ORIGINAL ARTICLE

Randomized Multicenter Investigation of Folate Plus Vitamin B_{12} Supplementation in Schizophrenia

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Importance: More effective treatments are needed for negative symptoms of schizophrenia, which are typically chronic, disabling, and costly. Negative symptoms have previously been associated with reduced blood folate levels, especially among patients with low-functioning variants in genes that regulate folate metabolism, suggesting the potential utility of folate supplementation.

Objectives: To determine whether folic acid plus vitamin B_{12} supplementation reduces negative symptoms of schizophrenia and whether functional variants in folate-related genes influence treatment response.

Design: Parallel-group, randomized, double-blind, placebo-controlled clinical trial of 16 weeks of treatment with 2 mg of folic acid and 400 μg of vitamin B_{12}.

Setting: Three community mental health centers affiliated with academic medical centers in the United States.

Participants: Outpatients with chronic schizophrenia who were psychiatrically stable but displayed persistent symptoms despite antipsychotic treatment. Eligible patients were 18 to 68 years old, were treated with an antipsychotic agent for 6 months or more at a stable dose for 6 weeks or more, and scored 60 or more on the Positive and Negative Syndrome Scale.

Intervention: One hundred forty subjects were randomized to receive daily oral folic acid plus vitamin B_{12} or placebo.

Main Outcome Measures: Change in negative symptoms (Scale for the Assessment of Negative Symptoms [SANS]), as well as positive and total symptoms (Positive and Negative Syndrome Scale).

Results: Folate plus vitamin B_{12} improved negative symptoms significantly compared with placebo (group difference, −0.33 change in SANS score per week; 95% CI, −0.62 to −0.05) when genotype was taken into account but not when genotype was excluded. An interaction of the 484C>T variant of *FOLH1* (rs202676) with treatment was observed ($P=.02$), where only patients homozygous for the 484T allele demonstrated significantly greater benefit with active treatment (−0.59 change in SANS score per week; 95% CI, −0.99 to −0.18). In parallel, we observed an inverse relationship between red blood cell folate concentration at baseline and 484C allele load ($P=.03$), which persisted until 8 weeks of treatment. Change in positive and total symptoms did not differ between treatment groups.

Conclusions: Folate plus vitamin B_{12} supplementation can improve negative symptoms of schizophrenia, but treatment response is influenced by genetic variation in folate absorption. These findings support a personalized medicine approach for the treatment of negative symptoms.

Trial Registration: clinicaltrials.gov Identifier: NCT00611806

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Schizophrenia is a chronic psychiatric illness characterized by positive symptoms (delusions, hallucinations, and disorganization), negative symptoms (apathy, social withdrawal, and loss of emotional expressiveness), and cognitive impairment. Antipsychotic medications control positive symptoms sufficiently to allow most individuals to live safely within the community. However, considerable disability is associated with negative symptoms and cognitive deficits, for which effective treatment is not available.[1]

Current neurodevelopmental models posit a complex and heterogeneous genetic vulnerability to schizophrenia that interacts with environmental factors.[2] One specific and well-studied gene × environment interaction, the interplay of dietary folic acid intake with common genetic variants that

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influence folate metabolism, has potential implications for schizophrenia pathogenesis and treatment.[3] Folate is a B vitamin that provides methyl donors for biosynthetic methylation reactions, including the synthesis of neurotransmitters and DNA, and contributes to gene expression through methylation of DNA and histones.

Folate deficiency has been identified as a risk factor for schizophrenia through epidemiologic, biochemical, and gene association studies. The regional incidence of schizophrenia transiently doubled approximately 2 decades after famines in the Netherlands[4] and China,[5] suggesting that reduced folate intake during early neurodevelopment predisposed to schizophrenia risk. Consistent with this finding, elevated concentrations of homocysteine (which is inversely related to folate) in serum obtained from women during the third trimester of pregnancy were associated with a more than 2-fold increased risk for schizophrenia in offspring.[6] Cross-sectional studies have indicated decreased blood folate levels in patients with schizophrenia.[7,8] We previously reported an inverse correlation between serum folate concentrations and negative symptoms among patients with schizophrenia, but positive symptoms were unrelated to folate levels.[9]

Several common missense single-nucleotide polymorphisms in genes that regulate folate and 1-carbon metabolism have been associated with schizophrenia phenotypes. The most widely studied is the 677C>T (222Ala>Val) variant in the methylenetetrahydrofolate reductase (*MTHFR*) gene, each copy of which reduces MTHFR activity by 35%.[10] The low-functioning 677T variant is slightly but consistently overrepresented among patients with schizophrenia (T allele frequency, 0.32 in patients and 0.30 in controls; odds ratio, 1.15; 95% CI, 1.04 to 1.26; n=10 202).[11] This variant, along with missense variants in 3 other genes that regulate 1-carbon metabolism—folate hydrolase 1 (*FOLH1*), methionine synthase (*MTR*), and catechol *O*-methyltransferase (*COMT*)—predict negative symptom severity in schizophrenia without affecting positive symptom scores.[12]

Two small placebo-controlled trials found therapeutic benefit with folate supplementation in patients with schizophrenia under conditions of folate deficiency. In a study conducted in the United Kingdom, Godfrey et al[13] reported generalized symptom improvement in 17 patients with low red blood cell (RBC) folate levels treated with methylfolate. Levine and colleagues[14] reported improvement of total symptoms in 42 subjects with hyperhomocystemia and schizophrenia treated with folate and vitamin B_{12} supplementation in a crossover study conducted in Israel. Because the prevalence of folate deficiency and hyperhomocystemia have dropped markedly in the United States since mandatory folate fortification of grain products was implemented in 1998,[15] the benefits of folate supplementation may be less readily detected and may depend on genotype. We previously conducted a placebo-controlled pilot trial of folate supplementation in 36 patients with schizophrenia who had prominent negative symptoms and found no treatment effect in the whole sample, but a significant interaction between treatment and *MTHFR* genotype emerged, reflecting improvement in negative symptoms among subjects with 677C/T or 677T/T genotypes who received folate.[16]

Herein, we report a larger multisite study of folate plus vitamin B_{12} supplementation vs placebo in medicated outpatients with schizophrenia. Vitamin B_{12} (cobalamin) is a cofactor for MTR, which remethylates homocysteine to methionine, which in turn is converted to the universal methyl donor *S*-adenosylmethionine. We included vitamin B_{12} in the active treatment arm to potentiate downstream effects of folate on methylation reactions and reduce the risk of masked pernicious anemia.[17] We hypothesized that folate plus vitamin B_{12} supplementation would improve negative symptoms but that treatment response would depend on the presence of hypofunctional genetic variants in the folate metabolic pathway.

METHODS

PATIENT CHARACTERISTICS

Subjects were outpatients with schizophrenia who were psychiatrically stable but displayed persistent symptoms despite antipsychotic treatment. Eligible patients were 18 to 68 years old, were treated with an antipsychotic agent for 6 months or more at a stable dose for 6 weeks or more, and scored 60 or more on the Positive and Negative Syndrome Scale (PANSS). Schizophrenia diagnosis was confirmed by a research psychiatrist using a structured clinical diagnostic interview using *DSM IV-TR* criteria and all available clinical records. Patients were excluded if they exhibited parkinsonism (score of ≥12 on the Simpson-Angus Scale), which can mimic negative symptoms. Patients were also excluded if they were taking supplemental folate or vitamin B_{12}, were medically unstable, had a history of significant neurological illness, reported abuse of alcohol or illicit substances within 3 months, were pregnant or nursing, tested positive on a baseline urine toxicology screen, or had an abnormal serum creatinine level. Prior to screening, all subjects provided written informed consent after the study was explained to them by a research physician.

STUDY DESIGN AND TREATMENT

This was a randomized, double-blind, placebo-controlled, parallel-group, 16-week trial of supplementation with folate and vitamin B_{12} conducted at 3 urban community mental health clinics (Boston, Massachusetts; Grand Rapids, Michigan; and Rochester, New York). The study was approved by institutional review boards affiliated with each site.

Following a 2-week, single-blind placebo lead-in, subjects who continued to meet entry criteria were randomly allocated in a 2:1 ratio to supplementation with 2 mg of folate and 400 μg of vitamin B_{12} or placebo in identical capsules and instructed to take 1 capsule of each daily. The randomization sequence was developed and assigned in the research pharmacy and was stratified by site. Given the previously reported relationship between serum folate level and negative symptoms,[16] randomization was also stratified by serum folate concentration at screening (< or ≥14.4 ng/mL [to convert to nanomoles per liter, multiply by 2.266], reflecting the mean serum folate value in our previous investigation). This ensured that baseline folate levels would be matched in the active treatment and placebo groups. Subjects returned to the clinic every 2 weeks to review their medical and psychiatric status. Adherence was monitored by pill counts at each visit. Fasting blood samples were drawn at baseline and weeks 2, 4, 8, 12, and 16 for assays of serum vitamin B_{12}, plasma homocysteine, and RBC folate concentrations. Clinical rating scales (Scale for the As-

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sessment of Negative Symptoms [SANS], PANSS, and Calgary Depression Rating Scale) were performed at baseline and weeks 2, 4, 8, 12, and 16. The Calgary Depression Rating Scale was included to rule out confounding effects of depression on negative symptoms, given their overlapping phenomenology and previous reports suggesting a therapeutic benefit of folate for depression.[18] To rule out the possibility that treatment effects could reflect dietary nutrient differences between treatment or genotype groups, dietary folate and vitamin B_{12} intake were determined using the Nutrition Data System for Research,[19] administered at baseline and weeks 8 and 16. The Nutrition Data System for Research is a Windows-based dietary analysis program that provides validated estimates of nutrient intake based on 24-hour recall of food and supplement consumption. All assessments were conducted by trained raters blind to treatment and genotype.

DNA extracted from whole blood samples was genotyped for 4 variants previously associated with negative symptom severity in a different cohort of patients with schizophrenia[12] (*FOLH1* 484C>T, rs202676; *MTHFR* 677C>T, rs1801133; *MTR* 1298G>A, rs1805087; and *COMT* 675G>A, rs4680) using the MassARRAY platform (Sequenom).

Several of these variants have previously been shown to affect blood folate and homocysteine levels.[20,21] Given the possibility that genotype effects on folate absorption or metabolism could influence treatment response, we evaluated whether these variants affected blood folate levels at baseline and throughout the duration of the trial. Further, to rule out the possibility that any such effects reflected confounds related to schizophrenia diagnosis or medication use, we studied a second cohort of 89 healthy individuals recruited from the Boston community. Participants were without current psychiatric illness, history of prior psychotic illness, or first-degree relative with psychosis. Healthy subjects underwent identical testing of RBC folate level, genotype, and dietary folate intake as did participants with schizophrenia.

STUDY END POINTS

The primary objective of the study was to assess the effects of adjunctive folate and vitamin B_{12} supplementation on positive and negative symptoms of schizophrenia. Based on pilot results,[16] we hypothesized that folate and vitamin B_{12} supplementation would specifically improve negative symptoms (SANS total score) and that this pattern would be influenced by genotype. Significant clinical findings were followed up by post hoc analyses of relevant subscales. We also determined relationships among genotype and baseline RBC folate levels, as well as changes in folate levels over time.

For clinical measures with significant treatment effects, we determined whether changes in blood chemistry levels predicted symptom changes, in conjunction with genotype. Following established methods,[22,23] we also examined the relationship between baseline symptoms and symptom change separately based on baseline RBC folate tertiles, given that cerebrospinal fluid (CSF) folate levels reach saturation at moderate blood folate levels.[23,24]

STATISTICAL ANALYSES

Effects of *FOLH1*, *MTHFR*, *MTR*, and *COMT* genotype on baseline blood chemistry levels were determined using multiple linear regression, based on 0, 1, or 2 copies of the low-functioning alleles.[10,12,20,25,26] All randomized subjects with 1 postbaseline visit or more were included in linear mixed model analyses of SANS total score, PANSS total score, PANSS positive symptoms score, and Calgary Depression Rating Scale score by treatment, incorporating change from baseline at weeks 2, 4, 8, 12, and 16. Secondary analysis of clinical outcomes included *FOLH1*, *MTHFR*, *MTR*, and *COMT* genotype, entered simultaneously as between-subject factors into the model. To maximize group size, subjects homozygous for the minor allele were grouped together with heterozygotes. Baseline RBC folate concentration was then entered as a planned covariate given previously reported relationships between folate levels and negative symptoms. Blood chemistry levels were log-transformed if their distribution deviated significantly from normality. Alpha (2-tailed) was set at .05.

RESULTS

PATIENTS

Between December 2007 and April 2011, 189 patients were screened, 149 were eligible, and 140 were randomized to folate plus vitamin B_{12} (n=94) or placebo (n=46) (**Figure 1**). The study continued until the enrollment goal, based on our pilot study,[16] was reached. A total of 121 subjects (86%) completed 16 weeks of treatment. Completion rates did not vary by site (P=.47). One randomized participant was subsequently found to have an ineligible baseline PANSS score (42) and was dropped from all analyses, although including the subject would not have altered any result. Baseline demographic and clinical characteristics were similar in both groups, as were medication use patterns and dietary folate and vitamin B_{12} intake (**Table 1** and eTable 1, http://www.jamapsych.com). Baseline blood chemistry levels were also similar except vitamin B_{12} levels, which were higher in the active treatment group; accordingly, all analyses were covaried by baseline vitamin B_{12} level. Genotype and RBC folate levels were available for 120 randomized patients, the remainder of whom declined to provide blood for genotype analysis or had missing samples or failed assays. Subjects without genotype did not differ from those with genotype on any baseline demographic variable, baseline clinical ratings, or change in clinical ratings (eTable 2). Genotype distribution did not differ significantly by treatment group (Table 1 and eTable 3).

BLOOD CHEMISTRY LEVELS

Baseline RBC folate levels correlated with *FOLH1* T (P = .03) and *MTHFR* T (P = .04) allele loads, despite equivalent dietary folate intake among genotype groups (eTable 4). Baseline serum vitamin B_{12} and plasma homocysteine levels were not influenced by genotype ($P > .17$). To validate genotype effects on folate levels, we studied a separate cohort of 89 healthy individuals, confirming the relationship between allele load and folate only for *FOLH1* (P = .03) (eTable 4).

Dietary folate and vitamin B_{12} intake did not differ significantly between treatment groups at baseline, week 8, or week 16 ($P > .24$) (eTable 1). Levels of RBC folate (**Figure 2**A) and serum vitamin B_{12} increased significantly over time in the active treatment group ($P < .001$) but not the placebo group ($P > .80$), yielding significant between-group differences ($P \leq .001$). Further, we observed a significant 3-way interaction among treatment, time, and *FOLH1* genotype (P = .006), where

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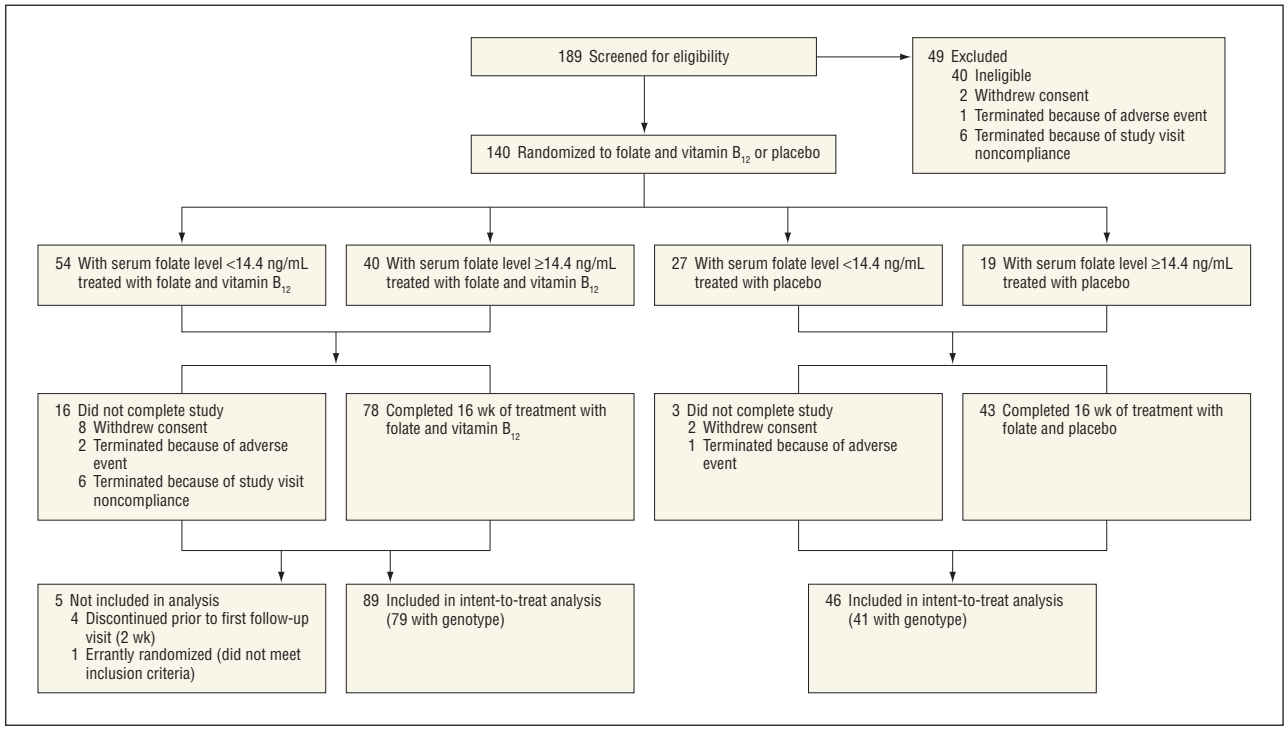

Figure 1. Consolidated Standards of Reporting Trials flow diagram. To convert folate to nanomoles per liter, multiply by 2.266.

Table 1. Study Participants

	Folate and Vitamin B_{12} (n = 93)	Placebo (n = 46)	Statistics	*P* Value
Demographics				
Age, y, mean (SD)	45.3 (1.1)	45.9 (1.6)	$t = 0.29$	.77
Male, %	71.0	71.7	$\chi^2 = 0.01$	>.99
White, %	62.4	67.4	$\chi^2 = 0.34$	.58
Duration of illness, y, mean (SD)	19.5 (1.2)	19.4 (1.6)	$t = 0.01$	>.99
Education level,[a] mean (SD)	4.2 (0.1)	4.1 (0.2)	$t = 0.40$	.69
Current smoker, %	55.3	56.5	$\chi^2 = 0.02$	>.99
Medications, %				
Typical antipsychotics	22.8	23.9	$\chi^2 = 0.02$	>.99
Atypical antipsychotics	83.7	89.1	$\chi^2 = 0.73$	.45
Antidepressants	54.3	56.5	$\chi^2 = 0.06$	.86
Anticonvulsants	38.0	28.3	$\chi^2 = 1.29$	.34
Clinical measures, mean (SD)				
SANS total score	35.2 (1.6)	33.2 (2.2)	$t = 0.72$	.47
PANSS positive score	16.8 (0.5)	16.7 (0.9)	$t = 0.06$	.95
PANSS total score	71.7 (1.0)	73.6 (1.9)	$t = 0.94$	.35
CDRS total score	3.7 (0.4)	3.2 (0.5)	$t = 0.79$	.43
Chemistry levels, mean (SD)				
RBC folate, ng/mL	465 (16)	439 (22)	$t = 0.91$	.37
Serum vitamin B_{12}, pg/mL	631 (26)	511 (29)	$t = 2.81$	.01
Plasma homocysteine, μmol/L	8.6 (0.3)	8.4 (0.3)	$t = 0.48$	.63
Genotype, %				
FOLH1 484TT/C carrier	52/48	46/54	$\chi^2 = 0.33$	.70
MTHFR 677CC/T carrier	52/48	63/37	$\chi^2 = 1.45$	.25
MTR 1298AA/G carrier	72/28	54/46	$\chi^2 = 4.10$	.07
COMT 675GG/A carrier	32/68	37/63	$\chi^2 = 0.30$	.37

Abbreviations: CDRS, Calgary Depression Rating Scale; *COMT*, catechol *O*-methyltransferase; *FOLH1*, folate hydrolase 1; *MTHFR*, methylenetetrahydrofolate reductase; *MTR*, methionine synthase; PANSS, Positive and Negative Syndrome Scale; RBC, red blood cell; SANS, Scale for the Assessment of Negative Symptoms.

SI conversion factors: To convert folate to nanomoles per liter, multiply by 2.266; homocysteine to milligrams per liter, divide by 7.397; and vitamin B_{12} to picomoles per liter, multiply by 0.7378.

[a] Education level scale: 0 = no schooling, 1 = kindergarten to seventh grade, 2 = completed eighth grade, 3 = some high school, 4 = high school diploma or equivalent, 5 = some college, 6 = bachelor's degree, 7 = some graduate school, 8 = graduate degree.

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C allele load predicted RBC folate increases over time in the active treatment group (β = 0.29; P = .03) but not the placebo group (β = 0.06; P = .74). Follow-up analyses within the active treatment group indicated that *FOLH1* genotype effects on RBC folate levels persisted until week 8 (Figure 2B). There was no effect of anticonvulsant use on RBC folate levels at baseline (t = 1.10; P = .27) and no effect on change in RBC folate levels over time in either treatment group ($P > .29$). Homocysteine levels fell significantly only in the active treatment group (P = .006) but not significantly more than in the placebo group (treatment group difference, P = .15).

SYMPTOM CHANGE

Intent-to-treat analysis (**Table 2**) examined symptom change in patients who completed 1 postbaseline visit or more (n = 135). Patients who received folate plus vitamin B_{12} exhibited a significant decline in negative symptoms (−0.19 change in SANS score per week; 95% CI, −0.35 to −0.03; P = .02) and those who received placebo did not change (+0.02 per week; 95% CI, −0.21 to 0.24; P = .88), although the difference between the folate and placebo groups did not reach significance (−0.21 per week; 95% CI, −0.49 to 0.07; P = .15). The same pattern was evident for PANSS positive scores. For PANSS total score, both the active and placebo groups improved significantly over time, but again, there was no difference in improvement between treatment groups. No significant effects of treatment were seen for depression scores.

When including *FOLH1*, *MTHFR*, *MTR*, and *COMT* genotype simultaneously into a linear mixed model of negative symptoms (n = 120), the main effect of treatment over time became significant (group difference, −0.33 per week; 95% CI, −0.62 to −0.05; P = .02), again reflecting a significant decline in negative symptoms in the active treatment group (−0.17 per week; 95% CI, −0.34 to −0.01; P = .04) but not the placebo group (+0.16 per week; 95% CI, −0.07 to 0.39; P = .18) (Table 2). To determine whether the between-group differences reflected genotype effects specifically within the active treatment or placebo groups, the groups were then considered separately, and each genotype was tested for an effect on negative symptoms. In the placebo group, no genotype was significantly associated with change in negative symptoms ($P > .10$). In the active treatment group, only *FOLH1* genotype significantly predicted change in negative symptoms (P = .04).

Direct comparison of the treatment groups indicated a significant *FOLH1* genotype × treatment interaction on negative symptom change (P = .02), with active treatment conferring a benefit over placebo in the T/T group (treatment group difference, −0.59 per week; 95% CI, −0.99 to −0.18; P = .005) but not among C allele carriers (treatment group difference, +0.09 per week; 95% CI, −0.29 to 0.47; P = .64) (**Figure 3** and eTable 5). For *MTHFR*, benefit of active treatment over placebo was seen among T carrier patients and not C/C patients, although the genotype × treatment interaction did not reach significance (P = .17). *MTR* and *COMT* genotype did not significantly influence treatment effects on negative symptoms over time (eTable 5). Including baseline RBC folate levels in the model did not influence the significance level of any result.

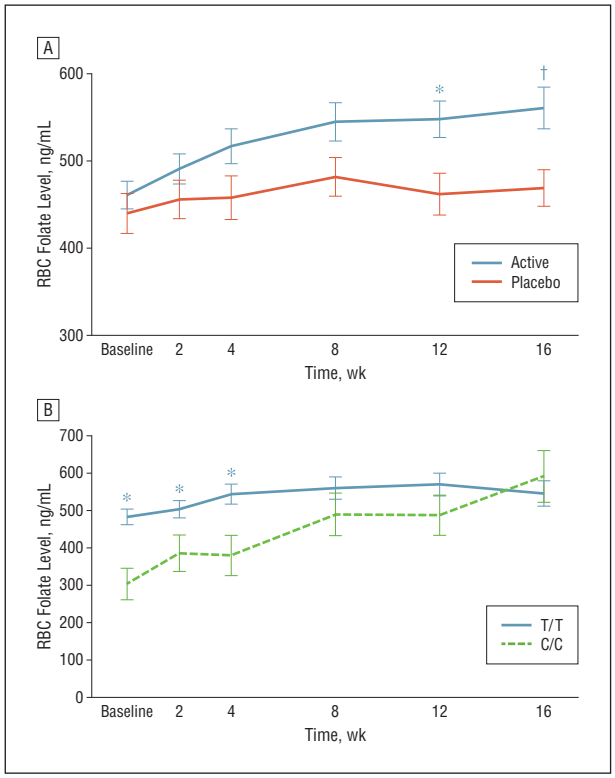

Figure 2. Change in red blood cell (RBC) folate level over time. A, Change in the active and placebo groups. B, Change among patients receiving active treatment, grouped by *FOLH1* 484T>C genotype. Error bars indicate standard error. *$P < .05$. †$P < .01$. To convert folate to nanomoles per liter, multiply by 2.266.

Analogous linear mixed models of PANSS positive, PANSS total, and Calgary Depression Rating Scale scores incorporating genotype did not detect significant differences between treatment groups (Table 2) nor were any genotype × treatment interactions observed (eTable 5).

NEGATIVE SYMPTOM SUBSCALES

Post hoc analyses indicated that, among negative symptom subscales, the alogia subscale most strongly contributed to between-group differences in SANS score (eTable 6). Similarly, when examining *FOLH1* genotype, a significant genotype × treatment interaction was present only for alogia (P = .03). Although alogia was the only subscale that showed a significant treatment effect, changes in alogia scores correlated significantly with changes in affective flattening and anhedonia ($r > .30$; $P < .001$) and also correlated at trend level with changes in avolition and attention ($r > .15$; $P < .10$).

RELATIONSHIP BETWEEN BLOOD CHEMISTRY LEVELS AND NEGATIVE SYMPTOMS

Although both RBC folate levels and negative symptoms each improved in the active treatment group, there was no significant overall correlation between change in folate levels and change in negative symptom ratings at any treatment visit. This pattern did not differ by *FOLH1* geno-

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Table 2. Intent-to-Treat Analysis

	Change in Symptoms/wk, Relative to Baseline							
	SANS Score		PANSS Positive Score		PANSS Total Score		CDRS Score	
	Mean (95% CI)	*P* Value	Mean (95% CI)	*P* Value	Mean (95% CI)	*P* Value	Mean (95% CI)	*P* Value
Before Inclusion of Genotypes in Model								
Active	−0.19 (−0.35 to −0.03)	.02	−0.06 (−0.12 to −0.01)	.02	−0.21 (−0.35 to −0.07)	.004	−0.02 (−0.06 to 0.02)	.25
Placebo	0.02 (−0.21 to 0.24)	.88	−0.04 (−0.11 to 0.03)	.28	−0.22 (−0.42 to −0.03)	.02	0.04 (−0.01 to 0.09)	.16
Difference	−0.21 (−0.49 to 0.07)	.15	−0.02 (−0.12 to 0.07)	.61	0.02 (−0.23 to 0.26)	.89	−0.06 (−0.12 to 0.00)	.07
After Inclusion of Genotypes in Model								
Active	−0.17 (−0.34 to −0.01)	.04	−0.08 (−0.13 to −0.02)	.01	−0.21 (−0.35 to −0.07)	.004	−0.00 (−0.04 to 0.04)	.84
Placebo	0.16 (−0.07 to 0.39)	.18	−0.01 (−0.10 to 0.07)	.74	−0.10 (−0.31 to 0.11)	.35	0.04 (−0.02 to 0.09)	.20
Difference	−0.33 (−0.62 to −0.05)	.02	−0.06 (−0.16 to 0.04)	.24	−0.11 (−0.36 to 0.14)	.39	−0.04 (−0.11 to 0.03)	.25

Abbreviations: CDRS, Calgary Depression Rating Scale; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for the Assessment of Negative Symptoms.

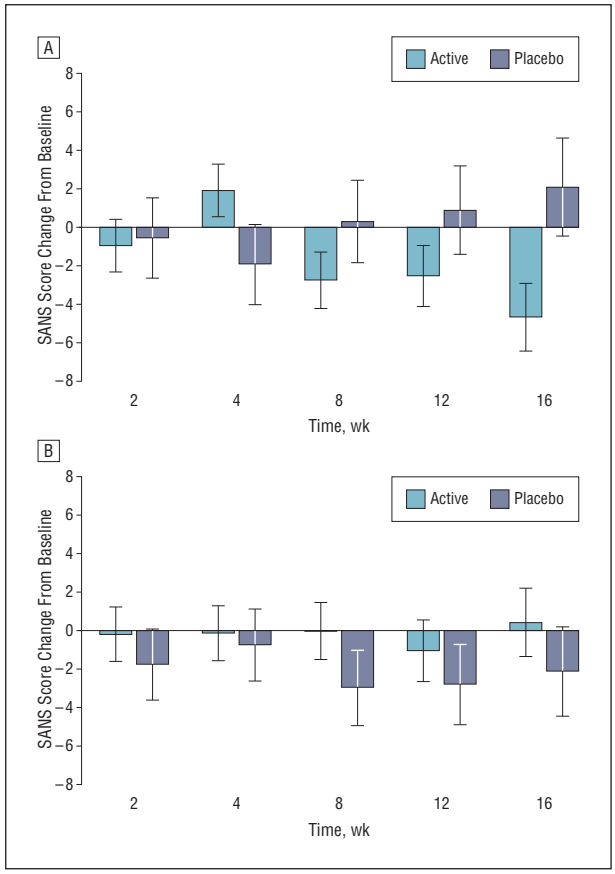

Figure 3. Change from baseline negative symptoms (Scale for the Assessment of Negative Symptoms [SANS] total score) among *FOLH1* 484T/T (A) and 484C carrier (B) patients, assessed throughout the course of treatment. For T/T patients, change over time was significant among those who received active treatment ($P = .003$) but not placebo ($P = .16$), with a significant difference between groups ($P = .005$). For C carrier patients, change over time was not significant in either the active ($P = .93$) or placebo ($P = .60$) groups, with no significant difference between groups ($P = .64$). Error bars indicate standard error.

type. Changes in negative symptoms were also unrelated to changes in vitamin B_{12} and homocysteine levels.

Secondary analyses considered the relationship between baseline RBC folate levels and negative symptoms according to baseline RBC tertiles. Baseline RBC folate level correlated inversely with baseline SANS score in the lowest tertile ($r = -0.39$; $P = .01$) but not in the middle ($r = -0.27$; $P = .09$) or upper ($r = -0.16$; $P = .35$) tertiles. In the active treatment group, baseline RBC folate level correlated positively with 16-week change in SANS score (ie, greater improvement with lower baseline RBC folate) in the lowest tertile ($r = 0.43$; $P = .04$) but not the middle ($r = 0.29$; $P = .20$) or upper ($r = 0.23$; $P = .29$) tertiles. In the placebo group, baseline RBC folate level did not correlate with 16-week change in SANS score for any tertile.

ADVERSE EVENTS

Treatment-emergent adverse effects did not differ substantially between the active and placebo groups (eTable 7). Three subjects were hospitalized for worsening psychosis (2 in the active group and 1 in the placebo group).

COMMENT

The present results indicate that supplementation of antipsychotic medications with folate and vitamin B_{12} improves negative symptoms of schizophrenia but only when accounting for common functional genetic variants in enzymes that regulate folate absorption and metabolism. Although 4 such variants have previously been associated with negative symptom severity,[12] the genotype that contributed most strongly to treatment response was *FOLH1* 484T>C. FOLH1 is a glutamate carboxypeptidase that is anchored to the intestinal brush border and facilitates transfer of dietary folate into the body.[27] The 484T>C variant, located in exon 2 of the structural transmembrane region, codes for a substitution of histidine for tyrosine at amino acid position 75 of the protein.

As with all 4 genetic variants included in the present study, it was the low-functioning variant of *FOLH1* (484C) that was associated with increased negative symptoms in a previously studied cohort of patients with schizophrenia.[12] These effects were mitigated, though, in the presence of higher blood folate levels, leading to the hypothesis that patients with low-functioning variants would tend to show a greater benefit from vitamin supplementation. However, these previous findings were limited by

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the fact that they were cross-sectional. Protracted exposure to high levels of folate may have been necessary for its protective effects among patients with hypofunctional variants. Moreover, the functionality of the *FOLH1* variant had not been as extensively characterized as the others.[20,21,25]

Herein, we found that it was the high-functioning variant of *FOLH1* (484T) that was required to show a benefit of vitamin supplementation for negative symptoms. While this was counter to our expectations, the effects of the *FOLH1* variant on RBC folate levels (both at baseline and throughout the study) may be instructive in understanding its relationship to negative symptoms. Baseline RBC folate levels were inversely related to 484C allele load, a pattern confirmed in a second cohort of healthy individuals. Dietary folate intake did not vary by genotype, suggesting that the 484C allele impairs folate absorption. Thus, the finding that only patients homozygous for the T allele exhibited improvement in negative symptoms after 16 weeks of folate supplementation could reflect diminished folate absorption, and briefer exposure to higher folate levels, among C allele carriers.

The present results are also consistent with previous work involving the hypofunctional *MTHFR* 677C>T variant, which confers increased schizophrenia risk,[11] as well as more pronounced negative symptoms.[12,28] Herein, only T allele carriers exhibited a significant benefit for active treatment over placebo for negative symptoms, reproducing results from our previous, smaller study of folate supplementation in schizophrenia.[16] However, the interaction of genotype and treatment did not reach statistical significance in the present study, possibly reflecting lower baseline symptom severity than the previous cohort.

After genotypes were entered into the model, the slope (SANS score change per week) for the placebo group changed more than the slope in the active treatment group, and this pattern contributed to the between-group difference in SANS response. This raises the question of whether gene effects within the placebo group were responsible for the between–treatment group differences that emerged after entering genotype into the model. However, unlike the active treatment group, there was no significant change in negative symptoms among patients in the placebo group, either before or after introduction of genotype; further, follow-up testing did not identify specific gene effects within the placebo group, as it did in the active treatment group. Still, we cannot rule out that folate-related genes influenced the placebo response, in addition to the response to active treatment.

We did not observe a significant overall relationship between change in RBC folate level and change in negative symptoms. This finding may reflect the nonlinear relationship between peripheral and central folate levels. Transport of folate across the blood-brain barrier is tightly regulated by specialized folate transporters within choroid plexus epithelial cells.[29] Accordingly, CSF folate levels generally fall within a narrower range than blood folate levels.[23,24] The CSF folate levels plateau at moderate blood folate levels.[23,24,30] Specifically, Obeid and colleagues[23] reported that blood and CSF folate levels correlated strongly only within the lowest tertile of blood folate level in 72 individuals. The lack of an overall relationship between changes in symptoms and RBC folate level may reflect the fact that folate supplementation increased blood folate levels more than the levels required for CSF saturation in most subjects. However, when we divided the cohort by baseline RBC folate tertiles, we observed that blood folate levels correlated inversely with baseline SANS scores, and positively with change in SANS scores, only for participants who fell within the lowest tertile. This pattern explains the lack of correlation across the entire cohort and is also very consistent with the known physiology of folate transport into the brain.

For patients with the low-functioning *FOLH1* 484C variant, response to treatment might have been delayed because it took longer to achieve CSF folate saturation. However, by the midpoint of the study, RBC folate levels among C carriers had reached the same level as T/T patients, and it is possible that C carrier patients would also have shown a benefit had the treatment period been extended. That said, the mechanism underlying the gradual timing of SANS response to vitamin supplementation across the entire cohort remains uncertain. It does not solely reflect the timing of RBC folate increases, since these measures were uncorrelated, potentially because of the saturation kinetics of the blood-brain barrier described earlier. Clinical changes related to vitamin supplementation may lag behind biochemical changes that are directly related to the intervention, as is the case for serotonin reuptake inhibitors and in depression. Rather, clinical improvement may be more directly related to slower downstream mechanisms such as altered gene expression.[31] In the case of folate supplementation, gene expression changes may be particularly relevant, given their dependence on DNA methylation status.

Patients with schizophrenia experience differing degrees of negative symptoms, and the present investigation did not include a minimum SANS threshold for study entry. However, all subjects presented with at least some negative symptoms at baseline, and all but 6 participants would have met inclusion criteria for the CONSIST trial,[32] a recent negative symptom treatment study that required a SANS total score of 20 or more or a SANS affective flattening or alogia subscale score of 3 or more. With regard to treatment effects on negative symptom subscales in the present study, while alogia was most strongly affected by vitamin supplementation, the correlation between alogia and other subscale scores suggests that the changes in alogia were part of a broader change in negative symptoms.

Folate and vitamin B_{12} supplementation did not confer a benefit for positive symptoms or depression scores, regardless of genotype. This finding echoes earlier observations that related blood folate levels specifically to negative symptoms[9] and genetic variants in folate metabolism specifically to negative symptom severity.[12,28] It also suggests that improvement in negative symptoms was not confounded by improvement in depression, a potential concern given their overlapping phenomenology.

Several limitations to the present study should be acknowledged. First, multiple outcomes were studied, although our main hypotheses related to negative symp-

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toms, reflecting the previous findings described earlier.[9,12,28] The additional ratings in total, positive, and depression symptoms were necessary to establish whether treatment effects were specific to negative symptoms. Four genetic variants were included, although all were again based on previous findings,[12,28] and the use of a single regression model incorporating all 4 genotypes per outcome variable reduced the number of comparisons. Second, the study population included a mixture of races and ethnicities, although the previous work on which genetic hypotheses were based[12] included only subjects of European ancestry. While population stratification artifact remains a possibility, outcomes did not differ by race, and the present results may generalize across a broader population than if only 1 racial group were studied. Third, the study sample size precluded the analysis of cumulative genotype effects, which have previously been shown to influence negative symptoms and their relationship to blood folate levels.[12] Finally, treatment effects were modest, with a 15% difference in SANS score change between the active treatment and placebo groups and 27% difference between *FOLH1* T/T vs C allele carriers. While there remains no gold standard for translating quantitative changes in negative symptom rating scales to qualitative measures of clinical and functional improvement,[33] the degree of SANS score change in the present study is consistent with other studies that have determined clinically detectible thresholds for PANSS total score change through cross-validation with global clinical impression scales.[34] Even small effects of folate and vitamin B_{12} supplementation could be clinically meaningful, though, given the disability associated with negative symptoms, the lack of available treatments,[35] and the minimal apparent adverse effects of vitamin supplementation.

With these limitations in mind, the present results have direct treatment implications not only for schizophrenia but also for folate-related interventions in other areas of medicine. Well-replicated associations of reduced folate and elevated homocysteine concentrations as risk factors for stroke,[36,37] cardiovascular disorders,[38,39] and dementia[40,41] have been tempered by large, prospective studies that have failed to find a benefit of folate supplementation on disease progression.[42-47] The current results suggest that individual differences in folate metabolism related to the presence of common functional genetic variants may have a bearing on treatment outcomes in these other disorders, as well as negative symptoms of schizophrenia.

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