MTR* 2756 AG/GG	9 (15)	−10.4 *P* = .04	−20.43 to −0.46	−1.36	62.5% *P* = .002	2
CACNA1C AG/AA + *DRD2* TC/TT	10 (17)	−9.9 *P* = .002	−16.32 to −3.55	−1.48	68.8% *P* < .001	1
MTHFR 677 CT/TT + BMI ≥ 30 kg/m²	13 (22)	−9.9 *P* = .001	−15.79 to −3.97	−1.45	50.0% *P* = .003	2
BMI ≥ 30 kg/m² + *DNMT3B* AG/AA	20 (34)	−9.8 *P* < .001	−13.67 to −5.94	−1.98	66.7% *P* < .001	1
FOLH1 AG/GG + *COMT* (rs4633) CC	16 (27)	−9.8 *P* = .001	−15.36 to −4.29	−1.47	58.3% *P* = .001	2
DNMT3B AA + *FOLH1* AG/GG	9 (15)	−9.7 *P* = .001	−15.23 to −4.08	−1.76	50.0% *P* < .001	2
GCHFR TA/TT + *DRD2* TC/TT	15 (25)	−9.7 *P* < .001	−15.03 to −4.40	−1.91	56.0% *P* < .001	2
BMI ≥ 30 kg/m² + *DRD2* TC/TT	10 (17)	−9.6 *P* = .006	−16.40 to −2.81	−1.46	68.8% *P* < .001	1
DRD2 TC/TT + *MTR 2756* AG/GG	8 (14)	−9.5 *P* = .001	−14.95 to −4.14	−2.29	66.7% *P* = .001	1
DNMT3B AA + *MTR* 2756 AG/GG	5 (8)	−9.5 *P* = .019	−17.42 to −1.53	−2.34	50.0% *P* < .001	2
CACNA1C AG/AA + *GCH1* TC/TT	17 (29)	−9.4 *P* < .001	−14.37 to −4.52	−1.48	59.2% *P* = .001	2
MTHFR 677 CT/TT + *CACNA1C* AG/AA	13 (22)	−9.0 *P* < .001	−13.59 to −4.39	−1.15	47.1% *P* = .015	2
CACNA1C AG/AA + *DRD2* 129 TT	8 (14)	−9.0 *P* = .03	−17.07 to −0.89	−1.48	50.0% *P* < .001	2
FOLH1 AG/GG + *MTR* 2756 AG/GG	18 (31)	−8.9 *P* < .001	−13.30 to −4.41	−1.22	45.8% *P* = .010	2
CACNA1C AG/AA + *DNMT3B* AG/AA	21 (36)	−8.8 *P* < .001	−11.94 to −5.73	−1.64	54.6% *P* = .001	2
FOLH1 AG/GG + *MTHFR* 1793 GA	8 (14)	−8.1 *P* = .002	−13.27 to −2.96	−1.68	33.3% *P* = .176	3
FOLH1 AG/GG + *RFC1* 80 AA	9 (15)	−8.1 *P* = .009	−14.18 to −1.99	−1.36	50.0% *P* = .012	2
GCHFR TA/TT + *MTHFR* 1793 GA	8 (14)	−8.1 *P* = .002	−13.27 to −2.96	−1.68	33.3% *P* = .176	3
CACNA1C AG/AA + *MTHFR* 1793 GA	6 (10)	−8.0 *P* = .048	−15.94 to −0.06	−1.32	40.0% *P* = .177	3
FOLH1 AG/GG + *DNMT3B* AG/AA	26 (44)	−7.7 *P* < .001	−10.85 to −4.65	−1.18	42.5% *P* = .004	2
FOLH1 AG/GG + *GCH1* TC/TT	20 (34)	−7.6 *P* < .001	−11.66 to −3.51	−1.32	51.2% *P* = .002	2
GCHFR TA/TT + *COMT* (rs4633) CC	17 (29)	−7.5 *P* = .001	−13.24 to −1.76	−1.16	58.3% *P* = .002	2
FOLH1 AG/GG + *DRD2* TC/TT	17 (29)	−7.2 *P* = .001	−11.53 to −2.82	−1.04	47.9% *P* = .001	2
GCHFR TA/TT + *DNMT3B* AG/AA	21 (36)	−7.2 *P* < .001	−10.58 to −3.92	−1.37	40.4% *P* = .010	2
GCHFR TA/TT + *RFC1* 80 AA	7 (12)	−7.1 *P* = .035	−13.69 to −0.50	−2.63	50.0% *P* = .016	2
BMI ≥ 30 kg/m² + *CACNA1C* AG/AA	26 (44)	−7.1 *P* < .001	−11.02 to −3.13	−1.05	40.2% *P* = .014	2
FOLH1 AG/GG + *RFC1* AA	9 (15)	−6.9 *P* = .012	−12.24 to −1.51	−1.31	50.0% *P* = .020	2
RFC1 815 TT + *FOLH1* AG/GG	8 (14)	−6.2 *P* = .023	−11.46 to −0.84	−1.19	50.0% *P* = .015	2
GCH1 TC/TT + *DNMT3B* AG/AA	18 (31)	−5.8 *P* = .110	−12.86 to 1.31	−0.76	41.2% *P* = .037	2
GCHFR TA/TT + *RFC1* AA	8 (14)	−5.6 *P* = .109	−12.47 to −1.2	−0.85	45.8% *P* = .014	2
GCHFR TA/TT + *RFC1* 815 TT	8 (14)	−5.6 *P* = .109	−12.47 to −1.2	−0.85	45.8% *P* = .014	2
MTHFR 677 CT/TT + *FOLH1* AG/GG	20 (34)	−4.5 *P* = .095	−9.69 to −0.7	−0.58	25.0% *P* = .148	4
BMI≥ 30 kg/m² + *GCHFR* TA/TT	23 (39)	−4.2 *P* = .035	−8.19 to −0.2	−0.74	35.0% *P* = .025	3
GCHFR TA/TT + *DRD2* 129 TT	18 (31)	−3.6 *P* = .117	−8.02 to −0.8	−0.56	22.6% *P* = .219	4

[a]Pooled across study phases with equal weights.
Abbreviations: BMI = body mass index, NA = not available.

folate metabolism. These results suggest that the presence of certain surrogate markers may help identify patients with SSRI-resistant MDD who are particularly responsive to adjunctive therapy with L-methylfolate 15 mg. Prospective, well-controlled, confirmatory trials with an adequate sample size are clearly needed to validate these findings.

Drug names: citalopram (Celexa and others), escitalopram (Lexapro and others), fluoxetine (Prozac and others), paroxetine (Paxil, Pexeva, and others), sertraline (Zoloft and others).

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