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The folate hydrolase 1561 C>T polymorphism is associated with depressive symptoms in Puerto Rican adults

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Abstract

Objective—To examine associations between variants of genes involved in the uptake, retention and metabolism of folate and depressive symptoms, and whether such associations are direct or through mediation by folate or homocysteine.

Methods—We performed a cross-sectional analysis of data from 976 Puerto Rican adults, aged 45–75 years, residing in the greater Boston area, Massachusetts. Twelve single nucleotide polymorphisms (SNPs) in genes involved in folate uptake, retention and metabolism were investigated. These include folate hydrolase (*FOLH1*), folate polyglutamate synthase (*FPGS*), r-glutamyl hydrolase (*GGH*), methylene tetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*), proton-coupled folate transporter (*PCFT*), and reduced folate carrier (*RFC1*). The Center for Epidemiologic Studies Depression Scale (CES-D) was used to measure depressive symptoms.

Results—The *FOLH1* rs61886492 C>T (or 1561C>T) polymorphism was significantly associated with lower CES-D score ($p = .0025$), after adjusting for age, sex, population admixture, smoking, and educational attainment. Individuals with the TT and TC genotypes were 49% less likely (odds ratio (OR): 95% confidence interval [CI]: 0.51: 0.29, 0.89) to report mild depressive symptoms (CES-D score ≥ 16 & ≤ 26) and 64% less likely (OR: 95% CI: 0.36: 0.18, 0.69) to report moderate to severe depressive symptoms (CES-D score > 26), compared to those with the CC genotype. No significant mediation effects by plasma folate or homocysteine on the associations between this SNP and CES-D score were observed.

Conclusions—The *FOLH1* 1561C>T polymorphism may be associated with risk of depressive symptoms.

Keywords

Depressive symptoms; genetics; polymorphisms; folate; folate hydrolase; glutamate carboxypeptidase II

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INTRODUCTION

Folate functions as a coenzyme in single-carbon transfers involving metabolism of amino acids and nucleotide synthesis. Low folate status has been observed in depressed patients (1-3), in severe (1, 2) and prolonged depressive episodes (3) and in those with poor response to antidepressants (2, 3). Supplementation with folic acid has been reported to improve depression on its own (4) and response to antidepressants (5). Total homocysteine, a functional biomarker of folate and vitamin B12 status, has also been linked with depression (6). There is also evidence to suggest that vitamin B12 (7) and vitamin B6 (7, 8) may be associated with depressive symptoms. Nevertheless, not all studies have found significant associations between folate (9-12), vitamin B6 (11), vitamin B12 (10-13) or homocysteine (9, 11-13) and depressive symptoms. Additional studies are needed to clarify the associations between these related vitamins, homocysteine and depression.

Several mechanisms have been suggested for the roles of folate and homocysteine in central nervous system function. Folate may be involved in bipterin-dependent neurotransmitter synthesis (14) and methylation of biogenic amines and phospholipids (15). Folate deficiency increases homocysteine concentration. Homocysteine or its metabolites may have a direct excitotoxic effect on the N-methyl-D-aspartate glutamate receptors (15). In addition, homocysteine can cause dysregulation of promoter-specific DNA methylation, which may directly influence monoaminergic neurotransmission (16).

Genetic association studies may further elucidate the role of folate in depression. The C>T transition at nucleotide 677 in exon 4 of the gene, encoding 5,10-methylenetetrahydrofolate reductase (*MTHFR*), has been associated with lower plasma folate concentrations and higher risk of depression in some (17, 18), but not all studies (19).

To our knowledge, there are few data on associations of depression with single nucleotide polymorphisms (SNPs) other than the C677T in the *MTHFR* gene. SNPs in other folate-metabolizing genes, such as those encoding folate hydrolase (*FOLH1*), proton-coupled folate transporter (*PCFT*), reduced folate carrier 1 (*RFC1*), folate receptor 1 (*FOLR1*), folate polyglutamate synthase (*FPGS*), L-glutamyl hydrolase (*GGH*), and methionine synthase (*MTR*), may also be important (20).

Therefore, we used the Center for Epidemiologic Studies Depression Scale (CES-D) to measure depressive symptoms in a sample of Puerto Rican adults residing in greater Boston area. We aimed to investigate: 1) associations between plasma folate, homocysteine, vitamins B6, B12 and CES-D score; 2) associations of the CES-D score and depressive symptoms with selected SNPs in folate uptake, retention and metabolism related genes (*FOLR1*, *FOLH1*, *RFC1*, *PCFT*, *FPGS*, *GGH*, *MTHFR* and *MTR*), and whether observed associations were mediated by folate and homocysteine and moderated by plasma vitamin B6 and B12.

METHODS AND MATERIALS

Study population

Data for the current analyses were obtained from the Boston Puerto Rican Health Study, which was initially started in 2004. This is an ongoing study to investigate the influence of social and physical environments and individual risk factors, and their interactions with genes on chronic disorders and diseases among Puerto Rican adults living in Massachusetts. Detailed information about study design and data collection has been described previously (21). Briefly, one Puerto Rican adult, aged 45-75 years, was randomly recruited from each qualified household, identified in the greater Boston area mainly through enumeration of

year 2000 Census blocks containing at least 10 Hispanics, within Census tracts containing at least 25 Puerto Rican adults aged 45-75 years. Most (77.4%) of the participants were recruited through this strategy; the remainder were recruited through partnerships with community organizations, random approach at community events, from calls responding to flyers, or from referrals of community members. A home interview was conducted to collect data on demographic variables, acculturation, depressive symptoms, health history, medication use, dietary intake, supplement use and anthropometric measures. Overnight fasting blood samples (12 h) were drawn the day after the home interview or as soon as possible thereafter. Aliquots were saved and stored at -80°C until processed. The Institutional Review Board of the New England Medical Center and Tufts University Health Sciences approved this study; written informed consent was obtained from each participant. A total of 976 participants with available genotype data were included in the current analyses.

The CES-D was used to measure depressive symptoms. This instrument has been shown to have good reliability and discriminating features in Puerto Ricans (22). Data also suggest that the CES-D has relatively high sensitivity and specificity for identification of patients with major depression among middle-aged and older Puerto Ricans (23). Consistent with previous studies, we classified participants as having no depressive symptoms (CES-D score <16), mild depressive symptoms (CES-D score ≥ 16 & ≤ 26), or moderate to severe depressive symptoms (CES-D score ≥ 26) (24). Educational attainment was dichotomized by years in school (≥ 9 vs. <9 years). Poverty status was defined as total household income was below the threshold released each year by the U.S. Department of Health and Human Services. Acculturation was assessed with seven questions regarding use of English and/or Spanish at work, to watch television, listen to the radio, read newspapers/books, and to talk with neighbors, friends and family members: a summed score ranged from 0 (fully unacculturated, only using Spanish) to 100 (fully acculturated, only using English) (25). Smoking status was categorized as never (<100 cigarettes in entire life), past and current smoking. Alcohol use was defined as not current, current moderate (≤ 1 drink daily for women or ≤ 2 drinks daily for men) or current heavy (>1 drink daily for women and >2 drinks daily for men). Physical activity was measured using a modified Paffenbarger questionnaire of the Harvard Alumni Activity Survey and a score was calculated (21).

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Blood pressure was measured at the beginning, in the middle and at end of the home interview; the latter two were averaged for final analysis. Hypertension was defined as taking antihypertensive medication or systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg. Diabetes was defined as taking hypoglycemic medication or a fasting glucose ≥ 7.0 mmol/L.

Blood metabolites

Plasma folate and vitamin B12 were measured with immunoassay kits (Siemens Medical Solutions, Diagnostics Division, Tarrytown, NY) on the Immulite 1000 analyzer system (Immulite DPC, Los Angeles, CA). Plasma pyridoxal-5'-phosphate (PLP), the active form of vitamin B6, was measured enzymatically using tyrosine decarboxylase (21). Total plasma homocysteine was assayed with Waters HPLC and detected with a Perkin Elmer 650-15 Fluorescence Spectrophotometer (Perkin Elmers., Waltham, MA) (21). Serum creatinine was determined with Olympus Creatinine Reagents on the Olympus AU400e (OSR6178) (Olympus America Inc., Melville, NY).

SNP selection and genotyping

Detailed information about SNP selection and genotyping has been described elsewhere (20). In brief, eight genes involved in folate uptake, retention and metabolism were selected, including *FOLR1*, *FOLH1*, *RFC1*, *PCFT*, *FPGS*, *GGH*, *MTHFR* and *MTR*. Selection of SNPs was based on several considerations including: 1) minor allele frequency ≥ 0.2 ; 2) no more than one SNP from a specific linkage disequilibrium block; 3) SNPs suggested from previous literature; and 4) the potential for altering transcription process and/or protein activity of the SNP. Final decisions were made based on the capability of a TaqMan probe to successfully detect a SNP. After being isolated from the buffy coat of blood samples, DNA was used for genotyping with custom and predesigned TaqMan SNP assays (Applied Biosystems, Foster City, CA).

Statistical analysis

Plasma folate, PLP, vitamin B12, homocysteine and serum creatine were log-transformed (base 10) to improve normality. The CES-D score was not highly skewed and no improvement of normality was observed when the CES-D score was log-transformed. Therefore, the original continuous CES-D score was used as our primary outcome. Characteristics of participants with mild and with moderate to severe depressive symptoms were compared to those without, using logistic regression for categorical variables as outcomes and general linear models for continuous variables as outcomes. Spearman partial correlation coefficients were calculated between CES-D score and plasma folate, PLP, vitamin B12 and homocysteine and other continuous covariates, adjusting for age and sex. Further, means of the CES-D score were compared across quartiles of plasma folate concentration, adjusting for covariates, with general linear models. First, age, sex, smoking and educational level were adjusted, as suggested in previous studies (17). Secondly, other socioeconomic and life style confounders including poverty, acculturation score, alcohol use and physical activity score were further adjusted. Finally, vascular risk factors including BMI, presence of hypertension and diabetes, and serum creatine (a marker of kidney function) were entered in the final multivariate model. Log-transformed plasma folate was included as an independent variable for the trend test. The same analysis approach was used to compare CES-D score across quartiles of plasma vitamin B12, PLP, and homocysteine, respectively.

Chi-square tests were conducted to examine status of the Hardy-Weinberg equilibrium of genotypes of each SNP. Pairwise linkage disequilibrium (LD) among SNPs was assessed as the squared correlation coefficient (r^2) using the genetics package for R (26). General linear regression was used to assess associations between individual SNPs and CES-D score, and multinomial logistic regression was used to assess associations between individual SNPs and mild and moderate to severe depressive symptoms, adjusting for age, sex, smoking, educational level and population admixture, estimated with informative ancestral markers (27). The initial analysis was conducted assuming additive, dominant and recessive genetic models for all SNPs except for *FOLH1* rs61886492 (dominant model only). Because Bonferroni correction of multiple testing is overly conservative if tested SNPs are not independent, we used the approach of Li and Ji (28), a modification of method by Nyholt (29), to calculate the effective number of SNPs based on pairwise LD correlation coefficients to correction for multiple testing of additive effects for all SNPs (dominant effect of *FOLH1* rs61886492 only) on CES-D score, and on mild and moderate to severe depressive symptoms, respectively. The effective number of SNPs was 11 in the current study. Therefore, the multiple adjusted significant p values should be $< .0047$, to keep type I error below the level of $\alpha = 0.05$.

To further examine whether the associations between SNPs and CES-D score were mediated by either folate or homocysteine, a bootstrapping technique suggested by Preacher and Hayes (30) was used for SNPs that remained significant after correction for multiple testing. This bootstrapping mediation model allows us to calculate the direct effect of SNPs on CES-D score, as well as indirect effects through either folate or homocysteine, after adjustment for potential confounding factors.

In addition, we tested whether associations between significant SNPs and CES-D score were moderated by plasma PLP. Plasma PLP was dichotomized to low and high groups at the median. Interaction terms, the product of dichotomized PLP and genotypes, were included in general linear models. We tested the significance of the interaction terms after adjustment for age, sex, population admixture, smoking, and education level. Further adjustments for other risk factors mentioned above were also conducted. We repeated the analysis to test potential interaction between vitamin B12 and genotypes.

A p value of $< .0047$ for SNP analysis and nominal $p < .05$ for other analyses were considered statistically significant. Statistical analyses were conducted with SAS version 9.1.3 (SAS Institute Inc, Cary, NC) and R version 2.10.1 (31).

RESULTS

Among the 976 Puerto Rican adults, only 0.1% had folate deficiency (<6.8 nmol/L), 2.8% had plasma vitamin B12 deficiency (<148 pmol/L) and 18.1% of men and 7.4% of women had elevated plasma homocysteine (>13 nmol/L). More than ten percent (10.3%) had vitamin B6 deficiency (PLP <20 nmol/L) and another 16.5 % had insufficient vitamin B6 (PLP ≥ 20 nmol/L and <30 nmol/L).

Approximately 30% had mild depressive symptoms (CES-D score ≥ 16 & ≤ 26), and another 30% had moderate to severe depressive symptoms (Table 1). Those with moderate to severe depressive symptoms were younger, more likely to be women, less educated, less acculturated, less physically active, to have lower income, and to smoke currently, when compared to those without depressive symptoms. Participants with moderate to severe depressive symptoms also had lower concentrations of plasma PLP.

Plasma PLP (Spearman partial correlation coefficient $r = -.09$, $p = .006$), but not plasma folate ($r = -.05$, $p = .10$), vitamin B12 ($r = -.05$, $p = .16$) or homocysteine ($r = .002$, $p = .95$), was significantly associated with lower CES-D score after adjustment for age and sex. Higher plasma PLP remained significantly associated with lower CES-D score after adjusting for age, sex, smoking and educational levels (p for trend = .003, Table 2). This trend remained marginally significant ($p = .08$) after further adjustment for poverty status, acculturation score, smoking, alcohol use, physical activity score, diabetes, hypertension, BMI and serum creatine. No significant associations between plasma folate, vitamin B12 and serum creatine and CES-D score were observed (Table 2).

SNPs of *FOLR1* were not successfully genotyped (20). All other 12 SNPs were in Hardy-Weinberg equilibrium ($p > .05$ by Chi-square test). *FOLH1* rs61886492 C>T was in weak LD with rs202712 C>T ($r^2 = .04$; $p < .001$) and rs647370 G>A ($r^2 = .02$; $p < .001$), respectively, whereas rs202712 C>T was in relatively strong LD with rs647370 G>A ($r^2 = .57$; $p < .001$). *GGH* rs11545076 T>G was in strong LD with rs3758149 T>C ($r^2 = .96$; $p < .001$). *MTR* rs16834521 A>G was in weak LD with rs1805087 A>G ($r^2 = .12$; $p < .001$), and *RFC1* rs2297291 A>G was in relatively strong LD with rs12659 C>T ($r^2 = .70$; $p < .001$).

The *FOLH1* rs61886492 C>T allele (or 1561C>T) was examined with the dominant genetic model because there were only two participants with the TT genotype (Table 3). The

FOLH1 rs61886492 C>T allele was associated with lower CES-D score after adjusting for age, sex, smoking, educational level and population admixture (nominal $p = .0025$). Participants with the T allele were almost 50% less likely to have mild depressive symptoms (nominal $p = .02$), and 64% less likely to have moderate to severe depressive symptoms (nominal $p = .0022$), compared to those with the CC genotype. *FOLH1* rs202712 C>T (dominant model) and *PCFT* rs2239907 G>A (recessive model) were also nominally significantly associated with lower CES-D score (nominal $p < .05$) while *MTR* rs1805087 A>G was nominally significantly associated with higher CES-D score under either additive or dominant genetic models (both nominal $p = .04$). However, only the *FOLH1* rs61886492 SNP remained statistically significant in relation to lower CES-D score and moderate to severe depressive symptoms after correction for multiple testing.

We further examined whether the association between the *FOLH1* rs61886492 C>T and CES-D score was mediated by folate or homocysteine, using the bootstrapping approach suggested by Preacher and Hayes (30). As shown in figure 1, the total effect of the *FOLH1* rs61886492 C>T (coefficient $[c] = -4.4$, $p = .003$) on CES-D was similar to the direct effect (coefficient $[c'] = -4.3$, $p = .004$) after control for the pathway mediated by folate, when age, sex, population admixture, smoking and educational level were adjusted. The mediating effect of folate was not significant (95% confidence interval of the bootstrapping coefficient: -0.47 to 0.03). The association between the *FOLH1* rs61886492 C>T and CES-D score remained unchanged and was not significantly mediated by folate, even when a series of confounding factors including lifestyle, social economic factors, chronic diseases and plasma PLP and vitamin B12 were considered (Table 4). Similarly, the association between the *FOLH1* rs61886492 C>T and CES-D score was not mediated by homocysteine (Figure 1 and Table 4).

We did not observe any significant interaction between the *FOLH1* rs61886492 C>T and plasma PLP (in dichotomized form) on CES-D score, when adjusted for age, sex, population admixture, smoking and educational level using general linear models ($p > .10$) (data not shown). Further adjustment for other covariates including lifestyle, social economic factors, chronic diseases and plasma folate did not change the result ($p > .10$ for interaction). There was also no interaction between the *FOLH1* rs61886492 C>T and vitamin B12 ($p > .10$ for interaction).

DISCUSSION

A variant of the *FOLH1* gene was significantly associated with CES-D score and with moderate to severe depressive symptoms. However, there were no observed significant associations between CES-D score or depressive symptoms and any of the other SNPs in these genes related to folate uptake, retention and metabolism, after correction for multiple testing.

The frequencies of genotypes of the *FOLH1* rs61886492 C>T polymorphism were similar to those observed in most previous studies of other populations (32). In contrast, Halsted et al. did not find a significant association between the *FOLH1* rs61886492 C>T polymorphism and depression score in the Hordaland Homocysteine Study in Norway (32). This inconsistency may be due to study design and characteristics of participants. For example, the Hordaland Homocysteine Study did not use the CES-D to estimate depressive symptoms. In addition, only participants aged 70-72 years were included in their analyses (32), whereas we recruited individuals with an age range of 45-75 years old and depressive symptoms was more common with younger age in our study.

Our previous study showed that those with the *FOLH1* rs61886492 T alleles (combined CT and TT genotypes) had significantly higher folate concentration than those with the CC genotype in the same sample of participants (20). That may partially explain why the *FOLH1* rs61886492 C>T was associated with lower CES-D score. Interestingly, the association of *FOLH1* with CES-D score was not mediated by either folate or homocysteine, and was not moderated by either vitamin B6 or vitamin B12 in this population. Alternatively, the hydrolase encoded by *FOLH1* possesses *N*-acetylated α -linked acidic dipeptidase activity, and is therefore also known as glutamate carboxypeptidase II (GCPII) (32). In the brain, *FOLH1*/GCPII catalyzes the cleavage of *N*-acetylated-*L*-aspartyl-*L*-glutamate to *N*-acetylated-*L*-aspartyl and glutamate (32). Recent studies have implicated GCPII as an important regulator of glutamatergic neurotransmission, and have observed dysregulation of brain GCPII expression in psychiatric disorders, including schizophrenia, bipolar states and depression (33). Reduced *N*-acetylated-*L*-aspartyl concentrations are thought to indicate neuronal or axonal loss, or dysfunction (34). Inhibiting GCPII is protective in some animal models of neuropsychological disorders, possibly by decreasing glutamate (35). In depression, glutamatergic transmission may be disturbed (36) and antidepressants can change serum glutamate concentrations (37). Together with these observations, our results raise the hypothesis that polymorphisms of *FOLH1* could modify the risk of depressive symptoms by modifying GCPII activity. Further studies are needed to verify this hypothesis.

No SNPs of other studied genes were significantly associated with CES-D score or depressive symptoms. One potential reason is that very few participants had folate deficiency in this population because the food supply is now fortified with folic acid, as observed in the NHANES 1999-2000 (38). Although some of the studied SNPs, such as *MTHFR* C677T, may have functional effects on folate-dependent metabolism and thereby on depression (17, 18), such effects may only be apparent when folate concentrations are limiting. In addition, only a small proportion of our cohort had low plasma vitamin B12 concentrations. This may explain why folate, vitamin B12, as well as homocysteine, were not associated with CES-D score.

On the other hand, plasma PLP, the active form of vitamin B6, was inversely associated with CES-D score after adjustment for age, sex and educational attainment. This is consistent with several previous studies, including one in Hispanic elders (7, 8) but not all (39). In contrast to adequate status of folate and vitamin B12, relatively high prevalence of vitamin B6 deficiency and insufficiency was observed in our study. Biologically, PLP is a key coenzyme in the metabolic pathways of neurotransmitters, including serotonin, dopamine and norepinephrine (40). The role of these monoaminergic neurotransmitters has been well established in the pathophysiology of depression and available antidepressants are based on such mechanisms (41). For example, selective serotonin re-uptake inhibitors and serotonin and norepinephrine re-uptake inhibitors work on serotonin to reduce depressive symptoms (42). The potential beneficial effects of vitamin B6 on depression may be exerted through influencing production of monoaminergic neurotransmitters, and vitamin B6 has been suggested as a therapeutic adjunct to treat conditions with neurotransmitter abnormalities (43).

Nevertheless, antidepressants based on monoaminergic systems require time (generally more than 3 to 4 weeks) to act, and are not effective in approximately 30% of patients with major depressive disorder (41, 44). In the past few years, new mechanisms have emerged, and a disturbed glutamatergic system has been implicated in the pathophysiological process of depression (41). Indeed, drugs targeting the glutamatergic system have shown therapeutic effects on depression (41, 45). Our finding of associations of the *FOLH1* (*GCPII*) gene with mild and moderate to severe depressive symptoms is consistent with the role of the

glutamatergic system in depression. More studies on other genes related to the glutamatergic system with depression will be useful to verify this hypothesis.

The prevalence of depressive symptoms (CES-D score ≥ 16 , 60.5%) was higher for the current sample than those observed in previous studies of Puerto Ricans and other Hispanic adults (46). However, a generally high prevalence of depressive symptoms (44.4%) was also observed in our previous study including Puerto Ricans aged 60 and over in Massachusetts (25). Due to limitations of the CES-D as a screening tool (47), some of those with a high CES-D score may be misclassified and upon closer examination, may not be clinically diagnosed with major depressive disorder. However, a previous study demonstrated that the CES-D scale has high consistency, reliability, and strong discriminating ability between older Puerto Ricans with depression and non-patients, supporting the CES-D as a useful measure in this group (22). To reduce the potential for false positive cases, we classified our participants with a higher cut-off point (>26) to define those with moderate to severe depressive symptoms, and results were stronger for this more severe level of depressive symptoms.

In conclusion, among this middle-aged and older population with high prevalence of mild and moderate to severe depressive symptoms, the *FOLH1* rs61886492 (or 1561) C>T, but not SNPs of other studied genes related to folate uptake and metabolism, was associated with CES-D score and presence of depressive symptoms. No mediation effects of folate or homocysteine, or moderation effects of PLP or vitamin B12, were observed. CES-D score was associated with plasma PLP, but not with vitamin B12, folate, or homocysteine. Further investigation of the role of *FOLH1* in depressive symptomatology in this and other populations is warranted.

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Acronyms

MTHFR	methylene tetrahydrofolate reductase
SNP	single nucleotide polymorphism
FOLH1	folate hydrolase
PCFT	proton-coupled folate transporter
RFC1	reduced folate carrier
FOLR1	folate receptor 1
FPGS	folate polyglutamate synthase
GGH	r-glutamyl hydrolase
MTR	methionine synthase
CES-D	Center for Epidemiologic Studies Depression Scale
BMI	body mass index
PLP	pyridoxal-5'-phosphate
LD	linkage disequilibrium
GCPH	glutamate carboxypeptidase II

SE	standard error
OR	odds ratio
CI	confidence interval

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Figure 1.

The mediating effects of plasma folate (A) and homocysteine (B) on the association between folate hydrolase gene (*FOLH1*) rs61886492 C>T and Center for Epidemiologic Studies Depression Scale (CES-D) score. Genotypes were coded as 0 = *CC* and 1 = *CT* or *TT*. c = coefficient for total effect of *FOLH1* rs61886492 genotype on CES-D score; c' = coefficient for direct effect of *FOLH1* rs61886492 genotype on CES-D score after control for the mediated effect of log-transformed folate (A), or log-transformed homocysteine (B). The bootstrapping coefficients (95% confidence interval) for the mediating effect of folate and homocysteine were -0.11 ($-0.47, 0.03$) and 0.03 ($-0.05, 0.26$), respectively. All coefficients are presented after the adjustment for age, sex, population admixture, smoking and educational level, using the Preacher and Hayes method (30). $*p < .1$; $**p < .05$; $***p < .01$.

TABLE 1

Characteristics of Participants by Depressive Symptoms

	No Depressive Symptoms (CES-D score <16)	Mild Depressive Symptoms (CES-D score ≥16, ≤26)	Mild Depressive Symptoms (CES-D score ≥16, ≤26)	p ^a
<i>n</i>	386	289	301	
CES-D score	7.4 ± 4.8 ^b	20.8 ± 3.3	36.7 ± 7.2	
Age (y) ^c	58.9 ± 7.5	57.7 ± 7.2	56.7 ± 7.0 ^d	< .001
Women (%) ^c	63.0	73.4 ^e	83.1 ^d	< .001
Education (<9 years, %)	45.5	51.9 ^f	53.7 ^e	.003
Acculturation score ^g	25.3 ± 21.9	23.9 ± 22.8	20.4 ± 20.8 ^e	.006
Poverty (%)	52.8	55.8	69.4 ^d	< .001
Smoking (%)				< .001
Never	51.2	42.4	44.4	
Past	30.7	36.8 ^f	24.2	
Current	18.1	20.8	31.3 ^d	
Alcohol use (%)				.19
Not current	59.3	61.2	67.0	
Current moderate	34.7	31.1	27.3 ^f	
Current heavy	6.1	7.7	5.7	
Physical activity score	32.1 ± 4.7	31.8 ± 5.1	30.6 ± 3.8 ^e	< 100
Diabetes (%)	41.9	41.0	40.7	.89
Hypertension (%)	71.4	69.9	69.0	.81
Body mass index (kg/m ²)	31.6 ± 6.3	32.0 ± 7.0	33.1 ± 7.2	.29
Plasma folate (nmol/L)	39.7 (37.9, 41.6) ^h	40.2 (38.2, 42.2)	39.2 (37.3, 41.1)	.63
Plasma vitamin B12 (pmol/L)	366 (348, 385)	352 (334, 372)	345 (327, 363)	.14
Plasma pyridoxal-5'-phosphate (nmol/L)	48.4 (45.0, 52.1)	45.1 (41.7, 48.7)	41.4 (38.4, 44.6) ^f	.02
Plasma total homocysteine (μmol/L)	8.83 (8.51, 9.16)	8.76 (8.38, 9.15)	8.14 (7.86, 8.44)	.26
Serum creatine (μmol/L)	61.9 (60.3, 63.5)	61.3 (59.2, 63.4)	58.6 (56.9, 60.4)	.45

^a *p* values were age and sex adjusted.^b Mean ± standard deviation for such values.^c Not adjusted for itself.^d *p* < .001,^e *p* < .01,^f *p* < .05, compared with those without depressive symptoms, using Tukey's multiple comparison test for continuous variables (PROC GLM in SAS 9.1.3), and the Wald-Chi Square test for categorical variables (PROC LOGISTIC in SAS 9.1.3).

^gHigher score means more acculturated.

^hGeometric mean (95% confidence interval) for plasma and serum variables. CES-D = Center for Epidemiologic Studies Depression Scale.

TABLE 2

Adjusted Means of CES-D Score by Quartiles of Plasma Folate, Vitamin B12, PLP, and Homocysteine ^a

Metabolites	n	CES-D Score					
		Model 1 ^b		Model 2 ^c		Model 3 ^d	
		Mean (SE)	p	Mean (SE)	p	Mean (SE)	p
Folate, nmol/L							
Q1: 5.9-30.0	242	19.4 (0.8)		19.1 (1.0)		19.2 (1.0)	
Q2: 30.1-39.5	237	20.8 (0.9)		20.8 (1.0)		20.6 (1.1)	
Q3: 39.7-52.4	241	18.7 (0.9)		18.6 (1.0)		18.5 (1.1)	
Q4: 52.6-198.6	241	19.0 (0.9)		19.4 (1.0)		19.5 (1.1)	
<i>p</i> for trend ^e			.23		.56		.62
Vitamin B12, pmol/L							
Q1: 57.6-256.1	242	20.5 (0.9)		20.5 (1.0)		20.3 (0.9)	
Q2: 256.9-340.2	238	18.4 (0.9)		18.3 (1.0)		18.1 (0.9)	
Q3: 345.0-503.3	242	20.5 (0.9)		20.2 (1.0)		20.0 (0.9)	
Q4: 504.8-1712.2	239	18.4 (0.9)		19.0 (1.0)		19.0 (0.9)	
<i>p</i> for trend			.08		.21		.29
PLP, nmol/L							
Q1: 5.5-28.8	242	20.0 (0.8)		19.4 (1.0)		19.3 (1.0)	
Q2: 28.9-43.6	242	20.2 (0.9)		20.0 (1.0)		20.0 (1.0)	
Q3: 43.9-66.5	244	19.4 (0.9)		19.8 (1.0)		19.9 (1.0)	
Q4: 66.7-737.4	244	18.1 (0.9)		18.5 (1.0)		18.5 (1.1)	
<i>p</i> for trend			.003		.07		.08
Homocysteine, μmol/L							
Q1: 3.9-6.7	242	20.3 (0.9)		20.4 (1.0)		20.5 (1.1)	
Q2: 6.8-8.2	246	19.4 (0.9)		19.8 (1.0)		19.6 (1.0)	
Q3: 8.3-10.2	242	19.1 (0.9)		19.2 (1.0)		19.3 (1.0)	
Q4: 10.3-69.4	242	19.2 (0.8)		18.8 (0.9)		18.7 (1.0)	
<i>p</i> for trend			.35		.17		.17

^a Adjusted means were calculated with general linear models (PROC GLM in SAS 9.1.3) using the LSMEANS statement.^b Model 1, adjusted for age, sex, smoking and educational level.

^cModel 2, further adjusted for poverty status, acculturation score, alcohol use and physical activity score based on model 1.

^dModel 3, further adjusted for diabetes, hypertension, body mass index and creatine based on model 2.

^e*p* for trend was calculated by including log-transformed plasma variables (e.g. folate) as an independent variable in general linear models (PROC GLM in SAS 9.1.3).

CES-D = Center for Epidemiologic Studies Depression Scale; PLP = pyridoxal-5'-phosphate; SE = standard error; Q = quartile.

TABLE 3
Genotype Distributions and Their Associations with CES-D Score, Mild, and Moderate to Severe Depressive Symptoms^a

Gene and SNP ID no.	CES-D Score			Mild Depressive Symptoms			Moderate to Severe Depressive Symptoms				
	Genotype	n	Model	β (SE) ^b	p	Model	OR (95% CI) ^c	p	Model	OR (95% CI) ^c	p
<i>FOLH1</i>											
rs61886492	CC/CT/TT	862/87/2	dom	-4.5 (1.5)	.003	dom	0.51 (0.29, 0.89)	.02	dom	0.36 (0.18, 0.69)	.002
rs202712	CC/CT/TT	350/387/81	add	-1.3 (0.7)	.06	add	0.86 (0.65, 1.13)	.27	add	0.82 (0.61, 1.09)	.17
			dom	-1.9 (0.9)	.04						
rs647370	GG/GA/AA	475/379/64	add	-1.2 (0.7)	.07	add	0.95 (0.73, 1.24)	.72	add	0.84 (0.63, 1.12)	.23
<i>FPGS</i>											
rs10106	AA/AG/GG	244/449/235	add	-0.2 (0.6)	.73	add	0.97 (0.77, 1.22)	.77	add	0.96 (0.76, 1.22)	.73
<i>GGH</i>											
rs11545076	TT/TG/GG	476/339/74	add	0.5 (0.7)	.48	add	0.95 (0.73, 1.24)	.72	add	1.08 (0.82, 1.42)	.61
rs3758149	TT/TC/CC	522/358/74	add	0.3 (0.7)	.68	add	0.89 (0.69, 1.16)	.39	add	1.00 (0.77, 1.31)	.99
<i>MTHFR</i>											
rs1801131	AA/AC/CC	492/363/51	add	-1.0 (0.7)	.15	add	0.85 (0.65, 1.12)	.26	add	0.82 (0.62, 1.10)	.18
<i>MTR</i>											
rs16834521	AA/GA/GG	437/412/100	add	0.3 (0.7)	.47	add	1.08 (0.84, 1.38)	.57	add	1.13 (0.88, 1.46)	.34
rs1805087	AA/AG/GG	592/291/49	add	1.5 (0.7)	.04	add	0.93 (0.70, 1.24)	.62	add	1.24 (0.93, 1.66)	.15
			dom	1.9 (0.9)	.04						
<i>PCFT</i>											
rs2239907	GG/AG/AA	325/461/169	add	-0.1 (0.6)	.90	add	0.90 (0.71, 1.14)	.38	add	1.00 (0.78, 1.28)	.99
			rec	-2.3 (1.1)	.03						
<i>RFC1</i>											
rs2297291	AA/AG/GG	260/462/200	add	-1.0 (0.6)	.10	add	0.85 (0.67, 1.08)	.18	add	0.90 (0.71, 1.16)	.42
rs12659	CC/CT/TT	283/488/176	add	-0.4 (0.6)	.54	add	0.95 (0.74, 1.20)	.77	add	1.04 (0.81, 1.33)	.65

^a Genetic additive model is the default model, with 0 assigned for wild type (homozygote with major allele), 1 for heterozygote, and 2 for homozygote with minor allele. Only nominal significant results (nominal $p < .05$) are shown for genotypes tested by either the genetic dominant model (0 assigned for wild type and 1 for combined groups with at least one minor allele) or the genetic recessive model (0 assigned for genotype with at least one major allele and 1 for homozygote with minor allele). The p values indicated are nominal p values.

^b β (SE) was calculated by general linear model (PROC REG in SAS 9.1.3) after adjusting for age, sex, population admixture, smoking and educational level.

^c Multinomial logistic models were used to calculate odds ratios and 95% confidence intervals (PROC LOGISTIC with link = glogit in SAS 9.1.3) after adjustment for the same covariates as for β (SE).
 CES-D = Center for Epidemiologic Studies Depression Scale; SE = standard error; OR = odds ratio; CI = confidence interval; dom = dominant genetic model; add = additive genetic model; rec = recessive genetic model.

TABLE 4
Mediation of the Effect of *FOLH1* rs61886492 C>T on CES-D Score through Plasma Folate and Homocysteine^a

Mediator	Model 1 ^b		Model 2 ^c		Model 3 ^d	
	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p
Folate						
a	0.03 (-0.01, 0.08)	.18	0.03 (-0.01, 0.08)	.17	0.03 (-0.02, 0.07)	.21
b	-2.0 (-6.5, 2.6)	.40	-1.7 (-6.3, 2.8)	.46	-0.5 (-5.4, 4.5)	.85
c	-4.6 (-7.5, -1.6)	.002	-4.8 (-7.8, -1.9)	.001	-4.8 (-7.8, -1.8)	.002
c'	-4.5 (-7.4, -1.5)	.003	-4.8 (-7.8, -1.8)	.002	-4.8 (-7.8, -1.8)	.002
Bootstrapping indirect effect ^e	-0.05 (-0.40, 0.06)	-	-0.05 (-0.40, 0.07)	-	-0.01 (-2.8, 0.13)	-
Homocysteine						
a	-0.01 (-0.04, 0.03)	.77	0.001 (-0.03, 0.03)	.94		
b	-4.5 (-10.2, 1.17)	.12	-4.7 (-11.0, 1.52)	.14		
c	-4.5 (-7.4, -1.6)	.003	-4.8 (-7.8, -1.8)	.002		
c'	-4.5 (-7.4, -1.6)	.002	-4.8 (-7.8, -1.8)	.002		
Bootstrapping indirect effect ^e	-0.02 (-0.11, 0.27)	-	-0.004 (-0.23, 0.15)	-		

^a Both plasma folate and homocysteine were log-transformed before being entered as mediators in each model.

^b Model 1, adjusted for age, sex, population admixture, smoking, educational level, poverty status, acculturation score, alcohol use and physical activity score.

^c Model 2, further adjusted for diabetes, hypertension, body mass index and creatine based on model 1.

^d Model 3, further adjusted for plasma vitamin B12 and pyridoxal-5'-phosphate based on model 2.

^e Indirect effects were assessed using the bootstrapping approach suggested by Preacher and Hayes (30); Results are statistically significant if the 95% confidence interval does not contain zero.

CES-D = Center for Epidemiologic Studies Depression Scale; CI = confidence interval.