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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 studies[1–5]

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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

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[2] BLT, CMM, and YPQ were supported by the CDC and in part by an appointment to the Research Participation Program at CDC administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and CDC.

[3] Supplemental Material and Supplemental Tables 1–9 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

[4] The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.

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[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.

Received September 22, 2014. Accepted for publication February 9, 2015.
First published online March 18, 2015; doi: 10.3945/ajcn.114.099994.

Supplemental Material can be found at:
http://ajcn.nutrition.org/content/suppl/2015/03/18/ajcn.114.099994.DCSupplemental.html

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 do 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.

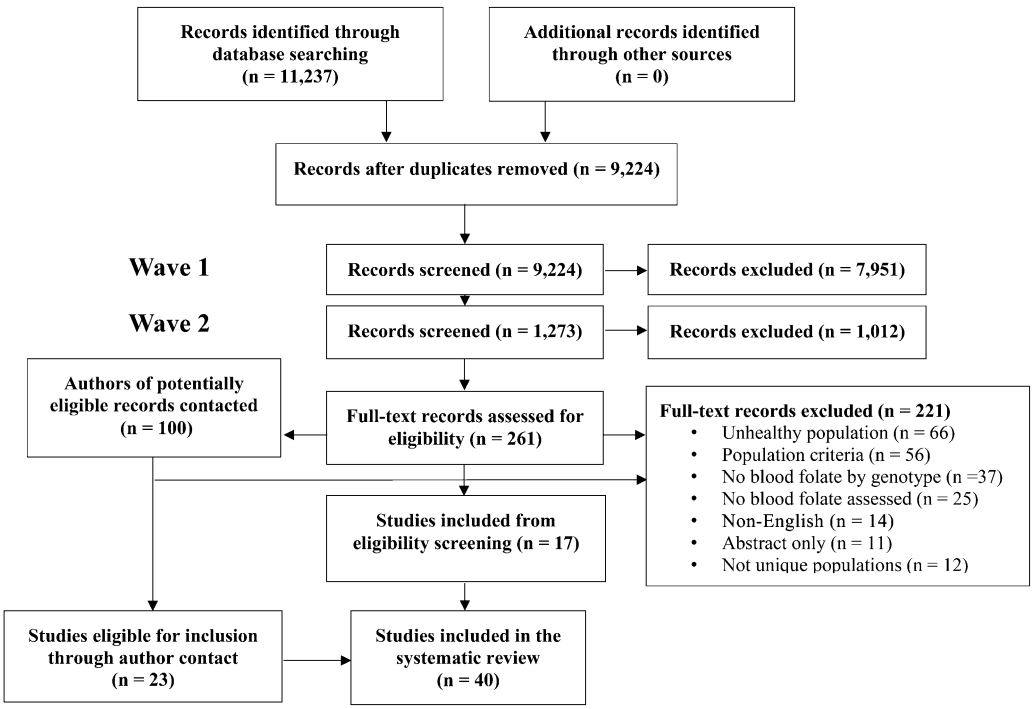

FIGURE 1 Record management and selection flow diagram for the systematic review and meta-analysis on the association between the *MTHFR* 677C>T polymorphism and blood folate concentrations. *MTHFR*, methylenetetrahydrofolate reductase.

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):

$$\text{Serum folate (nmol/L): } Y = 1.4209(95\%\ \text{CI: } 0.66, 2.18) \times X + 0.7854(95\%\ \text{CI: } 0.77, 0.80) \quad (1)$$

$$\text{RBC folate(nmol/L): } Y = 0.7297(95\%\ \text{CI: } 0.70, 0.76) \times X + 352.219(95\%\ \text{CI: } 308.12, 396.32) \quad (2)$$

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 folic 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

$$\log(\text{mBF}_{ij}) = \text{B0} + \text{B1} \times \text{CT} + \text{B2} \times \text{TT} + \text{B3} \times \text{FORT}_i + g_i \quad (3)$$

where the variable *CT* takes the value 1 if the mBF_{ij} result is associated with the *CT* genotype and 0 otherwise and *TT* is similarly defined for the *TT* genotype. The variable $FORT_i$ 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:

$$CC \text{ vs. } CT \text{ \% difference} = \exp(\text{B1}) - 1 \quad (4)$$

$$CC \text{ vs. } TT \text{ \% difference} = \exp(\text{B2}) - 1 \quad (5)$$

$$CT \text{ vs. } TT \text{ \% difference} = \exp(\text{B1} - \text{B2}) \quad (6)$$

We assumed noninformative normal prior distributions for the variables B0, B1, B2, and B3 with a mean of 0 and an SD of ~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 std_g. To complete the model specification, we assumed std_g 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 sig_g (32, 33). Estimates of the median *CC*, *CT*, and *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* C677T 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 plausible 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*

TABLE 1
Blood folate concentrations measured by using the microbiologic assay in women aged 12–49 y by genotype[1]

	RBC folate	S/P folate
No. of observations[2]	16	16
No. of studies[3]	6	6
Individuals across studies, *n*	908	1538
Percentage difference between estimated median blood folate concentrations[4] (95% CrI)		
CC > *TT*	16 (12, 20)	13 (7, 18)
CC > *CT*	8 (4, 12)	7 (1, 12)
CT > *TT*	9 (5, 13)	6 (1, 11)
Estimated median blood folate concentration, nmol/L (95% CrI)		
CC	759 (619, 1052)	15 (9, 24)
CT	697 (568, 971)	14 (9, 22)
TT	636 (518, 885)	13 (8, 21)
Probability that the difference in estimated median blood folate concentration by genotype is greater than zero, %		
CC > *TT*	100	100
CC > *CT*	100	99
CT > *TT*	100	99

[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]Included 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 = exp(B1) − 1.

and *CT* genotypes together for analyses) when determining the effect of *MTHFR* 677C>T polymorphism and its associated outcomes. Instead, adequate sample sizes for each genotype are needed to detect the polymorphism's additive effects.

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 intakes, 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-methenyl-THF, etc.).

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.

We thank all of the authors who provided additional data for this review including: Drs. Antonella Agodi, Martina Barchitta, and Annalisa Quattrocchi; Dr. Georg Alfthan; Dr. Marie Caudill; Dr. Inés García-García; Dr. Siobhan Hickling; Dr. Ileana Holst-Schumacher; Dr. Liia Iacoviello; Dr. Mohammad Perwaiz Iqbal; Dr. Linda Kelemen; Dr. Viktor Kozich; Dr. Patrick Liu; Dr. Su Peng Loh; Dr. Chizuko Maruyama; Drs. Helene McNulty and Leane Hoey; Dr. Denish Moorthy; Dr. Torbjörn Nilsson; Dr. Constantina Papoutsakis; Dr. Maria Pufulete; Dr. Rajiva Raman; Dr. Yvo Smulders; Dr. Ramasamyiyer Swaminathan; Dr. Luisa Elvira Torres-Sánchez; Dr. Bruno Zappacosta; and Dr. Andrea Zijno. We also thank Dr. Christine Pfeiffer of the National Center for Environmental Health at CDC for blood folate laboratory expertise and assay adjustment formulas, as well as our colleagues, Drs. Luz Maria De-Regil and Juan Pablo Peña-Rosas at the Evidence and Program Guidance Unit of WHO, for providing valuable input in the development of the protocol.

The authors' responsibilities were as follows—AMC, KSC, and HCH: designed the research; BLT, AMC, CMM, JM, PM, JG, YPQ, RJB, JR, and HCH: conducted the research; BLT, OJD, and JG: analyzed the data; BLT, OJD, KSC, and HCH: wrote the manuscript; and BLT and HCH: had primary responsibility for final content. None of the authors had any conflicts of interest.

REFERENCES

1. Institute of Medicine. Folate. In: Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, vitamin B12, pantothenic acid, biotin, and choline. Washington (DC): National Academies Press; 1998. p. 196–303.
2. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet 1991; 338(8760):131–7.
3. CDC. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Recomm Rep 1992;41(RR-14):1–7.
4. US Preventive Services Task Force. Folic acid for the prevention of neural tube defects: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2009;150:626–31.
5. Stover PJ. Physiology of folate and vitamin B12 in health and disease. Nutr Rev 2004;62(6 Pt 2):S3–12; discussion S3.
6. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr 2012;3:21–38.
7. Quinlivan EP, Davis SR, Shelnutt KP, Henderson GN, Ghandour H, Shane B, Selhub J, Bailey LB, Stacpoole PW, Gregory JF III. Methylenetetrahydrofolate reductase 677C->T polymorphism and folate status affect one-carbon incorporation into human DNA deoxynucleosides. J Nutr 2005;135:389–96.

8. Guéant-Rodriguez RM, Gueant JL, Debard R, Thirion S, Hong LX, Bronowicki JP, Namour F, Chabi NW, Sanni A, Anello G, et al. Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study in Mexican, West African, and European populations. Am J Clin Nutr 2006;83:701–7.
9. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol 2000;151:862–77.
10. Ogino S, Wilson RB. Genotype and haplotype distributions of MTHFR677C>T and 1298A>C single nucleotide polymorphisms: a meta-analysis. J Hum Genet 2003;48:1–7.
11. Yan L, Zhao L, Long Y, Zou P, Ji G, Gu A, Zhao P. Association of the maternal MTHFR C677T polymorphism with susceptibility to neural tube defects in offsprings: evidence from 25 case-control studies. PLoS ONE 2012;7:e41689.
12. Zhang T, Lou J, Zhong R, Wu J, Zou L, Sun Y, Lu X, Liu L, Miao X, Xiong G. Genetic variants in the folate pathway and the risk of neural tube defects: a meta-analysis of the published literature. PLoS ONE 2013;8:e59570.
13. Yadav U, Kumar P, Yadav SK, Mishra OP, Rai V. Polymorphisms in folate metabolism genes as maternal risk factor for neural tube defects: an updated meta-analysis. Metab Brain Dis 2015;30(1):7–24.
14. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. JAMA 1995;274: 1698–702.
15. Molloy AM, Daly S, Mills JL, Kirke PN, Whitehead AS, Ramsbottom D, Conley MR, Weir DG, Scott JM. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: implications for folate intake recommendations. Lancet 1997; 349:1591–3.
16. van der Put NM, Steegers-Theunissen RP, Frosst P, Trijbels FJ, Eskes TK, van den Heuvel LP, Mariman EC, den Heyer M, Rozen R, Blom HJ. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995;346:1070–1.
17. Crider KS, Zhu JH, Hao L, Yang QH, Yang TP, Gindler J, Maneval DR, Quinlivan EP, Li Z, Bailey LB, et al. MTHFR 677C->T genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation. Am J Clin Nutr 2011;93:1365–72.
18. Higgins J, Green SE, editors. Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0. The Cochrane Collaboration; 2011 [cited 2013 Apr 2]. Available from: www.cochrane-handbook.org.
19. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 2009;62:1006–12.
20. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
21. Fazili Z, Pfeiffer CM, Zhang M, Jain RB, Koontz D. Influence of 5,10-methylenetetrahydrofolate reductase polymorphism on whole-blood folate concentrations measured by LC-MS/MS, microbiologic assay, and bio-rad radioassay. Clin Chem 2008;54:197–201.
22. Institute of Medicine. Pantothenic acid, biotin, and choline. In: Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12. Washington (DC): National Academies Press; 2000.
23. Viswanathan M, Berkman ND. Development of the RTI item bank on risk of bias and precision of observational studies. J Clin Epidemiol 2012;65:163–78.
24. Souverein OW, Dullemeijer C, van't Veer P, van der Voet H. Transformations of summary statistics as input in meta-analysis for linear dose-response models on a logarithmic scale: a methodology developed within EURRECA. BMC Med Res Methodol 2012;12:57.
25. Pfeiffer CM, Zhang M, Lacher DA, Molloy AM, Tamura T, Yetley EA, Picciano MF, Johnson CL. Comparison of serum and red blood cell folate microbiologic assays for national population surveys. J Nutr 2011;141:1402–9.
26. Tamura T. Microbiological assay of folates. In: Picciano MF, Stokstad ELR, Gregory JF, editors. Folic acid metabolism in health and disease. New York: Wiley; 1990. p. 121–37.
27. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43–53.
28. O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathol 1992;45:344–7.
29. Gunter EW, Bowman BA, Caudill SP, Twite DB, Adams MJ, Sampson EJ. Results of an international round robin for serum and whole-blood folate. Clin Chem 1996;42:1689–94.
30. Owen WE, Roberts WL. Comparison of five automated serum and whole blood folate assays. Am J Clin Pathol 2003;120:121–6.
31. Flour Fortification Initiative Network [Internet]. Atlanta (GA): Food Fortification Initiative; c2014 [cited 2013 Jun 24]. Available from: ffinetwork.org.
32. Gelman A. Prior distributions for the variance parameters in heirarchical models. Bayesian Anal 2006;1:515–33.
33. Turner RM, Davey J, Clarke MJ, Thompson SG, Higgins JPT. Predicting the extent of heterogeneity in meta-analysis, using empirical data from the Cochrane Database of Systematic Reviews. Int J Epidemiol 2012;41:818–27.
34. Gelman A. A Bayesian formulation of exploratory data analysis and goodness-of-fit testing. Int Stat Rev 2003;71:369–82.
35. Thomas A, O'Hara B, Ligges U, Sturtz S. Making BUGS Open. R News March 2006;6(1):12–7. [cited 2014 Jun 2]. Available from: http://cran.r-project.org/doc/Rnews/Rnews_2006-1.pdf.
36. Prinz-Langenohl R, Bramswig S, Tobolski O, Smulders YM, Smith DE, Finglas PM, Pietrzik K. [6S]-5-Methyltetrahydrofolate increases plasma folate more effectively than folic acid in women with the homozygous or wild-type 677C→T polymorphism of methylenetetrahydrofolate reductase. Br J Pharmacol 2009;158:2014–21.
37. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. Nutrients 2011;3:370–84.
38. Shelnutt KP, Kauwell GP, Chapman CM, Gregory JF III, Maneval DR, Browdy AA, Theriaque DW, Bailey LB. Folate status response to controlled folate intake is affected by the methylenetetrahydrofolate reductase 677C→T polymorphism in young women. J Nutr 2003;133:4107–11.
39. Hung J, Yang TL, Urrutia TF, Li R, Perry CA, Hata H, Cogger EA, Moriarty DJ, Caudill MA. Additional food folate derived exclusively from natural sources improves folate status in young women with the MTHFR 677 CC or TT genotype. J Nutr Biochem 2006;17:728–34.
40. Owusu M, Thomas J, Wiredu E, Pufulete M. Folate status of Ghanaian populations in London and Accra. Br J Nutr 2010;103:437–44.
41. Alagratnam D, Wierzbicki AS, Swaminathan R, Turner C, Wickramasinghe SN. Serum homocysteine, folate and thermolabile variant of MTHFR in healthy Sri Lankans living in London. Atherosclerosis 2000;149: 221–2.
42. Holst-Schumacher I, Monge-Rojas R, Cambronero-Gutierrez P, Brenes G. Genetic, dietary, and other lifestyle determinants of serum homocysteine levels in young adults in Costa Rica. Rev Panam Salud Publica 2005;17:263–70.
43. Nilsson TK, Laanpere M, Altmae S, Serra-Majem L, Salumets A. A folate receptor alpha double-mutated haplotype 1816delC-1841A is distributed throughout Eurasia and associated with lower erythrocyte folate levels. Mol Biol Rep 2012;39:4471–8.
44. Choo SC, Loh SP, Khor GL, Sabariah MN, Rozita R. MTHFR C677T polymorphism, homocysteine and B-vitamins status in a sample of Chinese and Malay subjects in Universiti Putra Malaysia. Malays J Nutr 2011;17:249–58.
45. Guinotte CL, Burns MG, Axume JA, Hata H, Urrutia TF, Alamilla A, McCabe D, Singgih A, Cogger EA, Caudill MA. Methylenetetrahydrofolate reductase 677C -> T variant modulates folate status response to controlled folate intakes in young women. J Nutr 2003;133:1272–80.
46. Hung J, Abratte CM, Wang W, Li R, Moriarty DJ, Caudill MA. Ethnicity and folate influence choline status in young women consuming controlled nutrient intakes. J Am Coll Nutr 2008;27:253–9.
47. Chango A, Boisson F, Barbe F, Quilliot D, Droesch S, Pfister M, Fillon-Emery N, Lambert D, Fremont S, Rosenblatt DS, et al. The effect of 677C→T and 1298A→C mutations on plasma homocysteine and 5,10-methylenetetrahydrofolate reductase activity in healthy subjects. Br J Nutr 2000;83:593–6.
48. García-García I, Garcia-Fragoso L, Renta J, Arce S, Cadilla CL. Erythrocyte volume, folate levels, and the presence of methylenetetrahydrofolate reductase polymorphism. P R Health Sci J 2002;21:17–9.
49. Moriyama Y, Okamura T, Kajinami K, Iso H, Inazu A, Kawashiri M, Mizuno M, Takeda Y, Sakamoto Y, Kimura H, et al. Effects of serum B vitamins on elevated plasma homocysteine levels associated with the mutation of methylenetetrahydrofolate reductase gene in Japanese. Atherosclerosis 2002;164:321–8.

50. Smulders YM, Smith DE, Kok RM, Teerlink T, Gellekink H, Vaes WH, Stehouwer CD, Jakobs C. Red blood cell folate vitamer distribution in healthy subjects is determined by the methylenetetrahydrofolate reductase C677T polymorphism and by the total folate status. J Nutr Biochem 2007;18:693–9.
51. Taguchi T, Mori H, Hamada A, Yamori Y, Mori M. Serum folate, total homocysteine levels and methylenetetrahydrofolate reductase 677C>T polymorphism in young healthy female Japanese. Asia Pac J Clin Nutr 2012;21:291–5.
52. Yang Q, Bailey L, Clarke R, Flanders WD, Liu T, Yesupriya A, Khoury MJ, Friedman JM. Prospective study of methylenetetrahydrofolate reductase (MTHFR) variant C677T and risk of all-cause and cardiovascular disease mortality among 6000 US adults. Am J Clin Nutr 2012;95:1245–53.
53. Zijno A, Andreoli C, Leopardi P, Marcon F, Rossi S, Caiola S, Verdina A, Galati R, Cafolla A, Crebelli R. Folate status, metabolic genotype, and biomarkers of genotoxicity in healthy subjects. Carcinogenesis 2003;24:1097–103.
54. Torres-Sánchez L, Chen J, Diaz-Sanchez Y, Palomeque C, Bottiglieri T, Lopez-Cervantes M, Lopez-Carrillo L. Dietary and genetic determinants of homocysteine levels among Mexican women of reproductive age. Eur J Clin Nutr 2006;60:691–7.
55. McNulty H, McKinley MC, Wilson B, McPartlin J, Strain JJ, Weir DG, Scott JM. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. Am J Clin Nutr 2002;76(2):436–41.
56. Selhub J, Jacques PF, Dallal G, Choumenkovitch S, Rogers G. The use of blood concentrations of vitamins and their respective functional indicators to define folate and vitamin B12 status. Food Nutr Bull 2008;29(2 Suppl):S67–73.
57. Crider KS, Devine O, Hao L, Dowling NF, Li S, Molloy AM, Li Z, Zhu J, Berry RJ. Population red blood cell folate concentrations for prevention of neural tube defects: Bayesian model. BMJ 2014;349:g4554.
58. Molloy AM, Mills JL, Kirke PN, Ramsbottom D, McPartlin JM, Burke H, Conley M, Whitehead AS, Weir DG, Scott JM. Low blood folates in NTD pregnancies are only partly explained by thermolabile 5,10-methylenetetrahydrofolate reductase: low folate status alone may be the critical factor. Am J Med Genet 1998;78:155–9.
59. Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, Breteler MM, Bautista LE, Sharma P, Whittaker JC, et al. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. Lancet 2011;378:584–94.
60. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong LY, Gindler J, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med 1999;341:1485–90.
61. Arbour L, Christensen B, Delormier T, Platt R, Gilfix B, Forbes P, Kovitch I, Morel J, Rozen R. Spina bifida, folate metabolism, and dietary folate intake in a northern Canadian aboriginal population. Int J Circumpolar Health 2002;61:341–51.
62. Félix TM, Leistner S, Giugliani R. Metabolic effects and the methylenetetrahydrofolate reductase (MTHFR) polymorphism associated with neural tube defects in southern Brazil. Birth Defects Res A Clin Mol Teratol 2004;70:459–63.
63. Perez AB, D'Almeida V, Vergani N, de Oliveira AC, de Lima FT, Brunoni D. Methylenetetrahydrofolate reductase (MTHFR): incidence of mutations C677T and A1298C in Brazilian population and its correlation with plasma homocysteine levels in spina bifida. Am J Med Genet A 2003;119A:20–5.
64. WHO. Serum and red blood cell folate concentrations for assessing folate status in populations [Internet]. Vitamin and Mineral Nutrition Information System. [cited 2012 Dec 7.] Geneva (Switzerland): World Health Organization; 2012. Available from: http://apps.who.int/iris/bitstream/10665/75584/1/WHO_NMH_NHD_EPG_12.1_eng.pdf.
65. Molloy AM, Mills JL, Kirke PN, Whitehead AS, Weir DG, Scott JM. Whole-blood folate values in subjects with different methylenetetrahydrofolate reductase genotypes: differences between the radioassay and microbiological assays. Clin Chem 1998;44:186–8.
66. Clifford AJ, Noceti EM, Block-Joy A, Block T, Block G. Erythrocyte folate and its response to folic acid supplementation is assay dependent in women. J Nutr 2005;135:137–43.
67. Nakazato M, Maeda T, Emura K, Maeda M, Tamura T. Blood folate concentrations analyzed by microbiological assay and chemiluminescent immunoassay methods. J Nutr Sci Vitaminol (Tokyo) 2012;58:59–62.
68. Golding PH. Severe experimental folate deficiency in a human subject - a longitudinal study of biochemical and haematological responses as megaloblastic anaemia develops. Springerplus 2014;3:442.
69. Pfeiffer CM, Sternberg MR, Schleicher RL, Rybak ME. Dietary supplement use and smoking are important correlates of biomarkers of water-soluble vitamin status after adjusting for sociodemographic and lifestyle variables in a representative sample of U.S. adults. J Nutr 2013;143:957S–65S.