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RESEARCH PAPER

Effects of methionine synthase and methylenetetrahydrofolate reductase gene polymorphisms on markers of one-carbon metabolism

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Abstract Genetic and nutritional factors play a role in determining the functionality of the one-carbon (1C) metabolism cycle, a network of biochemical reactions critical to intracellular processes. Genes encoding enzymes for methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MTR) may determine biomarkers of the cycle including homocysteine (HCY), S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). MTHFR C677T is an established genetic determinant of HCY but less is known of its effect on SAM and SAH. Conversely, the relationship between MTR A2756G and HCY remains inconclusive, and its effect on SAM and SAH has only been previously investigated in a female-specific population. Folate and vitamin B_{12} are essential substrate and cofactor of 1C metabolism; thus, consideration of gene–nutrient interactions may clarify the role of genetic determinants of HCY, SAM and SAH. This cross-sectional study included 570 healthy volunteers from Kingston, Ontario, Ottawa, Ontario and Halifax, Nova Scotia, Canada. Least squares regression was used to examine the effects of MTR and MTHFR polymorphisms on plasma HCY, SAM and SAH concentrations; gene–gene and gene–nutrient interactions were considered with the inclusion of cross-products in the model. Main effects of MTR and MTHFR polymorphisms on HCY concentrations were observed; however, no gene–gene or gene–nutrient interactions were found. No association was observed for SAM. For SAH, interactions between MTR and MTHFR polymorphisms, and MTHFR polymorphism and serum folate were found. The findings of this research provide evidence that HCY and SAH, biomarkers of 1C metabolism, are influenced by genetic and nutritional factors and their interactions.

Keywords Methionine synthase · Methylenetetrahydrofolate reductase · Homocysteine · S-adenosylmethionine · S-adenosylhomocysteine · One-carbon metabolism

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Introduction

The one-carbon (1C) metabolism cycle is a network of biochemical reactions that provide 1C units required for critical intracellular processes (Mason 2003). Impairments of the 1C metabolism cycle have increasingly been implicated in numerous disease states including cancer, neurodegenerative disease and adverse reproductive outcomes (Mason 2003; Castro et al. 2003; MRC Vitamin Study Research Group 1991; Selhub et al. 1995; Malinow et al. 1993; Petri et al. 1996; Perry et al. 1995; Butterworth 1993; Kamei et al. 1993; Slattery et al. 1999; Zhang et al. 1999; Dekker et al. 1995; Rajkovic et al. 1997; Clarke et al. 1998). Disturbances to the cycle may be due to insufficient intakes of dietary cofactors such as folate and other B vitamins as well as common polymorphisms in genes involved in the 1C metabolism cycle.

Disruption of 1C metabolism may influence disease risk through impairments of DNA methylation and thymidine synthesis and influences on oxidative stress. Briefly, DNA methylation is an epigenetic mechanism

that most commonly involves the covalent addition of a methyl group to cytosine residues that precede guanine. DNA methylation is essential to the maintenance of cellular function since it plays a major role in the regulation of gene expression and cell signaling mechanisms (Molloy 2004). Thymidine is the precursor to thymine, one of the four nucleobases in the nucleic acid of DNA (Richards 2008). The downstream effects caused by a disruption in thymidine synthesis include genomic instability involving reduced nucleotide availability and the misincorporation of uracil into DNA leading to miscoding, strand breaks and increased micronucleus formation (Molloy 2004). Impairments in 1C metabolism also influence oxidative stress as the cycle provides metabolic intermediates that either react with pro-oxidants or promote antioxidant defense (Vijaya Lakshmi et al. 2011). Thus, the proper functioning of the 1C metabolism cycle is critical to a wide-spectrum of housekeeping functions within the cell.

Biomarkers of the pathways in 1C metabolism including homocysteine (HCY), S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) have often been used to represent the functionality of the cycle. Figure 1 depicts a simplified schematic of the 1C metabolism cycle; the following description focuses on the role of key enzymes in the pathways, and their associated genes are identified in brackets. The provision of methyl groups in 1C metabolism is through the metabolism of dietary methionine beginning with the activation of methionine by adenosine triphosphate (ATP) to produce SAM, the universal methyl donor. Transmethylation of SAM in which a methyl group of SAM is transferred to an acceptor-like DNA via DNA methyltransferases (*DNMTs*) results in the formation of SAH. Under ideal physiologic conditions, SAH is hydrolyzed to HCY and adenosine (James et al. 2002; Wagner and Koury 2007). HCY is then metabolized by two biochemical pathways: transsulfuration and remethylation. In the transsulfuration pathway, HCY is catabolized to form the amino acids cysteine and taurine in a series of irreversible reactions one of which is catalyzed by cystathione β-synthase (*CBS*) (Selhub 1999). Alternatively, to initiate a new cycle of 1C group transfer, HCY is remethylated back to methionine by methionine synthase (*MTR*) with vitamin B_{12} and SAM acting as cofactors and 5-methyltetrahydrofolate (5-methylTHF), a substrate. The remethylation of HCY also requires methionine synthase reductase (*MTRR*) to maintain activated vitamin B_{12} needed for the MTR-catalyzed remethylation reaction of HCY (Gaughan et al. 2001). 5-methylTHF is provided by the metabolism of dietary folate whereby methylenetetrahydrofolate reductase (*MTHFR*) catalyzes the conversion of 5,10-methylene-THF to 5-methyl-THF (Selhub 1999). MTHFR plays a central role in directing the provision of 1C groups toward methionine metabolism and away from thymidine synthesis (Jacobsen 2000). Thymidine synthesis is catalyzed by thymidylate synthase (*TYMS*) and converts deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), a precursor of thymidine. To ensure the proper functioning of the 1C-metabolism pathways, numerous levels of control are provided through allosteric regulation; for example, SAM is able to inhibit the action of MTHFR and stimulate CBS activity (Dekou et al. 2001). Further, SAH diminishes the SAM-mediated inhibition of MTHFR, is able to inhibit MTR, activates CBS and limits its own synthesis through inhibition of methyltransferases (Finkelstein 2007).

The investigation of the main effects of MTR and MTHFR polymorphisms on HCY, SAM and SAH concentrations was undertaken in this study. The role of MTHFR polymorphism on HCY (Ho et al. 2010), SAM and SAH (Ho et al. 2011) was previously investigated in a smaller subset of this study population. For the MTR gene, an A2756G polymorphism has been described resulting in a change of aspartic acid to glycine at position 919. The functional significance of this polymorphism is currently unknown, but it is postulated to be a functional mutation as it occurs in a protein domain involved in reductive methylation and regeneration of an active enzyme (Dekou et al. 2001). A common polymorphism in the MTHFR gene (MTHFR C677T) in which a substitution of alanine by valine at position 222 occurs in those homozygous for the MTHFR 'TT' genotype; the functional significance of this polymorphism is well established with the homozygous variant genotype (TT) conferring a decrease in enzymatic activity by 20–30 % (Trinh et al. 2002).

In this study, we investigated genetic determinants (MTR and MTHFR polymorphisms) of HCY, SAM and SAH concentrations in a healthy Canadian population between the ages of 20 and 50 years. Beyond genetic control, disturbances in nutrient intakes and metabolism can interfere with homeostasis of the 1C metabolism cycle and, in turn, HCY, SAM and SAH levels (Rasmussen et al. 2000). Thus, accordingly, interactions between polymorphisms and relevant nutrient cofactors involved in 1C metabolism (serum folate and vitamin B_{12}) on HCY, SAM and SAH concentrations were also examined. Investigating the influences of genetic and nutritional factors on metabolites of 1C metabolism will contribute to a better understanding of the role of 1C metabolism in the pathogenesis of disease states like cancer, cardiovascular disease and adverse pregnancy outcomes.

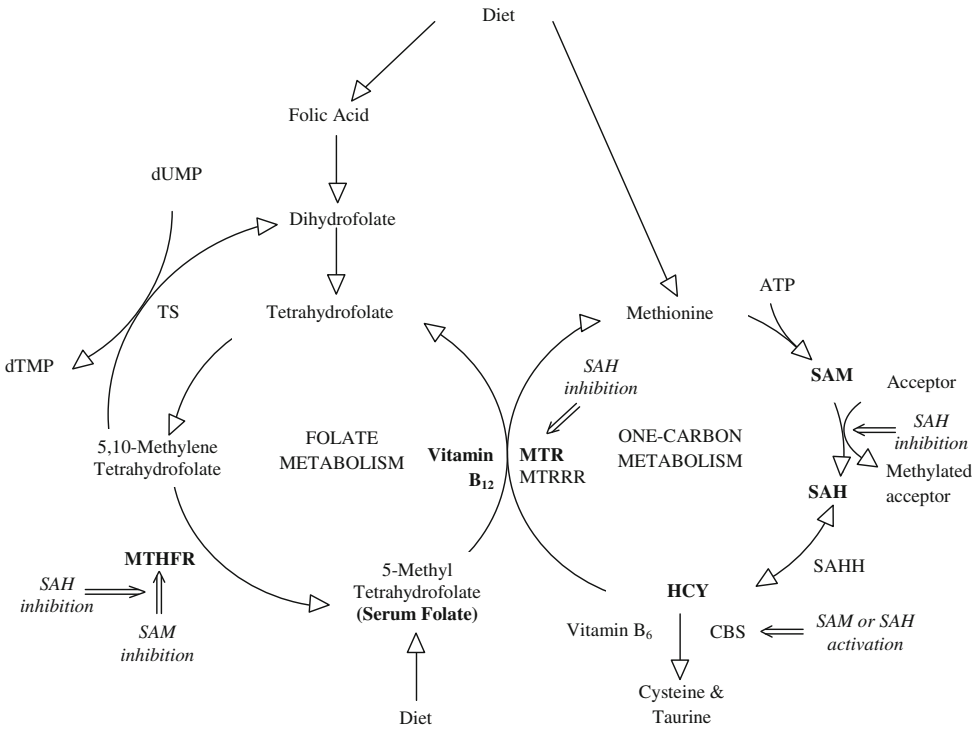

Fig. 1 One-carbon metabolism cycle (Jacobsen 2000) (*dUMP* deoxyuridine monophosphate, *TYMS* thymidylate synthase, *dTMP* deoxythymidine monophosphate, *MTHFR* methylenetetrahydrofolate reductase, *MTR* methionine synthase, *MTRR* methionine synthase reductase, *CBS* cystathione β-synthase, *ATP* adenosine triphosphate, *SAM* S-adenosylmethionine, *DNMT* DNA methyltransferase, *SAH* S-adenosylhomocysteine)

Materials and methods

Study population

The study methods and procedure have been described previously (King et al. 2012). Briefly, this cross-sectional study included 570 healthy male and female volunteers between the ages of 20 and 50 years from Kingston, Ontario, Ottawa, Ontario and Halifax, Nova Scotia, Canada. Subjects with health conditions known to be related to the biomarkers of interest (e.g., history of angina or other vascular disease, cancer or diabetes), who were pregnant or who had given birth in the previous year, were excluded from the study sample. Subjects provided a blood sample for genomic and biochemical analysis and completed a questionnaire. Ethics approval was granted by the Queen's University Health Sciences Research Ethics Board (Ethics #EPID 233-06), and all subjects provided written informed consent.

Laboratory analyses

Blood sampling, processing and aliquoting procedures were standardized between the three study centers; a total of 15 mL of venous blood were drawn from each participant. Specifically, fasting concentrations of plasma HCY, SAM and SAH were determined from 10 mL of blood drawn into tubes with ethylenediaminetetraacetic acid, and 5 ml of blood was obtained in a serum separator tube for measurements of serum folate and vitamin B_{12}. Serum and plasma samples were kept frozen at −70 °C prior to analysis. Plasma HCY, serum folate and serum vitamin B_{12} were measured at the Ottawa Hospital, Department of Pathology and Laboratory Medicine, Division of Biochemistry, Ottawa, Canada. Plasma SAM and SAH were measured at the Saskatchewan Disease Control Laboratory, Regina, Canada. All serum and plasma samples were shipped on dry ice.

Plasma HCY concentrations were quantified by the Abbott AxSYM immunoassay based on fluorescence polarization immunoassay technology (Abbott AxSYM, Abbott Laboratories, Abbott Park, IL, USA). This assay has an analytical range of 1–50 μmol/L and coefficients of variation (CV) of 5.5 % at 7.4 μmol/L, 6.2 % at 13.5 μmol/L and 5.4 % at 25.9 μmol/L. Plasma SAM and SAH were measured using a tandem mass spectrometry method developed by Struys et al. (2000) and Gellekink et al. (2005) (see King et al. (2012) for more details on the modifications to the published method). Briefly, modification to the published methods included the use of deuterium-labeled SAM ($^2H_3$-SAM) as an internal standard for both SAM and SAH analyses; otherwise, testing conditions of the assay were conducted as described. SAM and SAH were obtained from Sigma (St. Louis, MO, USA) and $^2H_3$-SAM from CDN Isotopes (Pointe-Claire, Quebec, Canada). All analyses were performed on an API 4000 triple quadrupole tandem mass spectrometer and binary HPLC pump (Agilent 1200 series). This assay has CV of 7 and 15 % for

SAM and SAH, respectively (King et al. 2012). Recoveries for both SAM and SAH were comparable to percentages published by Gellekink et al. (2005) (mean recoveries for SAM and SAH >90 %). Determination of serum folate was accomplished on a Beckman Coulter Access II (Beckman Coulter, Inc., Fullerton, CA, USA); this assay has analytical linearity of 1–45 nmol/L and a CV of 9.4 % at 3.4 nmol/L, 5.0 % at 9.3 nmol/L and 5.6 % at 22.0 nmol/L. Vitamin B_{12} was measured on the Beckman Coulter Unicel Dxl 800 (Beckman Coulter, Inc., Fullerton, California, USA), and the assay had an analytical range of 37–1,107 pmol/L with a CV of 10 %.

Genotype determinations

Genomic DNA was isolated from peripheral blood (200 µl) of the subjects using the Qiagen Blood Mini Kit. MTR A2756G genotyping was accomplished using Applied Biosystems (ABI) TaqMan® SNP assay. In brief, polymerase chain reactions (PCR) contained 20 ng of DNA template, 3.5 µl of H_20, 5 µl of 2× ABI TaqMan PCR Master Mix and 0.5 µl of 20× ABI SNP assay-by-design master mix (Assay ID: C 12005959-10) which contained 900 nmol/L forward primer, 900 nmol/L reverse primer, 200 nmol/L VIC-labeled MGB probe and 200 nmol/L FAM-labeled MGB probe. Real-time PCR reactions were conducted on the Eppendorf Mastercycler Realplex 4 following the conditions: 95 °C for 10 min, 92 °C for 15 s, 60 °C for 60 s and repeated for 40 cycles; FAM and VIC signals were detected at the end of each cycle. Positive controls and blank control were run on each batch. The MTHFR C677T genotyping method by PCR–RFLP has been reported previously (El-Sammak et al. 2004). To ensure reliability of the PCR–RFLP assay, genotyping was repeated for 10 % of the samples and two samples of each genotype were randomly selected and subjected to direct DNA sequencing to verify genotype; concordance was found to be 100 %.

Statistical analysis

Analysis of the effects of MTR polymorphism on HCY, SAM and SAH concentrations was assessed with the categorizations of MTR based on the dominant model (MTR: 'AA' vs. 'GA+GG') due to the small number of participants with the MTR 'GG' genotype. Sensitivity analysis was conducted with the exclusion of the MTR 'GG' genotype for all analyses (i.e., contrasting MTR 'AA' vs. 'GA'), and results were unchanged (data not shown). For the MTHFR polymorphism, all analyses were assessed as a codominant model, and analysis of the interaction between MTR and MTHFR polymorphisms examined the effect of MTHFR genotypes within strata of MTR 'AA' versus 'GA+GG' genotypes. For gene–nutrient interactions, serum folate status was dichotomized based on sex-specific 25th percentiles (males 28.1 nmol/L; females 28.7 nmol/L) and serum vitamin B_{12} status was dichotomized based on a clinical cut point of vitamin B_{12} deficiency where those below 148 pmol/L were considered 'Low' and those above were considered 'Normal' (Institute of Medicine 1998). All gene–nutrient analyses examined the effect of MTHFR and MTR genotypes within strata of folate and vitamin B_{12} on HCY, SAM and SAH.

Least squares regression was used to estimate adjusted means and 95 % confidence intervals (95 % CI) of HCY, SAM and SAH concentrations within MTR/MTHFR genotypes while controlling for predictors of each biomarker. Two-sided p values based on F tests are presented for each analysis. Covariates considered include age, sex, ethnicity, body mass index (BMI), smoking, alcohol consumption, tea consumption, coffee consumption and biochemical measures of dietary status [serum folate, vitamin B_{12}, creatinine, albumin, calcium, triglycerides and the ratio of total cholesterol and high-density lipoprotein (HDL)]. Determinants of each biomarker were assessed by backwards elimination with a liberal inclusion criterion ($p = 0.20$); predictors of HCY at the $p = 0.20$ level included age, sex, BMI, smoking, creatinine, vitamin B_{12}, calcium, albumin, ratio of total cholesterol and/HDL and folate. For SAM, predictors included age, BMI, alcohol, creatinine, vitamin B_{12}, triglycerides, ethnicity and tea consumption. For SAH, age, sex, BMI and creatinine were included as predictors in each model. A variable representing the three study centers was included as a random effects parameter in all analyses to account for the cluster sampling design. Interactions between MTR and MTHFR polymorphisms, and gene–nutrient interactions with serum folate and vitamin B_{12} levels on HCY, SAM and SAH were examined by inclusion of product terms into the regression model.

Results

The total sample of this study included 570 participants. Analyses of the effects of MTR and MTHFR polymorphisms on HCY concentrations included 570 and 566 participants, respectively. For SAM and SAH, analyses of the effects of MTR polymorphisms included 565 participants, and for the MTHFR polymorphism, analyses included 561 participants.

The frequency distribution and percentages for select characteristics of the study population are presented in Table 1. In the study population, there was a higher proportion of females (56 %), a higher proportion of those between the age of 20–29 (36 %) and higher recruitment

Table 1 Distribution of select characteristics and MTR and MTHFR polymorphisms in the study population

		Categories	*N*	Percentage (%)
Select characteristics	Sex	Female	318	55.8
		Male	252	44.2
	Age	20–29	207	36.3
		30–39	165	29.0
		40–49	198	34.7
	Study center	Kingston, Canada	233	40.9
		Halifax, Canada	266	46.6
		Ottawa, Canada	71	12.5
	Ethnicity	Caucasian	490	86.0
		Other	80	14.0
Genotypes	MTR	AA	363	63.7
		GA	192	33.7
		GG	15	2.6
	MTHFR[a]	CC	234	41.3
		CT	251	44.4
		TT	81	14.3

[a] Missing MTHFR genotype data on 4 individuals

from Halifax, Nova Scotia, Canada (47 %). The study population was primarily Caucasian (86 %). The distributions of MTR and MTHFR polymorphisms found in this study correspond well with those reported in the literature for Caucasian subjects (Ma et al. 1999; Silaste et al. 2001; Klerk et al. 2003; Chen et al. 2001; Jacques et al. 2003; Tsai et al. 2009; Summers et al. 2010; Fredriksen et al. 2007; Rodriguez-Oroz et al. 2009). The genotype distributions of all polymorphisms were in agreement with Hardy–Weinberg equilibrium ($p > 0.05$). Distributions of the biomarkers of interest in this study are presented in Table 2.

The main effects of MTR and MTHFR polymorphisms on HCY, SAM and SAH concentrations are reported in Table 3. For HCY, adjusted mean concentrations differed among the MTR ($p < 0.01$) and the MTHFR genotypes ($p = 0.04$) with higher levels of HCY in those with the MTR 'AA' or the MTHFR 'TT' genotypes. For SAM and SAH, no significant associations were found with the main effects of MTR or MTHFR polymorphisms.

Table 4 displays the gene–gene interaction between MTR and MTHFR polymorphisms on SAH levels ($p = 0.01$); no gene–gene interaction was observed for HCY and SAM (data not shown). Two perspectives on the interpretation of Table 4 are possible. That is, the effect of MTHFR polymorphism on SAH levels was found to differ according to MTR categories such that among those with

Table 2 Distribution of biochemical markers

	25th Percentile	Median	75th Percentile	Mean	Standard deviation
HCY (μmol/L)[a]	7.0	8.4	10.0	8.7	2.4
SAM (nmol/L)[b]	54.3	62.0	72.5	63.9	13.6
SAH (nmol/L)[b]	20.0	24.4	29.3	25.5	10.6
Folate (nmol/L)[a]	21.7	28.3	35.6	30.1	18.2
Vitamin B_{12} (ρmol/L)[a]	161	213	279	233	108

[a] $N = 570$

[b] $N = 565$

the MTR 'AA' genotype, SAH levels were highest in the MTHFR 'TT' genotype category; in contrast, among those with the MTR 'GA+GG' genotype, MTHFR 'CC' had the highest level of SAH. Alternatively, one may consider Table 4 and observe that the effect of MTR polymorphisms on SAH levels differed among MTHFR categories such that in the MTHFR 'CC' genotype, MTR 'GA+GG' had the highest level of SAH and in contrast, among the MTHFR 'TT' genotype, SAH levels were highest in those in the MTR 'AA' category.

Table 5 presents the gene–nutrient interactions between MTR/MTHFR polymorphisms and serum folate on SAH levels; no gene–nutrient interaction was observed for HCY and SAM (data not shown). A significant interaction was found between MTHFR polymorphism and serum folate ($p = 0.02$); specifically, among those with serum folate levels below the 25th percentile, the MTHFR 'CC' genotype was associated with higher SAH levels compared to those with serum folate levels greater than the 25th percentile. Finally, analysis of gene–nutrient interaction with vitamin B_{12} revealed no interaction with MTR/MTHFR polymorphisms on HCY, SAM and SAH concentrations (data not shown).

Discussion

In this study, we investigated the relationship between two common variants in genes encoding enzymes relevant to the regulation of 1C metabolism, MTR A2756G and MTHFR C677T, and fasting concentrations of HCY, SAM and SAH among 570 healthy Canadians aged 20–50 years. The role of genetic factors like MTHFR in determining HCY levels is well established in contrast to investigations of genetic determinants of SAM and SAH. Our results and those of others support MTR and MTHFR polymorphisms

Table 3 Adjusted mean concentrations of HCY, SAM and SAH associated with the main effects of MTR and MTHFR polymorphisms

	N	μHCY μmol/L (95 % CI)[a]	μSAM nmol/L (95 % CI)[b]	μSAH nmol/L (95 % CI)[c]
MTR				
AA	363	9.2 (8.9, 9.5)	64.0 (62.1, 66.0)	25.9 (24.6, 27.2)
GA+GG	207	8.7 (8.3, 9.0)	63.4 (61.2, 65.7)	25.8 (24.1, 27.4)
p value[d]		<0.01	0.57	0.88
MTHFR				
CC	234	8.8 (8.5, 9.2)	64.6 (62.5–66.8)	26.6 (25.0–28.1)
CT	251	9.1 (8.8, 9.5)	63.0 (60.9–65.2)	25.4 (23.9–26.9)
TT	81	9.5 (9.0, 10.0)	64.0 (60.9–67.0)	24.9 (22.5–27.3)
p value[d]		0.04	0.35	0.35

[a] $N = 570$ for analysis with MTR; $N = 566$ for analysis with MTHFR; predictors of HCY (expressed in μmol/L) include age, sex, BMI, smoking, calcium, ratio of cholesterol to HDL, albumin, creatinine, folate and vitamin B_{12}

[b] $N = 565$ for analysis with MTR; $N = 561$ for analysis with MTHFR; predictors of SAM (expressed in nmol/L) include age, BMI, alcohol consumption, tea consumption, creatinine, triglycerides, ethnicity and vitamin B_{12}

[c] $N = 565$ for analysis with MTR; $N = 561$ for analysis with MTHFR; predictors of SAH (expressed in nmol/L) include age, sex, BMI and creatinine

[d] Two-sided *p* values based on *F* test

Table 4 Multivariate analysis on mean SAH concentrations associated with the interaction of MTR and MTHFR polymorphisms

	N	MTR 'AA' μSAH (95 % CI)[a]	*N*	MTR 'GA+GG' μSAH (95 % CI)[a]	$P_{Interaction}$
MTHFR					
CC	156	25.5 (23.8, 27.3)	76	28.7 (26.2, 31.1)	0.01
CT	152	25.9 (24.0, 27.8)	96	24.6 (22.3, 26.8)	
TT	48	26.7 (23.6, 29.7)	33	22.4 (18.8, 26.0)	

[a] $N = 561$; predictors of SAH (expressed in nmol/L) include age, sex, BMI and creatinine

in having main effects on HCY levels. The main novel finding of this study was an interaction between MTR and MTHFR polymorphism in relation to SAH levels; as well, evidence of an interaction between MTHFR polymorphism and serum folate in determining SAH concentrations was also found.

Our results and those of previous investigations of the MTR–HCY relationship suggest the possibility that the MTR A2756G polymorphism may actually increase enzyme activity which would enhance the conversion of HCY to methionine, leading to lower levels of HCY. However, the biochemical effects of MTR A2756G polymorphism have not been examined in vitro due to the inability to express human MTR at sufficient levels in an active form (Harmon et al. 1999). Twenty-five studies have investigated the MTR–HCY association in healthy non-pregnant populations (Dekou et al. 2001; Ma et al. 1999; Silaste et al. 2001; Klerk et al. 2003; Chen et al. 2001; Jacques et al. 2003; Tsai et al. 2009; Fredriksen et al. 2007; Rodriguez-Oroz et al. 2009; Harmon et al. 1999; Bosco et al. 2003; Chung et al. 2010; D'Angelo et al. 2000; de Lau et al. 2010; DeVos et al. 2008; Diwakar 2008; Fillon-Emery et al. 2004; Geisel et al. 2001; Holmes et al. 2011; Jemaa et al. 2008; Kluijtmans et al. 2003; Naushad 2008; Palep-Singh et al. 2008; Summers et al. 2008, 2010; Yakub et al. 2012). However, of the 25 studies, only 10 were conducted among a study population of greater than 100 participants with similar age distribution and ethnic background (Ma et al. 1999; Klerk et al. 2003; Chen et al. 2001; Jacques et al. 2003; Tsai et al. 2009; Harmon et al. 1999; D'Angelo et al. 2000; Fillon-Emery et al. 2004; Geisel et al. 2001; Summers et al. 2008); two of the 10 comparable studies support the association found in this research (Chen et al. 2001; Harmon et al. 1999). In particular, sample size is of concern due to the low prevalence of the MTR polymorphism, which would limit the power of a study to detect an association if one truly exists; as well, it is unknown whether the association between MTR and HCY concentrations differs by age. In the literature, it is postulated that genetic factors may have more impact on HCY concentration among youths and that cumulative environmental factors may be more important in modifying HCY as individuals reach middle age (Kluijtmans et al. 2003). Finally, important lifestyle, environmental or additional genetic factors that may interact with MTR polymorphism could differ in their distributions between ethnic groups and thus limit comparison of results obtained across different ethnic populations. Furthermore, of the 10 studies that investigated the MTR–HCY relationship, four were female- (Summers et al. 2008) or male-specific (Ma et al. 1999; Chen et al. 2001; Harmon et al. 1999). Presently, it is unknown whether the genetic relationships under

Table 5 Adjusted mean concentration of SAH (95 % CI) according to MTR and MTHFR genotypes and serum folate level

		Serum folate ≤25th percentile μSAH (95 % CI)[a]		Serum folate >25th percentile μSAH (95 % CI)[a]	$P_{Interaction}$
MTR					
AA	95	26.7 (24.5–29.0)	265	25.5 (24.0–27.0)	0.72
GA+GG	45	27.3 (24.2, 30.3)	160	25.2 (23.4, 27.1)	
MTHFR					
CC	47	30.3 (27.3–33.4)	185	25.6 (23.9–27.3)	0.02
CT	67	26.1 (23.5–28.6)	181	25.2 (23.4–26.9)	
TT	24	22.4 (18.2–26.6)	57	26.0 (23.2–28.9)	

[a] $N = 565$ for analysis with MTR; $N = 561$ for analysis with MTHFR; predictors of SAH (expressed in nmol/L) include age, sex, BMI and creatinine

investigation are different between men and women. In the present study, no interaction between MTR polymorphism and sex on HCY levels was observed (data not shown).

For the MTHFR–HCY relationship, our results are in agreement with the established literature. In a recent meta-analysis of 59,995 individuals conducted by Holmes et al. (2011), HCY concentrations were 2.10 μmol/L higher among those with the 'TT' genotype than the homozygous wild-type genotype (CC) (Holmes et al. 2011). Only one of the 10 studies that have investigated MTR–HCY relationship examined the interaction between MTR and MTHFR polymorphisms on HCY concentrations (Harmon et al. 1999). Their results, like ours, report an absence of an interaction suggesting that polymorphisms in MTR and MTHFR may act independently to elevate HCY concentrations by compromising different parts of the pathway that do not interact directly with one another (Harmon et al. 1999).

In the literature, moderate elevations in HCY have been shown to be highly correlated with parallel elevations in SAH but not SAM. Thus, it was hypothesized that variations in MTR and MTHFR genes could affect SAH levels (James et al. 2002; Alonso-Aperte et al. 2008; Yi et al. 2000). Only one study conducted among pregnant women has investigated the associations between MTR polymorphism and SAM and SAH levels and reports no statistically significant association (Barbosa et al. 2008). Three studies have investigated the relationship between MTHFR polymorphism and SAM and SAH, two of which were conducted in women only and their results are consistent with those found in our main effect analyses (Barbosa et al. 2008; Davis et al. 2005). In our previous examination of the effect of MTHFR polymorphisms on SAM and SAH levels in a smaller subset of this study population ($N = 382$ for SAM and $N = 387$ for SAH), no association was found (Ho et al. 2011). No study to date has examined the gene–gene interaction between MTR and MTHFR polymorphisms on SAM and SAH levels; thus, this is the first study to report a significant interaction on affecting SAH concentrations. Like HCY, the relationships between MTR and MTHFR polymorphisms on SAM and SAH levels did not differ by sex (data not shown).

Serum folate and vitamin B_{12} are essential components of the 1C metabolism cycle; a priori, serum folate and vitamin B_{12} levels were postulated to modify the relationship between genetic susceptibility conferred by MTR and MTHFR polymorphisms and HCY concentrations and likewise SAM and SAH levels. Very few of the comparable 10 studies that examined the MTR–HCY relationship have investigated interactions between MTR polymorphism and serum folate (Dekou et al. 2001; Jacques et al. 2003; Harmon et al. 1999), and MTR polymorphism and vitamin B_{12} (Dekou et al. 2001; Jacques et al. 2003; Harmon et al. 1999; Kluijtmans et al. 2003) on HCY levels. Of the studies investigating the gene–nutrient interactions with the MTR polymorphism for HCY, none reports an association. Previously, no study has investigated the gene–nutrient interactions examined in this study in relation to SAM and SAH concentrations. We are the first to report an interaction between MTHFR polymorphism and serum folate on affecting SAH levels.

The gene–gene and gene–nutrient interactions observed for SAH levels cannot be readily explained but do illustrate the multiplicity of mechanisms that may affect the functionality of the 1C metabolism cycle. As discussed, numerous levels of controls within the 1C metabolism cycle exist; for instance, for healthy individuals on a normal diet, the metabolism of HCY is divided nearly equally between the transsulfuration and remethylation pathways. SAM levels determine which of the two pathways will be utilized for HCY since, through allosteric interactions; SAM inhibits the action of MTHFR while stimulating CBS activity (Dekou et al. 2001). SAH diminishes the SAM-mediated inhibition of MTHFR, and SAH is an inhibitor of MTR. As well, SAH can limit its own synthesis by inhibiting virtually all methyltransferases and simultaneously, increases in SAH could shift in the direction of hydrolysis and accordingly the concentration of HCY would increase (Finkelstein 2007). Thus, the directions of the observed interactions between MTR and MTHFR polymorphisms,

and MTHFR polymorphism and serum folate on SAH levels cannot be readily explained from the existing literature as it is likely that the multiple levels of control in these complex pathways is partly responsible. Another possible contributing factor to these findings includes confounding by other genetic variants affecting enzymes that play a role in these pathways; in a previous investigation of the effect of TYMS polymorphisms on SAH levels in a smaller subset of this study population, an interaction between polymorphisms in TYMS and serum folate was found (Ho et al. 2011). In any case, replication of these present findings is warranted to ensure that these are not spurious results.

As MTR is a vitamin B_{12}-dependent enzyme, an interaction between the MTR genotypes and vitamin B_{12} was initially hypothesized. However, the lack of an interaction may be explained by the location of this polymorphism in a domain of the protein that interacts with SAM and auxiliary proteins, a location that is distinct from the vitamin B_{12} cofactor-binding domain (Harmon et al. 1999). Harmon et al. (1999), who had previously reported on the absence of an interaction between MTR A2756G and serum vitamin B_{12} on HCY levels, speculated that the MTR 'A' allele may impair the binding of SAM and/or auxiliary proteins or possibly impair the stability of the protein; this hypothesis, however, was not demonstrated in in vitro studies (Harmon et al. 1999).

Limitations

The study population was aged 20–50, primarily Caucasian and exposed to folic acid food fortification since 1998; therefore, generalizability to other age groups, ethnic populations and unfortified populations may be limited. This study only examined the role of MTHFR C677T on HCY, SAM and SAH levels; future studies should investigate the influence of additional functional polymorphisms like MTHFR A1298C on metabolites of 1C metabolism. For the gene–nutrient analysis, it should be acknowledged that the intraindividual variability of serum folate and vitamin B_{12} concentrations may challenge the validity of a one-time measurement in representing an individual's average concentration and hence limit our ability to study these interactions. In addition, the present study population was comprised of healthy volunteers with relatively high serum folate and vitamin B_{12} levels; therefore, we had limited power to investigate the gene–nutrient interactions. This study investigated a several interactions between MTR and MTHFR polymorphisms, serum folate and vitamin B_{12} on biomarkers of 1C metabolism; the findings of this study should be interpreted with caution particularly in the absence of a biologic explanation since we cannot rule out multiple testing.

Conclusion

Our findings contribute to understanding the impact of genetic and nutritional factors on biomarkers of 1C metabolism. Specifically, this study found that MTR and MTHFR polymorphisms were independently associated with elevated HCY concentrations. Results are suggestive that the MTR A2756G polymorphism may increase enzyme activity and enhance the conversion of HCY to methionine leading to lower levels of HCY concentrations. As well, this study is the first to investigate the main effect of MTR polymorphism on SAM and SAH levels in healthy men and women and provides a novel examination of interactions with the MTHFR C677T polymorphism. We are the first to report a significant interaction between MTR and MTHFR polymorphism on determining SAH levels. The present study is one of few that have investigated gene–nutrient interactions between MTR and MTHFR polymorphisms, and serum folate and vitamin B_{12} on HCY levels; it is the first to examine these gene–nutrient interactions on SAM and SAH concentrations. In this healthy population, we did not observe any gene–nutrient interactions with the MTR polymorphism on biomarkers of 1C metabolism; however, an interaction between MTHFR polymorphism and serum folate on affecting SAH levels was observed. These interactions should be reinvestigated in different study populations; as well, further consideration of other genetic factors and nutrients relevant to the 1C metabolism cycle may further clarify underlying relationships.

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Conflict of interest Vikki Ho, Thomas E. Massey and Will D. King declare that they have no conflicts of interest.

Ethical standard All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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