Associations Between Single Nucleotide Polymorphisms in Folate Uptake and Metabolizing Genes with Blood Folate, Homocysteine, and DNA Uracil Concentrations

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Available at: https://works.bepress.com/zhenhua_liu/7/
Associations between single nucleotide polymorphisms in folate
uptake and metabolizing genes with blood folate, homocysteine,
and DNA uracil concentrations\textsuperscript{1,2,3,4}

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Abstract

\textbf{Background}—Folate is an essential nutrient that supports nucleotide synthesis and biological
methylation reactions. Diminished folate status results in chromosome breakage and is associated
with several diseases, including colorectal cancer. Folate status is also inversely related to plasma
homocysteine concentrations—a risk factor for cardiovascular disease.

\textbf{Objective}—We sought to gain further understanding of the genetic determinants of plasma folate
and homocysteine concentrations. Because folate is required for the synthesis of thymidine from
uracil, the latter accumulating and being misincorporated into DNA during folate depletion, the DNA
uracil content was also measured.

\textbf{Design}—Thirteen single nucleotide polymorphisms (SNPs) in genes involved in folate uptake and
metabolism, including folate hydrolase (\textit{FOLH1}), folate polyglutamate synthase (\textit{FPGS}), \(\gamma\)-glutamyl
hydrolase (\textit{GGH}), methylene tetrahydrofolate reductase (\textit{MTHFR}), methionine synthase (\textit{MTR}),
proton-coupled folate transporter (\textit{PCFT}), and reduced folate carrier (\textit{RFC1}), were studied in a cohort
of 991 individuals.

\textbf{Results}—The \textit{MTHFR} 677TT genotype was associated with increased plasma homocysteine and
decreased plasma folate. \textit{MTHFR} 1298A>C and \textit{RFC1} intron 5A>G polymorphisms were associated
with significantly altered plasma homocysteine concentrations. The \textit{FOLH1} 1561C>T SNP was
associated with altered plasma folate concentrations. The \textit{MTHFR} 677TT genotype was associated
with a \(\approx\)34\% lower DNA uracil content \((P = 0.045)\), whereas the \textit{G} allele of the \textit{GGH} – 124T>G
SNP was associated with a stepwise increase in DNA uracil content \((P = 0.022)\).

\textbf{Conclusion}—Because the accumulation of uracil in DNA induces chromosome breaks, mutagenic
lesions, we suggest that, as for \textit{MTHFR} C677T, the \textit{GGH} – 124 T>G SNP may modulate the risk of
carcinogenesis and therefore warrants further attention.

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\textsuperscript{2}Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily
reflect the view of the US Department of Agriculture.

\textsuperscript{3}This material is based on work supported by the US Department of Agriculture, Agricultural Research Service, under agreement no.
58-1950-7-707. Also supported by grants NIH P01 AG023394 (to KLT) and NIH K05 CA100048 (to JBM).
INTRODUCTION

Folate is an essential nutrient that is involved in biological methylation reactions and nucleotide synthesis. Severe folate depletion, although rare in developed countries, results in megaloblastic anemia. Diminished folate status of a magnitude insufficiently large to cause anemia results in hyperhomocysteinemia (1) and elevates the risk of congenital neural tube defects (2). Low folate status is also associated with an elevated risk of colorectal cancer (3) and possibly cancers of the breast (4), cervix (5), and lung (6). It has been estimated that those consuming the highest amounts of folate have a 30–40% lower risk of colorectal cancer than do those consuming the lowest amounts (3). Importantly, it is becoming clear that timing is crucial in this regard because there is evidence that, in individuals who harbor premalignant lesions, the progression from premalignant to cancerous lesions may be accelerated by high folate intake (7,8).

One of the crucial roles of folate is to provide one carbon units for the thymidylate synthase–mediated conversion of uracil (dUMP) to thymidine (dTMP). Folate depletion is known to restrict this conversion, resulting in an accumulation of intracellular uracil, the misincorporation of uracil into DNA, and subsequently, increased DNA breakage (9,10).

Natural food folates exist mostly in a polyglutamated state and are hydrolyzed by folate hydrolase (FOLH1) on the brush border membrane of the jejunum before absorption can occur. In contrast, folic acid from fortified foods and supplements is monoglutamated and does not require hydrolysis before uptake. Reduced folate monoglutamates and folic acid are absorbed from the gut by the recently identified proton coupled folate transporter (PCFT) gene (11).

Once inside intestinal cells, folate coenzymes of various oxidation states are converted to 5-methyl tetrahydrofolate (THF) before being released into the bloodstream. Circulating 5-methyl THF is taken up into tissues via the reduced folate carrier (RFC1) and the folate receptor (FOLR1), depending on the specific tissue. After entering cells, folate monoglutamates are polyglutamated by folylpolyglutamate synthetase (FPGS), which enables their retention within the cell and increases their affinity for folate-dependent enzymes. Because 5-methyl THF is a poor substrate for FPGS, it must first be demethylated by methionine synthase before it can enter the folate pool. Before folates can be released into the bloodstream, they must be hydrolyzed by γ-glutamyl hydrolase (GGH) (Figure 1).

Because folate has an important role in maintaining health, understanding the factors that govern folate status is essential. Much effort has been devoted to studying the effects of single nucleotide polymorphisms (SNPs), in 2 central enzymes of the folate pathway—methylene tetrahydrofolate reductase (MTHFR) and methionine synthase (MTR)—on plasma concentrations of folate and homocysteine. In the case of MTHFR, the much-studied 677 C>T SNP is associated with lower plasma folate concentrations, changes in folate form distribution, and elevated plasma homocysteine concentrations (12,13).

Of the genes involved in folate uptake and retention, FOLH1 has received the most attention with regard to its potential to modulate plasma folate status. Individuals heterozygous for the 1561C>T SNP were shown initially to have significantly lower plasma folate concentrations than major allele carriers (14), an association that was not subsequently replicated by the same investigators (15). Other studies have reported that the T allele of the SNP was associated with elevated plasma folate concentrations (16–18). Studies of the 80 G>A SNP (rs1051266) in the RFC1 gene have been much more consistent; 3 European cohorts reported no associations with plasma folate or homocysteine (15,18,19).

To the best of our knowledge, there are currently no published studies on the relation between SNPs in GGH, PCFT, FOLR1, or FPGS and plasma folate and homocysteine concentrations.
With regard to $GGH$, 2 SNPs ($-401C>T$, rs3758149, $-124T>G$, and rs11545076) have been shown to increase promoter activity when introduced into both HepG2 and MCF-7 cell lines (20).

To further our understanding of folate metabolism, we sought to determine whether selected SNPs in folate uptake and retention genes ($FOLR1$, $FOLH1$, $RFC1$, $PCFT$, $FPGS$, and $GGH$) as well as SNPs in $MTHFR$ and $MTR$ are associated with altered blood folate, homocysteine, and DNA uracil concentrations. Our a priori hypothesis was that the mutant alleles of selected SNPs would be associated with a reduced ability to take up, retain, and metabolize folate and hence result in reduced folate and increased homocysteine concentrations in plasma. Furthermore, because thymidine synthesis is dependent on folate, we also hypothesized that these SNPs would be associated with alterations in DNA uracil content. Samples from the ongoing Boston Puerto Rican Health Study were used to test these associations.

SUBJECTS AND METHODS

Subjects and study design

The current study was conducted in a nested fashion within the ongoing Boston Puerto Rican Health Study (BPRHS), which is described in detail elsewhere (21). Briefly, areas of high Hispanic density in the Boston metropolitan area were identified from the year 2000 census, and one Puerto Rican adult from households with at least one Puerto Rican person between 45 and 75 y of age was randomly selected for participation. Interviews were conducted in the home; in addition to a host of health-related and anthropometric data, detailed data were collected on dietary intake with the use of a questionnaire previously adapted from the National Cancer Institute/Block food-frequency form and validated for this population (22). Fasting blood samples were collected on the morning after the health interviews were conducted in the subjects’ home. Approval for the BPRHS was obtained from the Institutional Review Board of the New England Medical Center and Tufts University Health Sciences; the current nested study was declared exempt from such requirements because of the use of deidentified samples and data (National Institutes of Health exemption category 4). Demographic data, caloric intake, and plasma B vitamin concentrations of the study participants are shown in Table 1, and 991 people were genotyped for this study.

SNP selection

A list of SNPs identified within and mapping very near to each gene of interest was obtained from the National Center for Bio-technology Information SNP database, and a multipronged strategy was used to select those to be assayed. SNP selection was performed after weighing information gathered from published literature, public databases, and predictions from bioinformatics tools.

First, to have sufficient statistical power to detect potential differences in plasma folate and homocysteine between different genotypes, preference was given to SNPs with a reported minor allele frequency of $\geq0.2$. Second, linkage disequilibrium data retrieved for each gene from the website of the International HapMap project (23,24) was used to select Tag SNPs and preclude the selection of more than one SNP from a specific linkage disequilibrium block. Third, a thorough search of the literature was performed to identify SNPs that have previously been associated with folate status or that have some other functional indicator suggesting the potential for modulating folate status. In addition, multiple bioinformatics tools were used to predict whether a specific SNP had an indication of a functional effect and thereby the potential to modulate blood folate concentrations.
For SNPs lying upstream of the gene of interest, MAPPER was used to determine whether the SNP produces a predicted allele-specific transcription factor binding site (25). For exonic SNPs, both synonymous (no amino acid change) and nonsynonymous (amino acid change) changes were considered. For synonymous and nonsynonymous SNPs, changes in mRNA folding were predicted by using the GeneBee Molecular Biology Server (26). SNPs predicted to cause substantial changes in mRNA structure were considered to be of particular interest. For nonsynonymous SNPs, a prediction was made as to the nature of the amino acid change by using the Polyphen tool (27). In addition, SNPs reported to fall within splice sites were also considered for selection.

The final exclusion stage was based on the compatibility of the sequence surrounding each SNP with genotyping using Taqman probes. Several SNPs of interest were excluded at this stage because of the presence of surrounding nucleotide repeats (eg, rs1051266 at RFC1) based on analyses with RepeatMasker (28). A list of the SNPs selected for genotyping in our study is given in Table 2.

**DNA isolation and genotyping**

DNA was isolated from the buffy coat of blood samples by using QIAamp DNA Blood Mini kits according to the manufacturer’s instructions (Qiagen, Valencia, CA). Genotyping reactions were performed by using custom and predesigned TaqMan SNP genotyping assays according to manufacturer’s instructions (Applied Biosystems, Foster City, CA). Reaction master mix was added to 384 well reaction plates by using a Genesis RSP150 robotic liquid handling system (Tecan, Männedorf, Switzerland) followed by the addition of DNA with the use of a Rapid Plate workstation (Caliper Lifesciences, Hopkinton, MA). Thermal cycling was performed on 9700 thermalcyclers before plates were read with a 7900HT Sequence Detection System (both from Applied Biosystems).

**Biochemical measurements**

Total plasma homocysteine was measured by HPLC with fluorescence detection as previously described (33). Plasma pyridoxal phosphate (PLP) was determined using the radioenzymatic method of Camp et al (34). Plasma folate and vitamin B-12 were measured with Immulite chemiluminescent kits according to the manufacturer’s instructions (Diagnostic Products Corporation/Siemens, Los Angeles, CA). Plasma creatinine was measure with the use of a modified Jaffe method (35).

**DNA uracil measurement**

After the exclusion of persons taking medications that might potentially affect the DNA uracil content, a subset of 300 persons were randomly selected from the cohort. Specifically, persons taking certain antipurines (azathioprine), chemotherapy drugs (methotrexate), or antibiotics (sulfamethoxazole and trimethoprim) were excluded. DNA was extracted from 1 mL whole frozen blood by using standard phenol:chloroform extraction after proteinase K and RNase treatment; 5.0–10 μg DNA was used to determine the uracil content after uracil DNA glycosylase (New England Biolabs, Ipswich, MA) treatment according to the gas chromatography–mass spectrometry method of Blount and Ames (36) with modifications (37). Sufficient DNA for uracil analysis was recovered from 255 individuals. Within-run and between-run CVs were 7% and 10%, respectively.

**Statistical analyses**

All statistical analyses were performed by using SYSTAT 11 (San Jose, CA). Plasma folate, homocysteine, and uracil concentrations among different genotypes were compared by using multiple linear regression, with group comparison made with the Bonferroni method.
Associations between variables were studied by using log-transformed data (except age and alcohol intake) and Pearson correlation coefficients. Chi-square tests were conducted to examine whether the genotype frequencies of the selected SNPs were in Hardy-Weinberg equilibrium. All data are reported as adjusted means ± SEMs. Significance was accepted when \( P \leq 0.05 \) or \( \leq 0.004 \) when applying Bonferroni correction for multiple comparisons (alpha was divided by 12 because 2 of the 13 successfully assayed SNPs were in strong linkage disequilibrium). No further adjustment was made for testing multiple traits because the traits are mechanistically interrelated.

RESULTS

Plasma homocysteine was significantly correlated with plasma folate \((P < 0.001, r = -0.31)\), PLP \((P < 0.001, r = -0.17)\), plasma vitamin B-12 \((P < 0.001, r = -0.23)\), plasma creatinine \((P < 0.001, r = 0.44)\), alcohol intake \((P < 0.001, r = 0.14)\), and age \((P < 0.001, r = 0.19)\). Plasma folate was significantly correlated with age \((P < 0.001, r = 0.15)\) and intake of dietary folate equivalents \((P < 0.001, r = 0.2)\). None of the dietary or biochemical variables correlated significantly with DNA uracil content \((P > 0.05)\).

Blood folate, homocysteine, and DNA uracil concentrations did not have a normal Gaussian distribution; therefore, all associations with SNPs were performed with the use of log-transformed data.

Of the 17 SNPs tested, 13 were successfully assayed, whereas 4 were not, probably because the assay was not able to adequately amplify and distinguish both alleles. The average response rate for the 13 SNPs successfully genotyped was 94.3% (range: 83.2–97.6%). The minor allele frequencies of the 13 successful SNPs are reported in Table 3. All 13 SNPs were in Hardy-Weinberg equilibrium (by chi-square test). Although not listed as such in the HapMap database, the 2 SNPs tested in the \( GGH \) gene appeared to be in linkage disequilibrium in this population because the variant alleles almost always appeared together \((r^2 = 0.97)\).

After age, sex, alcohol intake, plasma folate, PLP, vitamin B-12, and creatinine were corrected for, the plasma homocysteine concentration was significantly associated with 3 of the tested SNPs (\( MTHFR 677C>T \), \( MTHFR 1298A>C \), and \( RFCint5A>G \)) at \( \alpha = 0.05 \); however, only one association retained significance at the \( \alpha = 0.0041 \) level (\( MTHFR 1298A>C \)). Two SNPs (\( MTHFR 677C>T \) and \( FOLH1 1561C>T \)) were associated with plasma folate after adjustment for age, sex, intake of alcohol, dietary folate equivalents, and smoking status at the 0.05 but not at the 0.0041 level. All of these SNPs conformed best to a dominant/recessive model (ie, the phenotype of heterozygotes was not different from either the homozygous wild-type or variant genotype).

Homozygosity for the \( T \) allele of the \( MTHFR 677C>T \) SNP was associated with significantly elevated plasma homocysteine compared with the pooled wild-type and heterozygote group. Homocysteine was 5.6% higher in \( TT \) subjects than in pooled \( CC \) and \( CT \) subjects \((P = 0.05; \text{Figure 2A})\). There was no significant interaction between plasma folate and the 677\( C \)\( T \) SNP for this homocysteine analysis. Plasma folate concentration was 4.6% lower in \( TT \) than in \( CC/CT \) individuals \((P > 0.02; \text{Figure 2A})\).

Plasma homocysteine was 16.7% \((P = 0.001)\) higher in those with the \( MTHFR 1298CC \) genotype than in those with the wild-type or heterozygous (\( AA/AC \)) genotype (Figure 2B). No significant association was detected for this SNP and plasma folate. Five subjects were homozygous for both \( MTHFR \) polymorphisms—an insufficient number to detect an elevation in homocysteine compared with those with the wild-type or heterozygous for both genes \((P > 0.05)\). Unlike most populations studied previously (38, 39), the \( MTHFR 677C>T \) and \( 1298A>C \)
SNPs did not appear to be in linkage disequilibrium in this population (linkage disequilibrium correlation $R = 0.002$; HelixTree v6.2.1, Bozeman, MT).

Individuals possessing one or more $T$ alleles of the $FOLH1$ 1561C>T SNP had 10.8% higher ($P = 0.03$) plasma folate concentrations but unchanged plasma homocysteine (Figure 2C) concentrations compared with those who were $CC$. The $RFC1$ intron5 A>G polymorphism was associated with $\approx 7\%$ lower plasma homocysteine concentrations in homozygous mutants than were the $AA$ and $AG$ genotypes combined ($P = 0.04$; Figure 2D). No significant associations between genotype and plasma folate or homocysteine concentrations were detected for any of the other SNPs.

In addition to the $MTHFR$ 677C>T polymorphism being associated with plasma folate and homocysteine concentrations, those with the $TT$ genotype had a significantly lower blood DNA uracil content. After correction for age, sex, and plasma folate, vitamin B-6, and vitamin B-12 concentrations, those with the $TT$ genotype had a DNA uracil content that was $33.8\%$ lower than that of those with the $CC$ and $CT$ genotypes combined ($0.49 \pm 0.18$ compared with 0.75 $\pm 0.07$ pg/ug DNA; $P = 0.045$).

The DNA uracil content was also associated with the $-124T>G$ polymorphism in the GGH promoter region. With the use of an additive model and after correction for the above-mentioned variables, heterozygosity was associated with a $30\%$ elevation in uracil, whereas homozygosity for the variant $G$ allele was associated with a $73\%$ elevation in DNA uracil ($P$ for trend $= 0.02$ and 0.06 for models with untransformed and log-transformed data, respectively) (Figure 3).

**DISCUSSION**

Because reduced blood folate concentrations are associated with several disease states, including colorectal cancer (3,40) and neural tube defects (41), we sought to further characterize the genetic determinants of folate status. We successfully tested for 13 SNPs in 7 genes involved in folate uptake and metabolism. These SNPs were also tested for associations with homocysteine—an amino acid whose conversion to methionine is dependent on folate and that has also been associated with cardiovascular disease (42,43).

Our observations for the $MTHFR$ 677C>T polymorphism are in good agreement with those of several studies that have shown that plasma homocysteine concentrations are elevated in those homozygous for the $T$ allele but not in those heterozygous for the $T$ allele (12,44). Consistent with previous reports (31), we also showed that $TT$ individuals have a significantly lower plasma folate concentration than do $CC$ individuals (Figure 2A)—an effect that is likely due to the reduced production of 5-methyl THF, the folate form that is released from tissues into the blood. Interestingly, in contrast with most of the literature to date (30,45,46), we showed that the $MTHFR$ 1298 A>C SNP has a stronger effect on plasma homocysteine than does the 677C>T SNP. Persons with the 1298 $CC$ genotype had plasma homocysteine concentrations almost 17\% higher than those with the $AA/AC$ genotype, whereas 677 $TT$ subjects had homocysteine concentrations only 5\% higher than $CC/CT$ subjects.

Another SNP to have received much attention in regard to plasma folate and homocysteine concentrations is the 1561C>T transition of the $FOLH1$ gene. Initially, the $T$ allele was associated with elevated plasma homocysteine and reduced folate concentrations in the elderly English people (14); however, this association was not confirmed in a larger study by the same group (15). Subsequently, 2 studies reported that the mutant $T$ allele was associated with significantly elevated plasma folate but not homocysteine concentrations (17,18), whereas a third reported the same effect in males but not in females (16). In our population, those with at least one copy of the $T$ allele had plasma folate concentrations that were 10.8\% higher than...
those in the wild-type group, but no change in homocysteine was observed (Figure 2C). Although the activity of the mutant enzyme was lower than that of the wild type when expressed in Cos-7 cells (14), the consensus of 4 independent human studies seems to suggest that the effect of the mutant allele may be the opposite in vivo. Also of interest was that these latter studies (16–18) all showed that, whereas the variant allele is associated with elevated folate, homocysteine is not. This finding indicates that elevated blood folate may not translate into increased tissue folate and thus the capacity for homocysteine remethylation.

We also observed an association between the RFC1 intron 5 G>A SNP with plasma homocysteine concentrations (Figure 2D). It is possible that the A allele that we tested for, or another SNP in linkage disequilibrium, significantly impairs the ability of cells to take up folate, which subsequently reduces their capacity to remethylate homocysteine, thereby causing it to accumulate and raise plasma concentrations. Whereas homocysteine was lower in GG individuals, it is unclear why plasma folate was unchanged.

The DNA uracil content was measured in a subset of the cohort, and associations with the 13 SNPs were tested. Of particular interest, the −124 G allele in the promoter region of the GGH gene was associated with significant stepwise elevations in DNA uracil content for each additional G allele (Figure 3). This polymorphism was previously shown to enhance the expression of luciferase compared with the wild type when inserted into a reporter vector in HepG2 and MCF-7 cells (20). It is not yet known whether this increase in promoter activity in vitro equates to higher mRNA and protein expression in vivo. An indication that these SNPs do have functional effects in vivo comes from studies with arthritis patients receiving methotrexate. Those homozygous for the −401 T allele were more likely to display a relative depletion of long-chain methotrexate polyglutamates than were those with the CC and CT genotypes, which indicates an increased glutamate hydrolysis capacity (47). On the basis of these findings, we suggest that the mutant genotypes of the −124T>G and/or −401C>T SNPs result in an increased expression of the GGH protein, which thereby increases the hydrolytic capacity of the cells and causes a relative depletion of intracellular folate polyglutamates. Because folylpolyglutamates are better substrates for most folate-dependent enzymes than monoglutamates, the GGH SNP may result in a functional depletion of the form of the cofactor that is most suitable for critical cellular reactions. Reduced availability of polyglutamated 5,10-methylene THF for the thymidylate synthase–mediated conversion of uracil to thymidine would result in a cellular accumulation of uracil that can be subsequently incorporated into DNA. It has been shown repeatedly that an elevated DNA uracil content is associated with increased DNA breakage (9,48)—a mutagenic event that may drive carcinogenesis. Therefore, this association warrants confirmation in a larger cohort and, if upheld, a study of the effect of these GGH promoter SNPs on markers of chromosome breakage, such as micronucleus formation. Because the accumulation of chromosome damage is a risk factor for carcinogenesis (49), it is also possible that these SNPs may be candidate risk factors for cancer.

It is interesting to note that, whereas the GGH −124T>G SNP was significantly associated with the DNA uracil content (Figure 3), it was not significantly associated with the plasma homocysteine concentration (P = 0.7; data not shown). We suggest that this phenomenon may be due to a differential sensitivity of these 2 processes to fluctuations in the intracellular abundance of polyglutamated folates. Several plausible explanations for this observation may exist. One, because the Michaelis constant of methionine synthase is estimated to be lower than the intracellular concentrations of its polyglutamated substrate, this reaction is always saturated under physiologic conditions (50). Conversely, the Michaelis constant for thymidylate synthase is higher than the intracellular concentration of its substrate and is therefore a concentration-dependent reaction (50). Because thymidylate synthesis is concentration-dependent, it may be more sensitive to small decreases in folate concentration than homocysteine remethylation.
In addition, we observed a significant 33% lower DNA uracil content associated with the MTHFR 677 TT genotype. This association will be described in more detail elsewhere (Chanson et al, preparation). It has been shown that, although total intracellular folate concentration is unchanged, the reduced MTHFR activity of TT subjects results in a relative depletion of 5-methyl THF, which is required for homocysteine remethylation, and an increased abundance of formylated folates (13,51). The reduced conversion of 5,10-methylene THF to 5-methyl THF is thought to increase its availability for thymidylate synthesis, which reduces the accumulation and subsequent incorporation of uracil into DNA. No significant association of the MTHFR 1298A>C SNP was seen with uracil, which is interesting because this SNP appears to have a stronger impact on plasma homocysteine than does the 677C>T polymorphism. It is unclear why this discrepancy exists; however, it should be noted that the minor allele frequency for the 1298A>C SNP is much lower than that for the 677C>T SNP (0.26 compared with 0.35). Thus, with only 12 persons of the 1298CC genotype in the subset tested for uracil, we may have insufficient power to detect effects on uracil in the current study.

In conclusion, we showed that 2 of the 13 SNPs studied were associated with altered plasma folate concentrations: 3 with altered plasma homocysteine concentrations and 2 with altered blood DNA uracil contents. Of particular interest was the observed association of the mutant alleles of the GGH promoter region with elevated DNA uracil concentrations. This is an intriguing association that warrants confirmation in a larger population. If the current association is upheld, it is possible that these SNPs may alter the risk of carcinogenesis by increasing the accumulation of chromosome aberrations.

Acknowledgments

We thank Jose Ordovas for facilitating the SNP analyses, Xian Adiconis and Bibiana Garcia-Bailo for their invaluable technical support, Jacob Selhub and Chao-Qiang Lai for their input during the preparation of the manuscript, and Gayle Petty and the Nutrition Evaluation Laboratory for the blood analyses.

The authors’ responsibilities were as follows—LDV and JWC: genotyping reactions; JWC, ZL, and AC: statistics; AC and EDC: uracil measurements; JWC and JBM: manuscript preparation; and KLT: human study. None of the authors had a conflict of interest.

References


FIGURE 1.
Proteins involved in the uptake, retention, and metabolism of folate. FOLH1, folate hydrolase 1 (γ carboxy peptidase II); FOLR1, folate receptor 1; FPGS, folate polyglutamate synthase; GGH, γ-glutamyl hydrolase; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; PCFT (SLC46A1), proton-coupled folate transporter; RFC1 (SLC19A1), reduced folate carrier 1; 1-C, one-carbon.
FIGURE 2.
Association of single nucleotide polymorphisms (SNPs) in methylene tetrahydrofolate reductase (MTHFR), reduced folate carrier 1 (RFC1), and folate hydrolase 1 (FOLH1) with plasma folate and homocysteine (Hcy) concentrations. Values are expressed as adjusted means ± SEMs. Hcy values were adjusted for age, sex, alcohol intake, plasma folate, plasma pyridoxal phosphate, vitamin B-12, and creatinine. Folate data were adjusted for age, sex, smoking status, alcohol intake, and dietary folate equivalent intake. *Log homocysteine and folate significantly different (P ≤ 0.05, general linear model) from other 2 genotypes combined (dominant/recessive) after adjustment for the abovementioned factors (n = 875–929). Note that for the FOLH1 1561C>T SNP (C), the CT and TT genotypes were combined because n = 2 for the TT genotype.
FIGURE 3.
Association of the γ-glutamyl hydrolase (GGH) single nucleotide polymorphisms rs11545076 genotype with blood DNA uracil concentrations. Values are expressed as means ±SEMs. n = 128, 92, and 18 for the TT, TG, and GG genotypes, respectively. Uracil data were adjusted for age, sex, plasma folate, vitamin B-12, and plasma pyridoxal phosphate. *P for trend (additive model) = 0.022 and 0.058 for the untransformed and log-transformed models, respectively.
### TABLE 1
Demographics, dietary intakes, and blood variables for the subjects

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.3 ± 6.6 (278)</td>
<td>58 ± 8.0 (713)</td>
<td>0.16³</td>
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<tr>
<td>Weight (kg)</td>
<td>83.6 ± 16.6 (275)</td>
<td>79.3 ± 18.6 (705)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>29.8 ± 6.6 (274)</td>
<td>33.2 ± 5.3 (705)</td>
<td>&lt;0.001³</td>
</tr>
<tr>
<td>Caloric intake (kcal/d)</td>
<td>2691.8 ± 1235.3 (272)</td>
<td>2140.5 ± 1234.7 (696)</td>
<td>&lt;0.001³</td>
</tr>
<tr>
<td>Multivitamin use (%)</td>
<td>18.3 (278)</td>
<td>21.6 (713)</td>
<td>0.26⁴</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td></td>
<td></td>
<td>&lt;0.001⁴</td>
</tr>
<tr>
<td>Never</td>
<td>33 (274)</td>
<td>52 (274)</td>
<td>—</td>
</tr>
<tr>
<td>Past</td>
<td>36 (274)</td>
<td>28 (274)</td>
<td>—</td>
</tr>
<tr>
<td>Current</td>
<td>31 (274)</td>
<td>20 (274)</td>
<td>—</td>
</tr>
<tr>
<td>Plasma folate (ng/mL)</td>
<td>17.4 ± 9.1 (276)</td>
<td>20 ± 9.0 (700)</td>
<td>&lt;0.001³</td>
</tr>
<tr>
<td>Plasma vitamin B-12 (pg/mL)</td>
<td>493.6 ± 240.9 (270)</td>
<td>528.8 ± 244.4 (706)</td>
<td>0.043³</td>
</tr>
<tr>
<td>Plasma pyridoxal phosphate (nmol/L)</td>
<td>61.9 ± 61.5 (278)</td>
<td>58.8 ± 61.3 (711)</td>
<td>0.472³</td>
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<tr>
<td>Plasma homocysteine (μmol/L)</td>
<td>10.6 ± 4.7 (278)</td>
<td>8.7 ± 4.8 (711)</td>
<td>&lt;0.001³</td>
</tr>
<tr>
<td>Plasma creatinine (mg/L)</td>
<td>9.8 ± 3.3 (277)</td>
<td>7.8 ± 2.7 (709)</td>
<td>&lt;0.001³</td>
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<tr>
<td>Blood cell DNA uracil (pg/μg DNA)</td>
<td>0.75 ± 0.81 (66)</td>
<td>0.67 ± 0.82 (189)</td>
<td>0.53³</td>
</tr>
</tbody>
</table>

¹ n in parentheses.
² ± SD (all such values).
³ General linear model.
⁴ Pearson chi-square test.
<table>
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<tr>
<th>Gene and mRNA ID no.</th>
<th>SNP ID no.</th>
<th>Nucleotide change</th>
<th>Location</th>
<th>Amino acid change</th>
<th>Common name</th>
<th>HUGO name</th>
<th>Bioinformatics predictions</th>
<th>Literature</th>
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<td>1561 C&gt;T</td>
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<td>exon 8</td>
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<td>FOLR2_E493A, A1298C</td>
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<td>G: NT</td>
<td>MTR-2.5-13 (Hs)</td>
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</table>

1. SNP names are GENE−position, where m indicates minus or upstream of mRNA start, i indicates intron and position from mRNA start, 3U indicates 3′-UTR and position in mRNA, the capital letters indicate an amino acid change, and fs indicates a frameshift.

2. Name of the transcription factor is listed, followed by the species in parentheses, e.g., NRF-2 (Hs).

3. SNP genotyping failed (undetermined genotype ≥ 25%).
**TABLE 3**

Observed minor allele frequencies (MAFs)

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</table>

All single nucleotide polymorphisms (SNPs) are in Hardy-Weinberg equilibrium (ch-square test). SNPs rs5819844 (PCFT), rs35179028 (FOLR1), rs7928531 (FOLR1), and rs41306702 (FPGS) could not be studied because of the failure of genotyping reactions. The average response rate for the 13 SNPs successfully genotyped was 94.3% (83.2–97.6%).