Assessing the association between the methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism and blood folate concentrations: a systematic review and meta-analysis of trials and observational studies1–5

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ABSTRACT
Background: The methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism is a risk factor for neural tube defects. The T allele produces an enzyme with reduced folate-processing capacity, which has been associated with lower blood folate concentrations.

Objective: We assessed the association between MTHFR C677T genotypes and blood folate concentrations among healthy women aged 12–49 y.

Design: We conducted a systematic review of the literature published from January 1992 to March 2014 to identify trials and observational studies that reported serum, plasma, or red blood cell (RBC) folate concentrations and MTHFR C677T genotype. We conducted a meta-analysis for estimates of percentage differences in blood folate concentrations between genotypes.

Results: Forty studies met the inclusion criteria. Of the 6 studies that used the microbiologic assay (MA) to measure serum or plasma (S/P) and RBC folate concentrations, the percentage difference between genotypes showed a clear pattern of CC > CT > TT. The percentage difference was greatest for CC > TT [S/P: 13%; 95% credible interval (CrI): 7%, 18%; RBC: 16%; 95% CrI: 12%, 20%] followed by CC > CT (S/P: 7%; 95% CrI: 1%, 12%; RBC: 8%; 95% CrI: 4%, 12%) and CT > TT (S/P: 6%; 95% CrI: 1%, 11%; RBC: 9%; 95% CrI: 5%, 13%). S/P folate concentrations measured by using protein-binding assays (PBAs) also showed this pattern but to a greater extent (e.g., CC > TT: 20%; 95% CrI: 17%, 22%). In contrast, RBC folate concentrations measured by using PBAs did not show the same pattern and are presented in the Supplemental Material only.

Conclusions: Meta-analysis results (limited to the MA, the recommended population assessment method) indicated a consistent percentage difference in S/P and RBC folate concentrations across MTHFR C677T genotypes. Lower blood folate concentrations associated with this polymorphism could have implications for a population-level risk of neural tube defects. Am J Clin Nutr 2015;101:1286–94.

Keywords: MTHFR, serum folate, plasma folate, red blood cell folate, neural tube defects

INTRODUCTION

Folate is a B vitamin (vitamin B-9) necessary for basic cellular functions (1) and is found naturally in foods such as dark-green leafy vegetables and legumes (1). A synthetic form, folic acid, is used in dietary supplements and fortified foods, including flour labeled as enriched and in ready-to-eat cereals (1). Research has indicated that adequate consumption of periconceptional folic acid can reduce the risk of a pregnancy affected by serious birth defects of the brain or spine (neural tube defects) (2). The Institute of Medicine, US Public Health Service, and US Preventive Services Task Force recommend that all women capable of pregnancy consume 400 µg folic acid daily for the prevention of neural tube defects (1, 3, 4).

The exact mechanism by which folate decreases the risk of neural tube defects is unknown; however, folate plays a major role in one-carbon metabolism, which is critical for cell division and other metabolic reactions (5). For example, in the one-carbon cycle, 5,10-methylenetetrahydrofolate is irreversibly reduced to 5-methyltetrahydrofolate (5-methyl-THF)6, the main circulating form of methionine.

6 Abbreviations used: BRQ II RIA, BioRad Quantaphase II radioimmunoassay; DFE, dietary folate equivalent; CrI, equal-tailed credible interval; HWE, Hardy-Weinberg equilibrium; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MA, microbiologic assay; MTHFR, methylenetetrahydrofolate reductase; PBA, protein-binding assay; RBC, red blood cell; S/P, serum or plasma; 5-methyl-THF, 5-methyltetrahydrofolate.
folate) in the presence of the enzyme methylenetetrahydrofolate reductase (MTHFR) (6). 5-Methyl-THF then donates its methyl group to homocysteine for the formation of methionine. Methionine can then undergo an enzymatic reaction to form S-adenosylmethionine, a universal methyl donor (5). The irreversible enzymatic reaction carried out by MTHFR plays a key role in regulating this cycle. However, a polymorphism of MTHFR, the 677C>T variant (rs1801133) in the gene encoding MTHFR, can reduce the enzymatic activity of MTHFR, resulting in decreased 5-methyl-THF concentrations, increased homocysteine concentrations, and reduced methylation capacity (7). The prevalence of the MTHFR 677TT genotype varies across ethnic groups and regions, ranging from <2% in West African and African American populations (8, 9) to >35% in some populations such as the northern Chinese and individuals of Mexican descent (8–10). Meta-analyses reported that mothers or infants with the TT genotype have significantly greater odds of a neural tube defect-affected pregnancy than those with the CC genotype (11–13).

Insufficient maternal blood folate concentrations have been associated with increased risk of neural tube defects (14). Given the association of the TT genotype with decreased blood folate concentrations (15, 16) and increased risk of neural tube defects (11, 12, 17), understanding the magnitude of the impact that the MTHFR 677C>T polymorphism may have on blood folate concentrations could be critical in assessing the population-level risk of neural tube defects. The objective of this systematic review was to assess the association between MTHFR genotype and blood folate concentrations among women of reproductive age (12–49 y) by applying Bayesian meta-analytic techniques to develop summary estimates of percentage differences between blood folate concentrations by genotype with the use of data from trials and observational studies.

METHODS

This systematic review followed guidelines from the Cochrane Handbook for Systematic Reviews (18) and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (19). We designed the protocol at the start of the review and documented any amendments thereafter (see Supplemental Material). The flow of record management steps is presented in Figure 1 and revisited in the Results.

Search strategy

With the assistance of a research librarian, we searched the following bibliographic databases for English-language literature from 1992 to March 2014: the Cochrane Library, CINAHL, Embase, POPLINE, PubMed, and Web of Science. Literature published before 1992 was not eligible because this is before the earliest documented genotyping of the MTHFR 677C>T mutation (20). The search strategy for Embase included search terms in the following areas: folic acid, blood folate [serum folate or red blood cell (RBC) folate or plasma folate], MTHFR (methylenetetrahydrofolate, MTHFR), dietary folate intake (intake or diet* or supplement*), folic acid intake, neural tube defects (neural tube defect or spina bifida or anencephaly), and women of childbearing age (Childbear* or wom*n or female* or girl* or pregnan*). This method was adapted for other databases as appropriate (for full search strategies for all databases, see Supplemental Material). We also searched reference lists of all articles in full-text review to identify additional potentially relevant studies.

FIGURE 1  Record management and selection flow diagram for the systematic review and meta-analysis on the association between the MTHFR 677C>T polymorphism and blood folate concentrations. MTHFR, methylenetetrahydrofolate reductase.
Controlled trials and cohort, case-control, and cross-sectional studies were eligible study designs. Population eligibility criteria were nonpregnant, nonlactating females 12–49 y of age. With the understanding that studies do not always provide the pregnancy and lactation status of their population, we considered studies for inclusion if status was not explicitly stated. Studies with mixed sex/age populations were included if data for nonpregnant, nonlactating females 12–49 y of age could be extracted separately or if >50% of the participants were female and the median/mean age was 12–49 y. Inclusion criteria for the reported study outcomes included blood folate concentrations [serum, plasma, or red blood cell (RBC) folate], blood folate assay method, and MTHFR C677T genotype frequency. Studies were excluded if they failed to meet any of the inclusion criteria or if they included unhealthy populations.

We contacted study authors for additional information (i.e., sex/age-stratified data, blood folate assay details, and blood folate concentrations by genotype), if it was missing or not presented in the format required for this review. Unpublished data received from contacted authors were also eligible for inclusion.

After author contact and receipt of additional data, studies were categorized into 2 tiers. Tier 1 was defined as studies with data limited to females aged 12–49, whereas tier 2 included studies with data in which <50% of the study population also included men or females outside our target age range. Studies without blood folate assay details or those using the Bio-Rad Quantaphase II radioimmunoassay (BRQ II RIA) were also classified as tier 2, because this assay has been shown to recover folate species differentially compared with microbiologic assay (MA) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods (21).

Selection of studies

The initial abstract review (wave 1) included criteria for this review and a related review by Marchetta et al. (CM Marchetta, National Center for Birth Defects and Developmental Disabilities at CDC, unpublished data, 2014). In wave 1, 3 teams of 2 reviewers (CMM and JR, RJB and HCH, PM and JM) independently reviewed one-third of all abstracts identified in the database results, screening records potentially relevant for inclusion in the 2 reviews. For clarification, the term “record” is used because multiple records may exist for a single study. Wave 1 abstracts underwent a second abstract review (wave 2) in which inclusion/exclusion criteria specific to this review were applied independently by 3 reviewers (BLT, JM, and PM). The same reviewers conducted full-text reviews to determine eligibility and to identify information necessary for author contact. Two attempts were made to contact all authors for additional information. In cases in which authors provided individual-level raw data, we did not conduct analyses and these data were not included in the meta-analyses. At each step, reviewers resolved any disagreement by discussion.

Data extraction

Three reviewers (BLT, JM, and PM) each abstracted one-third of the studies using a standardized abstraction form for the following data: study design, study location, sample size, study years, participant characteristics (age, ethnicity), intervention (if applicable; i.e., consumption of natural food folate or a folic acid–containing supplement), population selection methods, MTHFR C677T genotype, expected population genotype distribution (calculated and reported, if available) using the Hardy-Weinberg equilibrium (HWE), blood folate concentrations at baseline and follow-up by genotype, blood folate assay method, total folate intake (both natural food folate and synthetic folic acid), and statistical analyses. For any studies that did not report an HWE P value, we calculated it as a quality control measure.

Reported total folate intakes were converted to dietary folate equivalents (DFEs) by using the equation 1.7 μg DFE = 1 μg folic acid because of the greater bioavailability of folic acid than natural dietary folate (22). We converted all serum, plasma, and RBC folate concentrations to nanomolars per liter if presented originally as nanograms per milliliter using the equation 1 ng/mL = 2.266 nmol/L.

Quality assessment of studies

A risk-of-bias assessment was conducted for all included studies; for randomized controlled trials, risk of bias was assessed by using the Cochrane Handbook for Systematic Reviews of Interventions tool (18). Nonrandomized controlled trials, cohort-studies, and cross-sectional studies were assessed by using the Item Bank on Risk of Bias and Precision of Observational Studies from RTI International (23). Both tools were adapted to the study objectives with consensus from all reviewers and piloted before use (for the adapted RTI tool, see Supplemental Table 1). Two reviewers (YPQ and JG) independently conducted the risk-of-bias assessment and resolved any disagreements by discussion.

The risk-of-bias score (low, moderate, high) was assessed separately by outcome [serum or plasma (S/P) folate or RBC folate concentrations] by using several risk-of-bias categories or “domains.” Domain details for each respective tool are available elsewhere (18, 23). Domains were considered low risk of bias if all questions within a domain were graded as low, moderate risk of bias if ≥1 questions were graded as moderate, or high risk of bias if ≥1 questions were graded as high. The same convention was applied across all domains to assign an overall summary score for each study’s blood folate concentration outcome or outcomes. Most randomized controlled trials failed to provide sufficient information on 2 domains (sequence generation and allocation concealment) to assess the risk of selection bias. These domains were given a label of “unclear” and did not contribute to the overall risk-of-bias summary score for the study.

Data synthesis for statistical analysis

For the purposes of the meta-analyses, reported S/P folate concentration values were rounded to the nearest tenth decimal and RBC folate concentration values were rounded to the nearest integer. Reported measures of central tendency and sampling variability differed between studies. As a result, the reported measures from each study were standardized before analysis to correspond to the mean concentration on the natural log scale and the SE (also on the log scale) associated with that mean (24).

Because variations exist between MA methods (25), blood folate concentrations were standardized to increase comparability between studies; original values reported by authors are presented in
Supplemental Tables 2 and 3. All values from studies that used the Tamura assay method (denoted as “X” in the equation below) (26) were adjusted to the Molloy and Scott/O’Broin and Kelleher method (denoted as “Y” in the formula) (27, 28) by using the following weighted Deming regression equations (C Pfeiffer, National Center for Environmental Health at CDC, personal communication, 2014):

Serum folate (nmol/L): \[ Y = 1.4209(95\% CI: 0.66, 2.18) \times X + 0.7854(95\% CI: 0.77, 0.80) \]  
(1)

RBC folate (nmol/L): \[ Y = 0.7297(95\% CI: 0.70, 0.76) \times X + 352.219(95\% CI: 308.12, 396.32) \]  
(2)

To reflect the uncertainty in this conversion, the sampling variability of the coefficients in the above models was propagated to the standardized SEMs of the transformed log concentration by using Taylor series approximation.

Although it is known that blood folate concentrations measured by using commercial protein-binding assays (PBAs) are subject to wide variation (29, 30), the reported PBA values were used (i.e., no adjustment) because there are no validated standardization formulas across commercial kits. Although validated standardization formulas are available to convert BRQ II RIA to the Pfeiffer MA method (21) (which then could be converted to the Molloy/O’Broin MA method), we were unable to convert values from studies using the BRQ II RIA because those formulas require the knowledge of disaggregated blood folate concentration and MTHFR genotype data.

Stratifications and sensitivity analyses

Because of incomparability between MA and PBA results, all meta-analyses were stratified by the 2 folate assay method types. In addition to the primary outcome, we also conducted sensitivity analyses by stratifying on the study’s risk of bias (low vs. all studies) and study tier (tier 1 vs. both tier 1 and tier 2). In addition, the presence of mandatory folate acid fortification legislation in the country where the study took place was accounted for in the statistical modeling approach as described in the next section. Fortification status (mandatory legislation or no mandatory legislation present) was classified on the basis of the Food Fortification Initiative’s database on global fortification practice (31).

Statistical analysis

We conducted a series of Bayesian meta-analyses to develop summary estimates of the percentage differences in blood folate concentrations by MTHFR C677T genotype. In the analysis, the mean of the natural log of the observed folate concentration for each genotype in study \( i \) and genotype \( j \) (\( j \) corresponding to either CC, CT, or TT) was assumed to be a sample from a normal distribution with mean log blood folate (mBF\(_{ij}\)) and known SD based on the transformed value of the SEM log concentration as reported in the study results. The impact of genotype and fortification status on the mean log concentrations was modeled as

\[
\log(\text{mBF}_{ij}) = B0 + B1 \times \text{CT} + B2 \times \text{TT} + B3 \times \text{FORT}_i + g_i
\]

where the variable \( \text{CT} \) takes the value 1 if the mBF\(_{ij}\) result is associated with the CT genotype and 0 otherwise and \( \text{TT} \) is similarly defined for the TT genotype. The variable \( \text{FORT}_i \) takes the value 1 if the study was conducted in the period and setting with mandatory folic acid fortification and 0 otherwise. In the model, \( g_i \) corresponds to a random effect for study \( i \), reflecting unaccounted-for interstudy heterogeneity and inherent similarities between results reported in the same study. Note that with the use of this model, the estimates of the percentage difference between blood folate concentrations are given by the following formulas:

\[
\text{CC} \text{ vs. } \text{CT} \% \text{ difference} = \exp(B1) - 1
\]

(4)

\[
\text{CC} \text{ vs. } \text{TT} \% \text{ difference} = \exp(B2) - 1
\]

(5)

\[
\text{CT} \text{ vs. } \text{TT} \% \text{ difference} = \exp(B1 - B2)
\]

(6)

We assumed noninformative normal prior distributions for the variables \( B0, B1, B2, \) and \( B3 \) with a mean of 0 and an SD of \( \sim 32 \) [corresponding to a precision, i.e., 1 over the SE squared, of 1/(32\(^2\)) of 0.001]. The prior distribution for the study-level random effects was also assumed to be normal with a mean of 0 and an SD of \( \text{std}_w \). To complete the model specification, we assumed \( \text{std}_w \) to follow a uniform prior distribution bounded by 0 and 100. Because assumptions on the variance of the study-level random effects can have a significant impact on the resulting estimates, as a sensitivity assessment we also considered a half-t distribution and more informative prior distributions for \( \text{sig}_w \). Estimates of the median \( \text{CC}, \text{CT}, \) and \( \text{TT} \) blood folate concentrations [corresponding to the variables \( \exp(B0), \exp(B1), \) and \( \exp(B2) \)] are presented but the focus is on the estimated percentage difference between the genotypes, under the assumption that these differences are likely constant across populations. Posterior estimates of these percentage differences are summarized by using the median of the posterior samples and a 95% equal-tailed credible interval (CrI; defined as the range between the 2.5th and 97.5th percentiles of the posterior samples). In addition, we also estimated the posterior probability that the percentage differences between genotypes exceeds zero.

We used a Monte Carlo Markov chain approach to develop posterior estimates of the percentage difference in blood folate concentrations between genotype groups, with sampling chains conducted to a length of 100,000 iterations. Three chains were used for each analysis, with each chain starting at disparate initial values for all variables to allow for assessment of potential convergence. The first 50,000 samples from each chain were discarded to increase the potential of sampling from the posterior distribution, and every fifth sample was retained to reduce autocorrelation. Therefore, posterior estimates were based on 30,000 samples (10,000 samples from each of 3 chains). We
assessed convergence using visual inspection of variable-specific history plots for the chains, as well as Gelman-Rubin diagnostic plots (34). Comparisons of posterior predicted distributions for the percentage differences to the observed data indicated that the assumed model was consistent with the variation observed in the range of both RBC and S/P folate concentrations in the selected studies. Analyses were conducted by using OpenBUGS 3.2.2 (35).

RESULTS

Study characteristics

The search strategy yielded a total of 11,237 records; after removal of duplicate records, 9224 records remained for title and abstract review. A full PRISMA record management flow is presented in Figure 1. After contacting authors, we found a total of 40 studies that were eligible. All records identified were unique studies. Therefore, from this point forward, the term “study” will be used.

Overall, 29 studies were considered tier 1, and 11 studies were considered tier 2 (see Supplemental Tables 2 and 3). The countries represented in the included studies were Australia, Brazil, Canada, China, Costa Rica, the Czech Republic, Finland, France, Germany, Ghana, Greece, India, Ireland, Italy, Japan, Malaysia, Mexico, The Netherlands, New Zealand, Pakistan, Portugal, Spain, Taiwan, the United Kingdom, and the United States. There were 8 controlled feeding studies, 2 cohort studies, and 30 cross-sectional studies. Interventions ranged from 1 d (36) to 6 mo (37) (median = 14 wk), and daily dosages of total folate ranged from 115 μg DFEs (38) to 6800 μg DFEs (37).

Among all 40 studies, 3 studies included populations who were prescreened for genotype (CC or TT) (36, 38, 39), 2 studies were conducted in populations with a low prevalence of the T allele and thus had no women with TT genotype (40, 41), and 3 studies were conducted in population groups with an MTHFR 677TT genotype distribution that was significantly different from expected (HWE P value <0.05) (42–44).

Among the 19 studies assessing RBC folate concentrations, 3 studies were classified low risk of bias (17, 45, 46), 14 as moderate risk of bias, and 2 as high risk of bias (47, 48). Of the 39 studies assessing S/P folate concentrations, 3 studies were classified low risk of bias (17, 45, 46), 31 as moderate risk of bias, and 5 as high risk of bias. Detailed risk-of-bias results are shown in Supplemental Tables 4–5.

Among all 40 studies included in this review, 2 were excluded from meta-analyses for the following reasons: blood folate concentrations were not plausibly on the basis of reported total folate intake and verification was not possible (44) and missing statistical data (nonreport of an error estimate) (49). Of the remaining 38 studies, 7 studies reported using MAs, 28 reported using PBAs (e.g., chemiluminescent immunoassay), 1 reported use of both a PBA and LC-MS/MS to measure plasma folate and RBC folate, respectively (50), and 2 did not report assay method used (48, 51). The 2 unknown assay studies and 3 PBA studies that specifically used the BRQ II RIA (52–54) were restricted to tier 2 analyses. In the case of the study that used 2 methods, RBC folate values were not used in meta-analyses because it was the only study to use LC-MS/MS (50). However, plasma folate values were included in the PBA analyses.

Meta-analysis results

RBC folate and S/P folate concentrations

Results of studies using the MA indicated an additive effect of the MTHFR 677C>T polymorphism on RBC folate concentrations. The percentage difference between estimated RBC folate concentrations was greatest for the CC vs. TT genotype comparison, with CC exceeding TT by an estimated 16% (95% CrI: 12%, 20%). Similarly, concentrations in the CC group exceeded those with the CT allele by an estimated 8% (95% CrI: 4%, 12%), and individuals with the CT allele had concentrations in excess of the TT group by an estimated 9% (95% CrI: 5%, 13%). These results indicate an overall RBC folate concentration pattern of CC > CT > TT (Table 1). We estimate a 100% probability that the percentage difference in RBC folate concentrations was greater than zero for all genotype comparisons. The CC > CT > TT pattern was consistent after stratification by tiers (Supplemental Tables 6 and 7). S/P folate concentrations measured with the MA also indicated an additive effect of the MTHFR 677C>T polymorphism, CC > CT > TT, with estimated probabilities of CC concentrations being greater than CT and TT, and CT greater than TT, all exceeding 98% (Table 1). For both RBC and S/P folate evaluations, altering the assumptions on the prior distribution of the SD of the study-level random effects had virtually no impact on the resulting posterior estimates of percentage difference between genotypes.

Estimated summary median S/P and RBC folate concentrations and credible intervals were also modeled (Table 1), but given the highly heterogeneous study populations included in the systematic review (reflecting differing folate intake practices as well as nonrepresentative population sampling), these values should not be considered in any way representative of the populations included. The values instead serve as an illustrative example of the blood folate concentration patterns associated with the MTHFR C677T genotype.

PBAs

Results from PBA meta-analyses are presented in Supplemental Table 8. Compared with MA results, RBC folate concentrations measured with PBAs exhibited a clear reverse pattern of CC < CT < TT, as shown by a negative estimated percentage difference for genotype comparisons of CC vs. TT and CT, and CT vs. TT (RBC and S/P folate concentration patterns by individual PBA studies are shown in Supplemental Table 9). Unlike RBC folate concentration results, the pattern of genotype differences for S/P folate concentrations did not differ by assay. The magnitude of estimated percentage differences was greater with PBAs than was seen with MAs (e.g., PBA: CC > TT; 20%; MA: CC > TT, 13%).

DISCUSSION

Our analyses suggest that the association between MTHFR C677T genotype and blood folate concentrations in women aged 12–49 y shows an additive model, whereby blood folate concentrations follow a CC > CT > TT pattern, not a recessive CC/CT > TT pattern. The additive model indicates that the inheritance of 1 recessive allele (CT genotype) is also associated with lower concentrations, intermediate to CC and TT. We would caution against assuming a recessive model (grouping CC
The effect of and CT difference between TT a 16% increase in RBC folate concentration (the percentage pregnancy increases on the log scale (14, 57, 58). For example, concentrations decrease, the risk of a neural tube defect–affected risk is dependent on folate intake because as RBC folate con-
genotype. The MTHFR significantly higher (56); however, evidence has suggested that RBC folate con-
centrations are a result of both folate intake and MTHFR supplementation recommendations for the prevention of neural tube defects are folate-sensitive (60).

Implications for neural tube defect prevention
Clinical folate deficiency (associated with megaloblastic anemia) is generally defined as RBC folate concentrations <340 nmol/L (56); however, evidence has suggested that RBC folate concentrations for the prevention of neural tube defects are significantly higher (≥906 or 1000 nmol/L) (14, 57). RBC folate concentrations are a result of both folate intake and MTHFR genotype. The MTHFR genotype impact on neural tube defect risk is dependent on folate intake because as RBC folate concentrations decrease, the risk of a neural tube defect–affected pregnancy increases on the log scale (14, 57, 58). For example, a 16% increase in RBC folate concentration (the percentage difference between TT and CC genotypes found in our results) from 500 to 580 nmol/L (a lower folate intake setting) could result in an estimated 4.2–7.6 per 10,000 fewer neural tube defect–affected births. A corresponding 16% increase from 1100 to 1267 nmol/L (a higher folate intake setting) only results in an estimated 1.3–1.6 per 10,000 reduction and may have limited additional benefit for the reduction in risk of neural tube defects (57). Therefore, the polymorphism’s impact on neural tube defect risk would be greater in populations with lower folate in-
takes, consistent with other research on MTHFR and disease risk (59). However, research has indicated that not all neural tube defects are folate-sensitive (60).

It is unclear if the results presented here should affect folic acid supplementation recommendations for the prevention of neural tube defects. In a 6-mo supplementation trial of the recommended 400 μg of folic acid/d, women with the TT genotype were able to reach a mean RBC folate concentration of 927 nmol/L (95% CI: 857, 1002 nmol/L) (17). This suggests that some women with the TT genotype are able to reach RBC folate concentrations that are considered optimally protective of a neural tube defect–affected pregnancy [≥906 nmol (14)] with long-term, high-compliance supplementation. It is not clear if women who did not reach 906 nmol/L require additional folic acid intake, a longer intervention period, or other sustained interventions (e.g., staple food fortification with folic acid). However, a community intervention program in China showed that high-compliance use of 400 μg of folic acid/d did result in neural tube defect birth prevalence in a population with a high TT genotype prevalence that was similar to a population with low TT genotype prevalence (57, 60). There is limited evidence to conclude whether a mandatory folic acid fortification program could mitigate the risk conferred by the polymorphism (61–63). More research is needed to understand how to bridge gaps in blood folate concentrations because of the MTHFR 677C>T polymorphism.

PBA limitations
The WHO recommends the MA for population assessment of blood folate concentrations (64). Inconsistencies in blood folate assessment have been well documented when comparing across and within the 3 main analytic methods (MA, PBAs, LC-MS/MS) (21, 29, 30, 65–68). Differences between assay methods occur because blood folate concentrations are not a measure of a single folate species but a summary measure of many different folate species (e.g., 5-methyl-THF, 5,10-methyl-THF, etc.).

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td><strong>Blood folate concentrations measured by using the microbiologic assay in women aged 12–49 y by genotype</strong></td>
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<tr>
<td><strong>RBC folate</strong></td>
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<td>No. of observations</td>
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<td>No. of studies</td>
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<tr>
<td>Individuals across studies, n</td>
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<tr>
<td>Percentage difference between estimated median blood folate concentrations (95% CrI)</td>
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<td>CC &gt; TT</td>
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<td>CC &gt; CT</td>
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<td>CT &gt; TT</td>
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<tr>
<td>Estimated median blood folate concentration, nmol/L (95% CrI)</td>
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<td>CC</td>
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<td>CT</td>
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<td>TT</td>
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<td>Probability that the difference in estimated median blood folate concentration by genotype is greater than zero, %</td>
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<td>CC &gt; TT</td>
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<td>CC &gt; CT</td>
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<td>CT &gt; TT</td>
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1. Bayesian analyses were used for statistical estimates. RBC, red blood cell; S/P, serum or plasma; 95% CrI, equal-tailed credible interval (defined by the 2.5th and 97.5th percentiles of the posterior distributions for the estimated values).
2. Defined as a single measurement of blood folate concentrations in a study. In studies measuring blood folate concentrations by genotype, there may have been as many as 3 observations (RBC folate concentrations for CC, CT, and TT) for 1 study.
3. Including studies: references 15, 17, 38, 45, 46, and 55.
4. Percentage differences were estimated by using the exponentiated variable. For example, CC vs. TT % difference = \( \text{exp}(B1) - 1 \).
Chromatography-based assays (e.g., LC-MS/MS) are the only methods that quantify individual folate species; more commonly used assays (e.g., PBAs and MA) provide a summary measure of total folate.

Differential recovery across assay methods and differential distribution across MTHFR C677T genotypes of individual folate species can result in substantial variation in total blood folate concentrations. Both of these concepts have been shown previously (21, 65). Compared with MA and LC-MS/MS, the BRQ II RIA produces misleadingly low (or high) whole-blood folate concentrations because of under-recovery of 2 species (5-methyl-THF, 5-formyl-THF) and simultaneous over-recovery of non–methyl-THF species (21). Compared with MA, the BRQ II RIA underestimates whole-blood folate concentrations in individuals with CC and CT genotypes, on average, by 43–46% but only by 26–35% in individuals with the TT genotype (who have greater proportions of non–5-methyl-THF species), leading to a reversed CC < CT < TT whole-blood folate pattern (21). No assay differences by genotype were found with serum folate (21).

The reversed RBC folate concentration pattern by genotype still remained in meta-analyses, despite no data that used the BRQ II RIA, suggesting that a similar differential folate recovery mechanism exists in other PBAs. Other than for the BRQ II RIA, to our knowledge no other formulas exist to improve PBA comparability with MA. As shown here and elsewhere (30, 66–68), blood folate concentration assessment with PBAs has serious limitations that compromise interpretation. Considering the widespread use of PBAs in population assessment, there is a need for standardization between and among assays and a globally harmonized blood folate methodology applicable in multiple settings.

Strengths and limitations

Despite limited data, the use of a Bayesian approach in implementing the random-effects meta-analyses had a number of advantages. Incorporating the random effects increased the likelihood of accounting for interstudy heterogeneity and addressed the potential correlation among results reported in the same study. The flexibility of the Monte Carlo Markov chain estimation approach allowed direct sampling from the posterior distribution of the percentage differences in blood folate concentrations across genotype groups, without the difficulty in approximating the uncertainty associated with these estimates (if using other methods). Other strengths included standardization of blood folate concentrations across different MAs for increased comparability and the contribution of a considerable amount of data from the contacted study authors.

Limitations of our study include the inability to improve PBA interassay comparability (because validated adjustment formulas for PBAs do not exist), crude controlling for folic acid exposure via classifying countries by folic acid fortification legislation, and potential literature gaps that could have increased data for analyses [e.g., healthy control individuals from case-control studies, non–English-language studies, and unpublished (gray) literature]. Theoretically, studies in men and other age groups could have also contributed data, because it is estimated that blood folate concentrations are affected <15% by age and sex (69), but we focused our review on women of reproductive age given their risk of a neural tube defect–affected pregnancy.

Conclusions

On the basis of our findings, researchers should use caution when interpreting RBC folate concentrations assessed by PBAs. Our work supports previous literature that also suggested that PBAs may not reflect true blood folate concentrations without adjustments for differential folate recovery and MTHFR C677T genotype (21, 25, 65). There is a need for standardizing blood folate assay methods to improve comparability across ethnic/geographic populations.

Our meta-analyses indicate that blood folate concentrations differ between women aged 12–49 y by MTHFR C677T genotype and provide an estimate of the magnitude of the polymorphism’s impact on blood folate concentrations. Although folic acid interventions attenuate neural tube defect–affected pregnancy risk conferred by the C677T polymorphism, further research is necessary to identify programmatic details (e.g., supplement dosing durations, fortification amounts) to improve the effectiveness of such prevention efforts.

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