Does the MTHFR 677C→T variant affect the Recommended Dietary Allowance for folate in the US population?1–4

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ABSTRACT

Background: The MTHFR 677C→T variant is associated with reduced enzyme activity, abnormalities of folate metabolism, and potential increase in folate requirement. The effect of this variant on the Recommended Dietary Allowance (RDA) for folate is unclear.

Objective: The aim of this study was to assess the effect of the MTHFR 677C→T polymorphism on the current folate RDA for US adults aged ≥19 y (400 μg/d) by race and ethnicity.

Design: We calculated the projected RDA for folate for each racial and ethnic group according to the methods of the Institute of Medicine. We modeled the projected RDA with different hypothetical effect sizes ranging from 5% to 50%. The RDA value was then weighted according to the US prevalence of the TT (or the combined CT/TT) genotype in each racial and ethnic group.

Results: The projected RDA ranges were based on TT genotype frequencies and on different effect sizes (5–50%) that ranged from 400 to 421 μg/d for non-Hispanic whites, 401–436 μg/d for Mexican Americans, and 400–402 μg/d for non-Hispanic blacks.

Conclusions: Our findings suggest that the current RDA for folate differs little for non-Hispanic whites, non-Hispanic blacks, and Mexican Americans irrespective of the MTHFR 677C→T genotype, and, from a population perspective, the MTHFR 677C→T variant does not warrant modifications to the current RDA for dietary folate at this time.


INTRODUCTION

Disturbances in the folate metabolism pathway are associated with a wide range of conditions, and variants from genes involved in this pathway may play a role in the genesis of these conditions (1–7). Most research has focused on the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T variant (rs1801133) and has shown a significant association with methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism may play a role in the genesis of these with a wide range of conditions, and variants from genes in-

of the variant seems to be more pronounced under low- to normal-

The Recommended Dietary Allowance (RDA) for folate, which was set in 1998 by the Institute of Medicine (IOM), is 400 μg dietary folate equivalent (DFE)/d for men and women aged ≥19 y (16). At the time of this recommendation, the concentration of folate required to stabilize the MTHFR 677C→T variant and the proportion of the US population with this variant were unknown (16).

Since this recommendation was made, Gibney and Gibney (17) have suggested, within a hypothetical model, that the current recommendations for a hypothetical nutrient would be sufficient to cover genetic variations at an allele frequency ≤20% within the population, given a theoretical increase of 25% in nutrient requirements for a specific genotype. However, actual genotype variants and effect sizes might vary by population.

Testing the realities of including genetic information as part of an overall strategy to improve the population’s health will be critical as more genetic data are collected and used to develop recommendations such as nutrient requirements. In the current study, data from the third National Health and Nutrition Examination Survey (NHANES III) on the variant frequency of the MTHFR 677C→T polymorphism were used to assess the possible effect of this genetic variation on the current folate RDA for adults aged ≥19 y by race and ethnicity (18). We hypothe-
sized that, if the RDA for folate increased by ≥25% after ac-

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2 The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

3 Supported in part by a Fellowship in Genetics and the Public Health Research and Practice Program, which are jointly sponsored by the Centers for Disease Control and the American Society of Human Genetics (JR), and by a fellowship award from the Canadian Institutes of Health and Research (JR).

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counting for the MTHFR 677C→T variant, a review of the current RDA, with the inclusion of genetic variation data, could be warranted.

SUBJECTS AND METHODS

To estimate the folate RDA on the basis of the prevalence of the MTHFR 677C→T genotype in US adults, we used recently published data on the prevalence of the genotypes from NHANES III phase II (1991–1994) and a wide range of possible effect sizes of the MTHFR genotypes on folate requirements (18).

The National Health Examination Surveys have been conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention, since 1960 to estimate the prevalence of and risk factors for common diseases in the US population. A nutrition component was added in 1971, and the survey name was changed to NHANES. NHANES III was conducted in 2 phases: 1988–1991 and 1991–1994. Each phase was nationally representative and oversampled certain populations, including non-Hispanic blacks and Mexican Americans. Population weights were calculated to account for oversampling, multistage sampling design, and nonresponse to the household interview and the examination. Genotype data were available for phase II of NHANES III. Methods used for determining the MTHFR 677C→T genotype were described previously (18).

We used the method described by the IOM (16) to calculate projected RDAs. Actual RDA values are based on the estimated average requirement (EAR), which is set to meet the nutrient requirement needs for 50% of healthy individuals within each life stage and sex group (16). Using the EAR, the IOM assumed a CV of 10% for folate requirements and estimated the RDA to be 120% of the EAR (RDA = 1.2 × EAR) (16). The RDA should represent a sufficient daily dietary intake to meet the nutrient requirement needs for 97–98% of healthy individuals in a particular life stage and sex group (16).

The hypothetical calculations reported by Gibney and Gibney (17) were used as a template to test the effect of the MTHFR 677C→T variant on folate requirements (18). The IOM recommends that all non-pregnant adults aged ≥19 y consume 400 μg/d folate from foods and supplements with one exception: All women capable of becoming pregnant should consume 400 μg/d of folic acid from supplements, fortified foods, or both, in addition to consuming food folate from a varied diet to prevent the occurrence of neural tube birth defects (16). However, the EAR and RDA for all other life stages and sex groups are given in DFEs, which take into account the greater bioavailability of folic acid. For this study, we focused on the RDAs in DFEs and did not attempt to convert folic acid into DFEs for women capable of becoming pregnant.

We used the IOM’s EAR of 320 μg/d folate to calculate the projected RDA for each racial and ethnic group while taking into account a wide range of the possible effects of the MTHFR 677C→T variant on folate requirements. We modeled the projected RDA calculations with different effect sizes (Eg), which ranged from 5% to 50% (the actual effect sizes are unknown). Five-percent increments were used to model the projected RDA calculations. The RDA value was then weighted according to the prevalence of the TT (or the combined CT/TT) genotype in each racial and ethnic subgroup of the US population as reported by Yang et al (18).

The formula used for RDA calculations was as follows:

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\text{RDA}_w = F_g \{1.2[\text{EAR} (1 + E_g)]\} + \text{RDA} (1 - F_g) \tag{I}
\]

RDAw is the weighted RDA that is based on the MTHFR TT (or CT/TT) genotype effect and the frequency of the TT (or CT/TT) genotype. EAR is the estimated average requirement as set by the IOM (EAR = 320 μg DFE) (16). Eg is the frequency of the MTHFR TT (or CT/TT) genotype. Eg is the effect size of the MTHFR 677C→T variant. In this study, we used a range of 5–50%; (1 + Eg) is the factor by which the EAR needs to be increased further to compensate for the effect of the MTHFR 677C→T variant. When we modeled the effect of TT alone, we assumed that the CT genotype would have no effect on the RDA.

We assumed that a significant change of the RDA would be warranted if the RDA for folate increased by ≥25%. This value was chosen on the basis of data from the study of neural tube defects and folate intake. In fact, it was estimated that food fortification with folic acid would provide an average 100 μg/d additional folic acid (=25% of the RDA) (19, 20), which has had a public health effect by reducing the prevalence of neural tube defects by 26% in the United States (21, 22).

RESULTS

Overall, DNA was available for 7195 (71.6%) NHANES III participants aged ≥12 y examined in 1991–1994. Demographic characteristics of the participants have been described elsewhere (23). Data on the MTHFR 677C→T genotype were available for 7140 of those participants (18).

The prevalence of the MTHFR 677C→T genotype variant differed significantly by racial and ethnic group in the US population aged ≥12 y (18). The TT and CT genotype frequencies were highest among Mexican Americans (TT: 20.3%; CT: 49.8%), followed by non-Hispanic whites (TT: 11.7%; CT: 42.6%) and non-Hispanic blacks (TT: 1.3%; CT: 19.5%). The MTHFR 677C→T genotype frequency did not vary by age (23).

For non-Hispanic white adults, the RDAw based on a TT genotype frequency of 11.7% ranged from 400 to 421 μg/d (Figure 1A). On the basis of a genotype frequency of 20.3%, Mexican Americans had an RDAw that ranged from 401 to 436 μg/d. Among non-Hispanic blacks, the RDAw ranged from 400 to 402 μg/d for a TT genotype frequency of 1.3%.

Considering a 50% increase in folic acid requirement due to the presence of the TT genotype, the RDAw would be 35.7 μg or 8.9% higher than the actual RDA for Mexican Americans. The additional folate required was not as high for non-Hispanic whites or for non-Hispanic blacks (RDAw: 20.6 and 2.3 μg, respectively; actual RDA increase: 5.2% and 0.57%, respectively).

The frequency of the combined CT/TT genotypes was 20.8% for non-Hispanic blacks, 54.3% for non-Hispanic whites, and 70.1% for Mexican Americans. Among non-Hispanic blacks, the combined CT/TT genotype frequency was not common enough to see any change in the RDAw (Figure 1B). Even with an effect size of 50%, the RDAw would be only 8.4% higher than the actual RDA. Similarly, no significant changes were observed among non-Hispanic whites because an effect size of 50% resulted in an increase in RDAw <25% (exactly 23.9%). However, a >25% increase in the RDAw (27.5%) could be observed among Mexican Americans with an effect size of at least 45%.
MTHFR $677C\rightarrow T$ AND DIETARY FOLATE RECOMMENDATIONS

**DISCUSSION**

Data from our calculations suggest that the current RDA for dietary folate differs little for non-Hispanic whites, non-Hispanic blacks, and Mexican Americans irrespective of the presence of the *MTHFR TT* genotype. Findings from this study suggest that, from a population perspective, the *MTHFR 677C→T* variant does not warrant modifications to the current RDA for dietary folate on the basis of a hypothesis that revision would be warranted if genetic variations increased the RDA by $\geq 25\%$. The findings also support the conclusions of Gibney and Gibney (17). However, we cannot rule out the possibility that advances in genetics and nutrition research, resulting in a better comprehension of the one-carbon metabolism pathway, could lead to different conclusions in the future.

This newly calculated RDA considered the frequency of the *TT* genotype in the population as well as a wide range of effects of the *MTHFR 677C→T* genotype on the requirement for folate. On the basis of our calculations, even with an effect size of 50% of the *MTHFR 677C→T* variant, the increases in the requirement for folate would not warrant changes to the RDA for any of the racial and ethnic groups. The most profound increase in RDA was observed among Mexican Americans due to the higher frequency of the *TT* genotype (A) or on the combined *MTHFR CT and TT* genotype frequencies (B) for non-Hispanic whites (squares), non-Hispanic blacks (circles), and Mexican Americans (triangles) for a wide range of effects of the *TT* (A) or the combined *CT/TT* (B) genotype on folate requirement (5–50%).

We used a wide range of effect sizes (5–50%) because the estimation of the magnitude of the *MTHFR 677C→T* variant on the EAR for folate that is based on previous metabolic studies is unclear. To determine the EAR for folate, the IOM used evidence from 4 metabolic studies (24–27). The metabolic maintenance study by O’Keefe et al (24), in which 3 of 5 women became folate deficient after consuming a diet that contained 319 $\mu$g DFE/d for 70 d, was given the greatest weight in estimating the EAR. Deficiency was based on the combined evidence of 3 biochemical measurements: low red blood cell (RBC) folate (305 nmol/L), low serum folate (7 nmol/L), and high homocysteine (16 $\mu$mol/L) (24).

We found 6 metabolic studies that compared average concentrations of serum folate, RBC folate, or homocysteine before and after controlled folate intake according to the *MTHFR 677C→T* variant for $\geq 7$ wk (28–33). Only 3 of these studies examined the proportion of the population that became deficient (28, 29, 31), and only 1 of those 3 followed participants for $\geq 7$ wk (29). In this study, conducted by Solis et al (29), 29 Mexican American men with the *TT* genotype and 31 men with the *CC* genotype, all aged 18–55 y, were fed a diet that contained 438 $\mu$g DFE/d for 12 wk. At the end of 12 wk, 34% of the men with the *TT* genotype had serum folate $< 6.8$ nmol/L, and 79% had homocysteine concentrations $> 14.0$ nmol/L compared with 16% and 7%, respectively, among men who had the *CC* genotype. There were no genotype-folate interactions with RBC folate, and RBC folate did not approach deficiency in either group. One of the assumptions of our model is that 400 $\mu$g DFE/d is acceptable for people without the genetic variant of interest. The findings of Solis et al (29) suggest that 400 $\mu$g DFE/d may be inadequate for Mexican American men without the *MTHFR 677C→T* variant; however, RBC folate remained in the normal range for all study participants. In addition, results from previous studies (24, 27) suggest that 400 $\mu$g DFE/d is adequate to meet requirements. Thus, at this point, we believe our assumption is correct. In addition, given these limited data, we cannot make any conclusions from metabolic studies regarding the magnitude of the effect of the *MTHFR 677C→T* variant on folate requirement.

Also, it is unclear whether the effect of the genotype on the folate requirement would be the same for each racial and ethnic group.
group when considering the possible effect of different environmental and genetic backgrounds. The effect of the MTHFR 677C→T variant appears to be more deleterious in a low-folate environment (15) and thus may differ across racial and ethnic groups. Accordingly, data from NHANES 2001–2002 suggest that folic acid intake among nonpregnant women of childbearing age in the United States is inadequate: only 21% of Hispanic women consume ≥400 µg/d of folic acid from fortified foods and supplements compared with 41% of non-Hispanic white women (34). Other data from NHANES 1988–1994 and 1999–2000 have shown similar trends among the US population: non-Hispanic blacks and Mexican Americans have lower reported total folate intake than non-Hispanic whites (35).

Some caveats apply to the interpretation of the findings. In our calculations, only a single genetic variant from the folate metabolizing pathway was included. In addition to the MTHFR 677C→T variant, other factors, including bioavailability and other nutritional and genetic factors, could affect folate requirement. Also, this article does not address the RDA for folic acid for women capable of becoming pregnant. It is hypothesized that the increased need by racial and ethnic group and genotype frequency would mirror the results presented for folate, but more studies are needed to confirm this. Finally, with ≈20% of Mexican Americans harboring the TT genotype, and with the knowledge that the effect of the MTHFR TT genotype would be more important in a low-folate environment at the individual level, this proportion of the Mexican American population could justify a higher recommendation for folic acid intake. However, this increased need could be more critical when looking at nutrient requirements on an individual basis (or subpopulation basis) rather than on a population-wide scale. In that sense, rethinking the way RDAs are derived might be appropriate.

In conclusion, findings from this study provide the first evidence that the MTHFR 677C→T variant might not have a significant effect on the dietary folate recommendations for the US population. Although the frequency of the MTHFR TT varied across racial and ethnic groups, the current RDA for dietary folate differs little for non-Hispanic whites, non-Hispanic blacks, and Mexican Americans irrespective of the presence of the MTHFR TT genotype. However, the high frequency of the CT/TT genotype might result in an increased RDA for folate in Mexican Americans, but only if the effect size of this genotype reaches 45%. Further models incorporating information on the interactions between genotype and folate are needed to assess an overall effect of genetic variants on folate requirements for the US population.

The authors’ responsibilities were as follows—JR: conception of the study; data collection, analysis, and interpretation; and manuscript preparation; HCH: data interpretation, manuscript revision, and editing; MEC: data interpretation, manuscript revision, and editing; and QY: data collection, analysis, and interpretation; and manuscript revision and editing. The authors had no conflicts of interest.

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