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Genetic Variation: Impact on Folate (and Choline) Bioefficacy

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Abstract: Folate and choline are water-soluble micronutrients that serve as methyl donors in the conversion of homocysteine to methionine. Inadequacy of these nutrients can disturb one-carbon metabolism as evidenced by alterations in circulating folate and/or plasma homocysteine. Among common genetic variants that reside in genes regulating folate absorptive and metabolic processes, homozygosity for the MTHFR 677C>T variant has consistently been shown to have robust effects on status markers. This paper will review the impact of genetic variants in folate-metabolizing genes on folate and choline bioefficacy. Nutrient-gene and gene-gene interactions will be considered along with the need to account for these genetic variants when updating dietary folate and choline recommendations.

Key words: genetic variation, MTHFR, homocysteine, folate, choline, serum folate, folate bioefficacy, folate bioavailability, dietary recommendations, folate RDA

Introduction

Elucidating how nutrients are digested, absorbed, and metabolized in the human body is a fundamental endeavor of nutritional science. Nutrient bioavailability is considered to be a function of absorptive and metabolic processes that are influenced by such factors as nutrient form, dietary intake, and individual genetic variation [1, 2]. Other concepts that fall under the broader umbrella of bioavailability, as summarized in Gregory *et al.* [2], are:

- Bioaccessibility: fraction available for absorption after release from food matrix
- Bioconversion/bioefficacy: fraction of absorbed nutrient converted to the bio-active form
- Functional bioefficacy: fraction of nutrient that impacts a functional parameter

Folate, a water-soluble B-vitamin, is widely recognized for its role in preventing neural tube defects (NTDs), its inverse relationship with numerous chronic diseases (e.g., coronary heart disease, stroke, cancers, osteoporosis, and dementia), and its usefulness as a chemotherapeutic target. The health importance of choline, a "quasi" essential micronutrient commonly grouped with the B vitamins, is less certain; however its effects on fetal development and programming [3], non-alcoholic fatty liver [4–6], and its intermingling with folate are attracting attention.

Folate and choline intersect at the metabolic network involved in the transfer of one-carbon units, referred to as one-carbon metabolism, which is required for the biosynthesis of nucleotides, the interconversions of select amino acids, and cellular methylation [7]. Homocysteine, formed in the methionine cycle,

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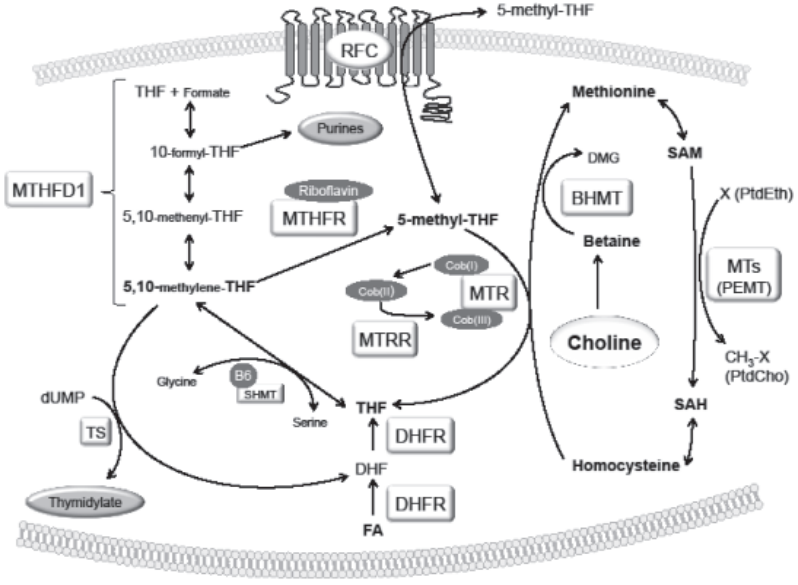

Figure 1: Folate-metabolizing genes, with common genetic variants, that may impact folate (and choline) bioefficacy. Abbreviations: BHMT, betaine:homocysteine methyltransferase; Cob(I), cob(I)alamin; Cob(II), cob(II)alamin; Cob(III), methylcob(III)alamin; DHF, dihydrofolate; DHFR, dihydrofolate reductase; DMG, dimethylglycine; dUMP, deoxyuridine monophosphate; FA, folic acid; MT, generic methyltransferase; MTHFD1, methylenetetrahydrofolate dehydrogenase; MTHFR, 5,10-methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; PEMT, phosphatidylethanolamine *N*-methyltransferase; PdtEth- phosphatidylethanolamine; PtdCho, phosphatidylcholine; RFC, reduced folate carrier; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase

is an indicator of cellular methylation stress (when elevated) and is associated with a plethora of health derangements. Methyl groups from folate or the choline derivative, betaine, can be used to convert homocysteine to methionine. In subsequent reactions, the nutrient-derived methyl group can be donated by S-adenosylmethionine (SAM) to acceptor molecules with effects on lipid metabolism, neurotransmitter biosynthesis and degradation, energy homeostasis, and gene expression and stability (e.g., DNA and histone methylation). Phosphatidylethanolamine *N*-methyltransferase (PEMT), a main consumer of SAM, catalyzes the sequential methylation of phosphatidylethanolamine to phosphatidylcholine (PtdCho) and provides a route for *de novo* synthesis of choline following its hydrolysis from PtdCho. As S-adenosylhomocysteine is a product of all SAM-dependent methyltransferases, the PEMT pathway is also a main producer of homocysteine (see Figure 1).

Folate bioefficacy can be assessed by measurements of serum/plasma and red blood cell (RBC) folate concentrations, well-established markers of folate status [8]. 5-Methyl-tetrahydrofolate (THF) is the main folate form in blood and its polyglutamated derivative is the principal bioactive form in tissue. Under normal physiologic conditions, circulating folate concentrations reflect the fraction of absorbed nutrient that is available to tissue and that can be converted to bioactive co-enzymes. Plasma total homocysteine (tHcy) concentration reflects folate availability for homocysteine remethylation to methionine and thus may be considered an indicator of functional folate bioefficacy [9].

Driven by the goal of discovering genetic polymorphisms that impact NTD development, homocysteine disposal, and the toxicity/efficacy of antifolate drugs, genetic variation within folate-metabolizing genes has been widely studied over the past decade. From this body of literature and more targeted studies, the impact of candidate genes and their polymorphisms on folate (and choline) bioefficacy can be examined. This paper provides examples of polymorphisms in folate-metabolizing genes that can impact folate (and choline) bioefficacy and explores the need to consider these variants when updating dietary recommendations.

Candidate Genes and Their Variants

Methylenetetrahydrofolate reductase (MTHFR)

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible reduction of 5,10-methylene-THF to 5-methyl –THF (see Figure 1) and thereby plays a critical role in regulating one-carbon flux between nucleotide biosynthesis and cellular methylation. Moreover, as the enzyme producing the main form of folate in blood, MTHFR directly impacts circulating folate concentrations. The 677C>T variant allele in the MTHFR gene has garnered prodigy-like status over the past decade with approximately 300 human papers published per year on this single nucleotide polymorphism (SNP) (or ~75 % of all publications on variants of genes in folate metabolism) [10]. In addition to having robust independent effects on folate status and health biomarkers, the MTHFR

variant 677T allele frequently interacts with other one-carbon metabolizing SNPs to affect folate and choline status biomarkers.

MTHFR 677C > T

Discovered in 1995, this non-synonymous exonic SNP (rs1801133) is the most common genetic cause of mild hyperhomocysteinemia and is associated with a 35 or 65 % loss in enzyme activity when one or two T alleles are present, respectively [11, 12]. The 677C > T polymorphism causes a substitution of valine for alanine in the protein's catalytic domain and yields a thermolabile enzyme that is more likely to dissociate from its riboflavin-derived FAD cofactor [13, 14]. The likelihood of having one or two copies of the 677C > T allele varies by ethnicity and geographic location [15, 16]. Within the United States, the prevalence of 677TT genotype averages 11 % but is approximately 20, 12, and 1 % among Hispanics, whites, and blacks, respectively [17]. Epidemiologic and controlled experimental studies consistently demonstrate lower folate status among individuals with the 677TT genotype, especially when folate status is compromised.

NHANES III data that pre-dates folic acid fortification of the food supply in the United States reported 22 % lower serum folate and 25 % higher tHcy in those with the 677TT versus 677CC genotype [17]. Among an adult Danish population, the 677TT genotype increased the likelihood of low serum folate by over two times (< 6.8 nmol/L; OR 2.24, $p < 0.001$) [18]. Similarly, in an adult Norwegian population, the 677TT genotype is associated with a 32 % increase in tHcy, a 29 % decrease in serum folate, and a 10 % reduction in the choline derivatives, betaine and dimethylglycine as compared to the 677CC genotype [19]. Although homozygosity for the 677T allele exerts the strongest effect on folate biomarkers, even a single copy of the 677T allele is associated with reduced folate status [17–19].

Highly controlled dose-response intervention studies have demonstrated a higher folate requirement in adults with the MTHFR 677TT genotype. Serum folate concentrations in Hispanic premenopausal women with the 677TT genotype were significantly lower throughout folate depletion (135 μg/day as dietary folate equivalents, DFEs) and during folate repletion with 400 μg DFE/day compared to those with the 677CC genotype [20]. These results paralleled a similar study conducted in non-Hispanic premenopausal women in which the 677TT genotype resulted in lower serum folate (pre- and post-repletion) and lower post-repletion RBC folate [21]. In men, consumption of the folate Recommended Daily Allowance (RDA), 400 μg DFE/day, for 12 weeks yielded significantly lower serum folate and three times higher plasma tHcy in the 677TT versus the 677CC genotype [22].

The effects of genetic polymorphisms on folate bioefficacy can interact with nutrient intakes of folate, choline, vitamin B12, vitamin B6, and/or riboflavin [23–25]. The most striking example is in the case of MTHFR 677C > T and folate. Although lower serum folate concentrations were observed in women with the MTHFR 677TT genotype consuming either 135 or 400 μg DFE/day as discussed above [20], no differences in serum folate were detected between MTHFR C677T genotypes in another treatment arm consuming 800 μg DFE/day. Other studies have also shown that with an intake of ≥ 600 μg DFE/day, serum folate concentrations did not significantly differ between the MTHFR C677T genotypes [26–27].

MTHFR 1298A > C

A second, common non-synonymous SNP in the Methylenetetrahydrofolate reductase (MTHFR) gene, 1298A > C (rs1801131), causes the substitution of an alanine for glutamate in the regulatory domain of the MTHFR protein [28–29]. The 1298A > C variant has been shown to reduce MTHFR enzyme activity in human lymphocytes; however, not to the extent that 677C > T diminishes MTHFR activity [28–29]. Notably, experiments using bacterial, mammalian, and yeast expression systems have failed to observe a functional impact of the 1298A > C SNP [14, 25, 30]. The prevalence of the 1298C allele varies by geographic region and ethnicity with 10 % of whites and ~ 3.5 % of blacks and Hispanics having the 1298CC genotype in the US [17].

The impact of the MTHFR1298A > C SNP on folate status is ambiguous. The majority of studies, including the NHANES III study described above, have found no significant associations between the 1298A > C variant and markers of folate status [17, 18, 29]. However, a large-scale epidemiologic study found that compared with the homozygous wild type genotype, the 1298CC genotype was associated with a 5 % increase of tHcy and a decrease of 9 % and 3 % for serum folate and betaine, respectively [19]. Thus, while there is evidence that the 1298CC genotype impacts folate bioefficacy, the current body of evidence indicates that the MTHFR 677TT genotype has a much greater effect on both metabolic bioefficacy, (i.e., generation of 5-methyl-THF) and functional bioefficacy (i.e., plasma tHcy concentrations).

MTHFR Haplotypes: 677C>T + 1298A>C

The 677C>T and 1298A>C SNPs are rarely found on the same allele (i.e., these SNPs are in strong linkage disequilibrium) [29, 31], which implies that the metabolic impact of the MTHFR A1298C genotype should be considered within the context of the MTHFR C677T genotype. A MTHFR genotype meta-analysis calculated Caucasian population MTHFR haplotype frequencies as follows: 677C/1298A, 37 %; 677C/1298C, 31 %; 677T/1298A, 32 %; and 677T/1298C, 0.23–0.34 % [31]. Notably, heterozygous MTHFR genotypes are of interest due to widespread prevalence and evidence of functional consequences (i.e., penetrance). The 677CT plus 1298AC genotype is associated with reduced MTHFR enzyme activity, reduced plasma folate, and increased tHcy [28, 29, 32]. A large-scale epidemiologic study (n=10,034) ordered MTHFR genotypes by decreasing levels of serum folate as follows: 677CC/1298AA (highest) > 677CC/1298AC > 677CC/1298CC ~ 677CT/1298AA > 677CT/1298AC > 677TT/1298AA (lowest) [32]. After comparing these genotypes with a model that included six MTHFR protein configurations of varying functionality and stability, the investigators found that the least functional/stable enzyme configurations corresponded with particular genotypes and lower serum folate concentrations [32]. These results indicate that although the effect of the heterozygosity for the 1298A>C and 677C>T SNPs do not always emerge as significant predictors of status endpoints, depending on genotype and folate exposure, these genetic polymorphisms can have an important impact on MTHFR enzyme function and folate bioefficacy.

Reduced Folate Carrier (RFC)

Reduced folate carrier (RFC), the product of the SLC19A1 gene, is a bidirectional folate transporter that is ubiquitously expressed and represents a main route of folate entry into most systemic tissues. However, despite being expressed in enterocytes, RFC does not appear to play a significant role in intestinal folate absorption. Instead, absorption of folate across the intestinal epithelium is mediated by the more recently identified proton-coupled folate transporter (PCFT) [33]. Mutations in PCFT are a cause of hereditary folate malabsorption [34]; however, putative common PCFT genetic variants have not yet been reported.

RFC spans the plasma membrane 12 times and has a relatively high affinity for reduced folates, including 5-methyl-THF, but a low affinity for oxidized synthetic folic acid. A common SNP resides at position 80 in exon 2 of RFC which changes a guanine to adenine (RFC 80G>A; rs1051266); although 80 A>G is sometimes reported [35]. The 80G>A SNP causes a substitution of histidine for arginine and is predicted to reside in the first transmembrane domain, a region implicated as important to carrier function [36]. The prevalence of the variant RFC 80A allele is ~42 % in Europeans [19]. Existing data show null [19, 35] or nominal [37] effects of the RFC 80AA genotype on markers of folate status including serum folate, RBC folate, and plasma homocysteine. A study conducted in Ireland reported higher RBC folate in women homozygous for the variant 80A allele; nevertheless, the genotype explained only a minor percentage, 5 %, of RBC folate variation [37]. Some studies report an interaction between the RFC G80A and MTHFR C677T genotypes [35] [38, 39] but due to discrepancies in nomenclature, the relationship of the interaction is unclear. Like MTHFR 1298A>C and MTRR 66A>G (see next section), the RFC 80G>A SNP in isolation does not appear to have a strong effect but may synergistically interact with the MTHFR 677C>T SNP to impact folate bioefficacy.

Methionine Synthase (MTR) & Methionine Synthase Reductase (MTRR)

Upon cellular entry, THF is generated from 5-methyl-THF when it is used to convert homocysteine to methionine in a reaction catalyzed by the vitamin B-12 (cobalamin)-dependent methionine synthase (MTR). MTR-bound cob(I)alamin can be methylated by 5-methyl-THF to generate the methylcob(III)alamin intermediate, which serves as the direct methyl donor in conversion of homocysteine to methionine. Alternatively, MTR-bound cob(I)alamin can be oxidized to the nonfunctional cob(II)alamin form which is subsequently reduced and methylated to the methylcob(III) alamin intermediate via the action of SAM-dependent methionine synthase reductase (MTRR) [40].

Genetic variation in MTR and MTRR has the potential to interfere with the production of methionine and THF, thereby disrupting folate homeostasis and elevating tHcy [41]. The MTR 2756A>G SNP (rs1805087) results in a glycine-for-aspartic acid substitution and appears to have a potentially protective effect, as it is associated with reduced tHcy concentrations [19, 41, 42], an effect that is independent of the MTHFR C677T genotype. Conversely, the nonsynonymous MTRR 66A>G SNP (rs1801394) is as-

sociated with increased tHcy but only when combined with the MTHFR 677TT genotype [17, 42, 43]. Given the high prevalence of the MTR 2756G and MTRR 66G variant alleles, 19 % and 25–60 %, respectively [17, 19], these SNPs have the potential to impact folate bioefficacy in a large number of individuals.

Dihydrofolate Reductase (DHFR)

Dihydrofolate, a product of thymidylate synthase, is reduced to THF by the enzyme dihydrofolate reductase (DHFR) (see Figure 1). Thus, DHFR plays a critical role in maintaining the cellular supply of reduced folates required for DNA synthesis. Folic acid is an additional substrate of DHFR, which via two consecutive reductions, enters the folate cycle as THF. Thus, DHFR is essential for converting folic acid, the synthetic folate used in food supply fortification and vitamin supplements, into a form that is usable by the body. Given these two functions, polymorphisms in the DHFR gene are of considerable interest in the folate bioefficacy discussion.

To date, there have been no SNPs identified in the coding region of DHFR; however, a 19-base pair deletion (19-bp del) polymorphism in intron 1 of the DHFR promoter (reference sequence NC_000005.8) results in the loss of a transcription factor binding site that may alter protein expression [44]. The 19-bp deletion allele is common with measures of the deletion/deletion (del/del) genotype of 17–33 % [44–47]. The 19-bp del polymorphism appears to be functional; however, it is unclear whether gene expression is increased [46, 48] or decreased [44, 49].

Reports of the association between the 19-bp del variant and folate status have also been mixed. One study reported that the homozygous 19-bp del/del and heterozygous 19-bp del/wild type genotypes were associated with 14.4 % and 2.5 % reductions in tHcy, respectively [45]. Another study that seems to support the former, found that among young Irish women the homozygous 19-bp del/del genotype was marginally associated with increased RBC and serum folate levels [50]. A third study found interactions between the 19-bp del/del genotype, folic acid dose, RBC folate, and circulating unmetabolized folic acid [47]. The homozygous 19-bp del/del genotype was associated with lower RBC folate on a folic acid dose of <250 μg/day, but higher plasma unmetabolized folic acid with a dose of ≥ 500 μg/day [47]. These findings suggest impaired functional capacity of the 19-bp del DHFR enzyme to convert folic acid to a physiologic form.

Genetic variation in DHFR may contribute to the nearly five-fold variation in DHFR enzyme activity measured in human liver samples ($n=6$) [51]. Of particular note in discussing bioconversion is that the reduction reaction involving synthetic folic acid and DHFR was significantly slower than the reduction reaction involving physiologic dihydrofolate and DHFR [51]. Although further research is warranted, these findings suggest that the 19-bp del DHFR variant, and perhaps other DHFR variants, have the potential to impact folate bioefficacy.

Genetic Polymorphisms and Choline Bioavailability

The interplay of folate and choline in one-carbon metabolism has been well described [7] and recently highlighted by data showing that genetic variants in folate-metabolizing genes altered biomarkers of choline status.

Methylenetetrahydrofolate dehydrogenase (MTHFD1)

The methylenetetrahydrofolate dehydrogenase (MTHFD1) gene codes for a tri-functional enzyme that enables entry of mitochondrial one-carbons into the cytosol with its 10-formyl THF synthetase activity and facilitates the interconversions of 10-formyl THF, 5,10-methenyl THF and 5,10-methylene THF with its cyclohydrolase and dehydrogenase activities [52]. A non-synonymous guanine-to-adenine SNP at nucleotide 1958 prompts the substitution of a glutamine for arginine amino acid in the 10-formyl THF synthetase domain (MTHFD1 1958G > A, rs2236225) [53]. Prevalence of the variant MTHFD1 1958AA genotype is about 20 % in Europeans [19]. However, frequency of the variant allele may be lower in blacks and higher in Hispanics [54, 55].

Although the 1958G > A SNP has not been associated with impaired folate status in epidemiological studies [19, 53], one or two copies of the variant 1958A allele increases the risk of exhibiting signs of choline deficiency (i. e., organ dysfunction) in response to dietary choline depletion ($OR=7$, $p=0.007$) [54]. This risk is further enhanced if the choline-deficient diet is coupled with a low folate intake. Premenopausal women carrying one or two copies of the variant 1958A allele were especially susceptible to increased risk of organ dysfunction under these dietary regimens ($OR=85$, $p<0.0001$). The investigators hypothesized

that the variant 1958A allele results in a dysfunctional MTHFD1 enzyme that reduces the flow of folate one-carbon units towards homocysteine remethylation. This in turn places a greater burden on one-carbon units derived from the choline metabolite betaine, leading to choline deficiency. Additionally, it may reduce the pool of SAM that is available for *de novo* biosynthesis of PtdCho through the PEMT pathway. PEMT is up-regulated by estrogen, which enables premenopausal women to make more choline endogenously (relative to men and postmenopausal women) [8, 56]. Thus, a dysfunctional MTHFD1 may impair the PEMT reaction and contribute to the excess susceptibility of premenopausal women with the variant 1958A allele to choline depletion.

The tendency for tHcy to increase more in premenopausal women if they had the MTHFD1 1958AA genotype [relative to the 1958GA ($p = 0.033$) and 1958GG ($p = 0.085$) genotypes] supports the hypothesis that the 1958G > A SNP has a functional effect on cellular methylation [55]. It should be noted that this effect was only observed on a folate-restricted diet and disappeared with subsequent folate repletion [55]. *In vitro* studies show the MTHFD1 1958A > G protein is thermolabile with reduced enzyme activity; however, added folate restores the 1958A > G protein to wild type enzyme activity levels [57]. Collectively, these findings indicate that under conditions of sub-optimal folate/choline intake, the MTHFD1 1958A > G SNP impacts folate and choline bioefficacy by modulating the availability of 5-methyl-THF.

MTHFR

Several studies have shown that MTHFR deficiency alters the metabolism of choline [58–61]. In young men consuming sub-optimal folate intakes along with one of four choline intakes (range: 300–2200 mg/day), lower concentrations of plasma PtdCho were observed in the 677TT versus CC genotype regardless of choline intake [59]. Diminished PtdCho in the MTHFR 677TT genotype under these dietary constraints may arise from increased use of choline as a methyl donor. This working hypothesis was recently supported by a tracer study involving a sub-sample of these men in which a greater flux of choline towards betaine (and other oxidative products) was detected in the MTHFR 677TT [62]. Although, the MTHFR C677T genotype modifies choline metabolism in premenopausal women, the relationship is less clear, likely due to engagement of compensatory mechanisms. For example, in premenopausal women that completed a study with constant choline intake of 349 mg/day and folate depletion and repletion stages ([20] discussed above), plasma PtdCho decreased significantly during folate depletion. However, women with the MTHFR 677TT genotype tended to be resistant to the decline ($p = 0.089$) [58]. When serum folate was included as a covariate in the statistical model, MTHFR genotype emerged as a predictor of plasma PtdCho ($p = 0.027$) and PtdCho was significantly higher in women with the 677TT genotype ($p = 0.032$) [58].

Discussion

Genetic variants in folate-metabolizing genes have been associated with risk of a variety of diseases, including birth defects, cancer, and cardiovascular disease. Mechanisms and causation are yet to be proven; however, proposed pathways involving DNA stability and one-carbon partitioning have been suggested [63]. Implicit in genetic variation and disease risk associations is that genetic polymorphisms alter nutrient bioefficacy such that protein and cellular functions are altered, ultimately conferring either increased or attenuated disease risk. Although there is debate about what can be concluded from associations between common genetic polymorphisms in folate-metabolizing genes and disease risk [64, 65], stronger conclusions about the impact of genetic polymorphisms on folate bioefficacy can be made.

Homozygosity for the MTHFR 677C > T variant allele is the most robust modifier of folate status markers among the folate-metabolizing SNPs investigated thus far. However, the severity of 677TT genotype on markers of folate metabolism depends to a large degree on folate intake because relatively high intracellular concentrations of folate can stabilize the functional impairment caused by the 677C > T SNP. Due mainly to folic acid fortification of the food supply, the average folate intake for men and women in the United States is 813 and 724 μg DFE/day, respectively [66], which is well above the current US folate RDA for adults (400 μg DFE/day) and above the level at which strong effects of the 677TT genotype are observed (< 600 μg DFE/day). Thus, given current folate intakes in the US (and other fortified populations) along with the marginal effects of common genetic variation on folate and choline bioefficacy (reviewed in part herein), it is unlikely that SNPs in folate-metabolizing genes, even the MTHFR 677C > T, detrimentally impact folate and choline status at the population level. A US epidemiological study examining serum folate and tHcy

levels, pre- and post-folic acid fortification stratified by MTHFR C677T genotype reveals as much: those with the 677TT genotype have significantly higher tHcy at both time points; however, the difference in means goes from 2.5 μmol/L pre-fortification to ≤0.7 μmol/L post-fortification [67].

Although there is little evidence that population-level folate status is compromised by common genetic polymorphisms in folate-metabolizing genes in the US, the issue of whether dietary recommendations should consider genetic variation remains [68, 69]. Research examining the current RDA of 400 μg DFE/day has shown it to be adequate for women with the 677TT genotype [20, 21], yet insufficient for men with the 677TT genotype [22]. In a recent study, 34 % of men with the 677TT genotype had serum folate concentrations <6.8 nmol/L, and 79 % had tHcy >14 μmol/L after 12 weeks of consuming the folate RDA [22]. Interestingly, these same signs of folate deficiency were exhibited in 16 % and 7 % of men with the 677CC genotype [22]. This small yet highly controlled study raises uncertainty as to whether the current folate RDA, 400 μg DFE/day, is sufficient for meeting the requirement of 97 % of men. Using a model that projected folate RDA ranges based on the 677TT genotype and different functional effect sizes (5–50 %), Robitaille *et al.* [70] concluded that the MTHFR 677TT genotype does not warrant changes to the current folate RDA. This type of statistical approach in assessing whether common SNPs affect nutrient requirements is highly useful but in this particular case [70], the adequacy of the folate RDA for the MTHFR 677CC genotype is questionable given the results of the controlled feeding study conducted by Solis *et al.* [22].

It has long been recognized that folate metabolism is under genetic control and that genetic heterogeneity explains a large percentage (i.e., >40 %) of variation in metabolism [8]. Historically, this genetic heterogeneity has been accounted for when setting RDAs by adding two coefficients of variation (CV) of 10 percent each to the Estimated Average Requirement (i.e., EAR x 1.2). However, the assumption of a 10 % CV is based on variation in basal metabolic rate [8] and may not adequately capture the impact of genetic variation in folate-metabolizing genes on bioefficacy and requirements. The plethora of studies published over the past decade on SNPs and aspects of folate metabolism afford the opportunity to refine estimates of variability such that effects of genetic background (as well as other factors) could be more thoroughly addressed. This type of analysis may in turn alleviate the need to individualize dietary recommendations on the basis of genetic sub-groups.

Conclusion

The impact of genetic variation on folate and choline status markers is well studied. Homozygosity for the variant MTHFR 677T allele is a strong modifier of folate (and choline) bioefficacy, particularly under conditions of sub-optimal folate intake. Gene-gene interactions are also apparent such that certain combinations (e.g., MTHFR 677TT and MTRR 66GG) may exacerbate metabolic disturbances. A nutrient-specific estimate of population-wide variability in folate bioefficacy would provide a background from which to determine the need to account for functional SNPs (within the context of complex interactions) in revising dietary recommendations for folate and choline.

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