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Polymorphisms in Serine Hydroxymethyltransferase 1 and Methylenetetrahydrofolate Reductase Interact to Increase Cardiovascular Disease Risk in Humans[1–3]

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Abstract

The enzymes serine hydroxymethyltransferase 1 (gene name *SHMT1*) and methylenetetrahydrofolate reductase (gene name *MTHFR*) regulate key reactions in folate-mediated one-carbon metabolism. Common genetic variants with the potential to influence disease risk exist in both genes. A prior report from the Normative Aging Study indicated no association of the *SHMT1* rs1979277 SNP with cardiovascular disease (CVD), but a strong gene-gene interaction was detected with *MTHFR* rs1801133. We investigated the effect of the *SHMT1* rs1979277 SNP and the *SHMT1* rs1979277-*MTHFR* rs1801133 interaction in 2 epidemiologic cohort studies. In the Nurses' Health Study (NHS), the *MTHFR* rs1801133 variant genotypes were associated with an increased CVD risk and there was an interaction between *SHMT1* and *MTHFR* such that the association of the *MTHFR* rs1801133 *CT* genotype (vs. *CC;* the *TT* genotype could not be evaluated) was stronger in the presence of the *SHMT1* rs1979277 *TT* genotype (OR = 4.34, 95% CI = 1.2, 16.2; $P = 0.049$). In the Health Professionals Follow-Up Study, the *MTHFR* rs1801133 genotype was not associated with CVD risk, nor was there an interaction with *SHMT1* rs1979277. The association of genetic variation in the *SHMT1* gene, alone and in interaction with *MTHFR*, in relation to CVD risk is relatively understudied at the population level and results in the NHS confirmed a past report of gene-gene interaction, which is consistent with mechanisms suggested by basic science studies. J. Nutr. 141: 255–260, 2011.

Introduction

Folate, a B vitamin fortified in the U.S. food supply since 1998, is involved in many cellular processes that influence disease risk. As tetrahydrofolate polyglutamates, folate coenzymes carry and activate one-carbon units for use in metabolic pathways, including generation of methylation potential and synthesis of purine and pyrimidine nucleotides (1). Evidence from basic science studies suggests genetic variants that alter the function of folate coenzymes have the potential to affect disease risk. The SHMT1 enzyme plays a key role in the folate metabolic pathway by catalyzing the interconversion of serine and tetrahydrofolate to glycine and 5,10-methylenetetrahydrofolate (5,10-methyleneTHF)[7] (2). The negligible concentration of free intracellular folate implies competition for the product of this reaction, 5,10-methyleneTHF; thus, SHMT1 is poised to mediate the flow of one-carbon units through thymidylate synthesis and the methionine cycle; both pathways are highly sensitive to folate status (3,4). The MTHFR enzyme, which catalyzes a metabolic step linked to SHMT1, functions to convert 5,10 methyleneTHF to 5-methylTHF, thereby committing one-carbon units to methylation reactions at the expense of thymidylate synthesis (1,5). A common genetic variant in the *SHMT1* gene, rs1979277 (*1420 C→T*), results in a modified protein (L474F). Although this

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[3] Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.
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[7] Abbreviations used: CHD, coronary heart disease; CVD, cardiovascular disease; HPFS, Health Professionals Follow-Up Study; 5,10-methyleneTHF, 5,10-methylenetetrahydrofolate; NAS, Normative Aging Study; NHS, Nurses' Health Study.

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mutation does not affect catalytic activity, it impairs SHMT1 nuclear transport and subsequent thymidylate synthesis and results in accumulation of the altered SHMT1 protein in the cytoplasm, where it may inhibit cellular methylation reactions by sequestering 5-methyl-THF (3,6,7). A common genetic variant in the *MTHFR* gene, rs1801133 (*677 C→T*) results in an altered protein (A222V) with impaired ability to partition one-carbon units to the remethylation pathway (5,8). Because the MTHFR and SHMT1 enzymes catalyze linked metabolic steps, the accumulation of the variant SHMT1 L474F protein in the cytoplasm and sequestration of 5-methylTHF, coupled with the impaired 5-methylTHF synthetic activity of the MTHFR A222V protein, suggests the potential for an interaction between the *SHMT1* rs1979277 and *MTHFR* rs1801133 genotypes. Such an interaction could have important metabolic consequences on cellular remethylation reactions and/or nucleotide synthesis and ultimately on disease risk.

The association of the *MTHFR* rs1801133 variant with cardiovascular disease (CVD) risk has been well studied (9–11); the increased risk of heart disease associated with the *MTHFR* rs1801133 *TT* vs. *CC* genotype is most pronounced in unfortified populations (10). Few studies have examined genetic variation in *SHMT1* in relation to CVD risk and the majority of publications investigating genetic variation in *SHMT1* focus on cancer (28 of 31 studies published prior to 8/2009). One of 3 studies of the *SHMT1*-CVD association (12–14) investigated the *SHMT1-MTHFR* gene-gene interaction in relation to CVD risk (12), and the risk associated with the *MTHFR* rs1801133 *CT* and *TT* genotypes (vs. *CC*) was stronger in the subgroup of men with the *SHMT1* rs1979277 *TT* genotype.

Given the key role of the SHMT1 and MTHFR enzymes in the generation of cellular methylation potential and nucleotide biosynthesis, further studies examining the *MTHFR-SHMT1* interaction in relation to CVD risk are needed. Thus, a candidate gene association study was conducted to investigate the hypothesis that the *SHMT1* rs1979277 and *MTHFR* rs1801133 genotypes interact to increase heart disease risk in the Health Professionals Follow-Up Study (HPFS) and the Nurses' Health Study (NHS). Because the association between these genotypes and CVD risk may be mediated by homocysteine and/or modified by B vitamins, these questions were also investigated.

Methods

To compute expected *SHMT1* genotype frequencies in European-ancestry populations, a literature search was conducted in August 2009 by using standardized methods. Genetic association studies reporting the association of *SHMT1* variants and chronic disease were identified on the PubMed search engine at the National Center for Biotechnology Information (15). Studies reporting original research on humans published in English were included if they reported the frequency of the *SHMT1* rs1979277 *TT* genotype and studied any of the following outcomes: CVD, coronary artery disease, coronary heart disease (CHD), and cancers (including the precancerous condition, colorectal adenoma). Eighteen case-control studies conducted in European-ancestry populations were identified (12,13,16–31), and by using data from controls only (total n = 12,883), we extracted genotype counts to compute expected *SHMT1* genotype frequencies in European-ancestry populations.

Data from 2 cohort studies comprised the study population. The HPFS enrolled 51,529 male health professionals aged 40–75 y in 1986, and the nested case-control study of CHD has been previously described (32). Cases comprised 266 men with incident nonfatal myocardial infarction or fatal CHD; case events occurred between the date of blood draw (about 1994 for all participants providing blood samples) and the return of the year 2000 questionnaire (32). Using risk-set sampling, 532 controls matched for age, smoking status, and date of blood sampling were randomly selected from the subgroup of participants free of CVD at the time of diagnosis of the cases. Only case-control sets in which all men had successful genotyping on both *SHMT1* rs1979277 and *MTHFR* rs1801133 were included [245 cases and 474 controls comprising 229 triads (1 case: 2 controls) and 16 pairs]. Blood samples were analyzed for total cholesterol (32) and RBC folate (33) as described elsewhere.

The NHS enrolled 121,700 female registered nurses aged 30–55 y in 1976, and a nested case-control study comprising 227 incident cases of nonfatal myocardial infarction and fatal CHD occurring between 1990 and 1998 was conducted (34). All women provided a blood sample prior to the case accrual period, thus in the prefolate fortification era; controls were matched to each case on age, smoking, month of blood draw, fasting status, and reported problems with blood drawing, and were randomly selected using risk-set sampling from participants with the matching criteria and free of CHD at the time of case diagnosis (34). Only case-control sets in which all women had successful genotyping on both *SHMT1* rs1979277 and *MTHFR* rs1801133 were included [227 cases and 425 controls comprising 198 triads (1 case:2 controls) and 29 pairs]. Blood samples were analyzed for total cholesterol (34), RBC folate, plasma folate, vitamin B-6, vitamin B-12, and homocysteine, as previously described (33).

Genotyping for the studied polymorphisms in both cohorts, *SHMT1* rs1979277 (*1420 C→T*) and *MTHFR* rs1801133 (*677 C→T*), was conducted as part of a larger genotyping effort (20). Laboratory personnel were unaware of case-control status and genotyping was repeated to exclude errors when genotype distributions were found to be out of Hardy-Weinberg equilibrium (HWE). The median genotyping success rate across all polymorphisms assayed was 95%, and concordance between the 10% quality control samples and genotyped variants was 100%.

SAS software (SAS Institute) was used for all statistical analyses. Genotype frequencies in controls were compared with those expected in HWE and Monte Carlo estimates of the exact *P*-values for the disequilibrium tests were computed (10,000 permutations). Conditional logistic regression analysis was used; adjusted regression models considered known risk factors for CHD. To test for the *SHMT1* rs1979277-*MTHFR* rs1801133 gene-gene interaction and for gene-nutrient and gene-gene-nutrient interactions, product terms involving the relevant genes and/or nutrients were included in regression models. The *SHMT1* rs1979277 SNP was coded as recessive and the *MTHFR* rs1801133 SNP was coded using dummy variables to allow for nonlinear associations. Nutrients and metabolites related to folate metabolism (RBC folate, plasma folate, plasma vitamins B-6 and B-12, and homocysteine) were considered as mediators and/or effect modifiers. An α level of 0.05 was used for main effects and HWE tests, an α level of 0.15 was used for interactions, and point estimates and 95% CI are shown. The study was approved by the Cornell University Committee on Human Subjects.

Results

In the HPFS, the frequency of the *MTHFR* rs1801133 *TT* genotype was higher in controls (13.3%) than in cases, and the prevalence in controls was greater than the expected frequency of 10.7% based on a past meta-analysis (10). In the NHS cohort, the frequency of the *MTHFR* rs1801133 *TT* genotype was similar between cases and controls and in the range expected. The expected *SHMT1* rs1979277 genotype frequencies were computed using data on controls from 18 studies conducted in European-ancestry populations, as follows: 48.9% *CC* (95% CI = 48.1, 49.8), 41.0% *CT* (95% CI = 40.2, 41.9), and 10.0% *TT* (95% CI = 9.5, 10.5). In comparison, the prevalence of the *SHMT1* rs1979277 *TT* genotype in HPFS controls (12.0%) was high, whereas the NHS genotype prevalence was closer to expected (**Table 1**). In the NHS, cross-classification of the 2

TABLE 1 Genotype frequencies for *SHMT1* rs1979277 (*1420 C→T*) and *MTHFR* rs1801133 (*677 C→T*) in the HPFS, 1994–2000 (*n* = 719) and the NHS, 1990–1998 (*n* = 652)[1]

		SHMT1 rs1979277 (*1420 C→T*)			*MTHFR* rs1801133 (*677 C→T*)		
	n	*CC*	*CT*	*TT*	*CC*	*CT*	*TT*
NHS				%			
Cases	227	46.3	46.7	7.1	43.2	46.7	10.1
Controls	425	48.5	43.1	8.5	48.9	41.2	9.9
HPFS							
Cases	245	49.0	44.9	6.1	42.0	48.2	9.8
Controls	474	45.6	42.4	12.0	39.0	47.7	13.3

[1] Values are percentages for cases and controls, respectively, and are presented separately for each study.

genotypes revealed an absence of the double variant homozygote genotype (rs1979277 *TT* and rs1801133 *TT*) in the cases. Genotype distributions for *SHMT1* rs1979277 and *MTHFR* rs1801133 did not differ significantly from HWE expectations in either cohort.

Genotype-outcome associations. Based on the biological hypothesis that the *SHMT1* rs1979277 *TT* genotype leads to increased cytoplasmic protein levels and therefore less remethylation of homocysteine to methionine, the first analysis goal was to establish an *MTHFR*-heart outcomes association given the well-known effects of common variants in *MTHFR* on homocysteine accumulation. Paradoxically, the *MTHFR* rs1801133 *T* allele was associated with a lower risk of CHD in the HPFS, and estimates of association were similar in simple models adjusting for demographics only and in models fully adjusting for multiple cardiovascular risk factors. In the NHS, the *MTHFR* rs1801133 *T* allele was associated with an increased risk of CHD, although there was no gradient in risk per the *MTHFR* allele. Thus, women with the *MTHFR CT* genotype had a 30% increased risk of CHD (vs. *CC* genotype: OR = 1.30, 95% CI = 0.9, 1.8) and women with the *MTHFR TT* genotype had a 22% increased risk (vs. *CC* genotype: OR = 1.22, 95% CI = 0.7, 2.1).

Following the hypothesis, the next analysis tested for a genotype-genotype interaction between *MTHFR* rs1801133 and *SHMT1* rs1979277. In the HPFS, where no main effects of the *MTHFR* rs1801133 genotype were evident, the risk associated with the *MTHFR* rs1801133 genotype did not differ by *SHMT1* rs1979277 genotype ($P = 0.47$ for interaction, likelihood ratio test statistic = 1.5, 2 *df*) (**Table 2**; **Supplemental Table 1**).] However, in the NHS, the risk associated with the *MTHFR* rs1801133 *CT* genotype varied by *SHMT1* rs1979277 genotype ($P = 0.049$ for interaction, likelihood ratio test statistic = 3.9, 1 *df*) (Table 2; Supplemental Table 1). In women with the *SHMT1* rs1979277 *TT* genotype, the risk of CHD in the *MTHFR* rs1801133 *CT* genotype group was ~4 times the risk in the *MTHFR* rs1801133 *CC* genotype group (OR = 4.34, 95% CI = 1.2, 16.2). In contrast, in women with the *SHMT1* rs1979277 *CC/CT* genotype, there was little or no association of the *MTHFR* rs1801133 *CT* (vs. *CC*) genotype with CHD (OR = 1.18, 95% CI = 0.8, 1.7). Risk associated with the *MTHFR* rs1801133 *TT* genotype in women with the *SHMT1* rs1979277 *TT* genotype could not be estimated, because there were no cases with the double homozygote genotype. In both the HPFS and NHS, minimally and fully adjusted models yielded very similar estimates of association for genotype.

Potential mediation of the gene-disease association by homocysteine was evaluated only in the NHS where homocysteine data were available. Only the *SHMT1 1420 C→T TT* genotype was associated with homocysteine in the NHS, and this association was not diminished after adjusting for the association of the *MTHFR* variant and the *SHMT1-MTHFR* interaction ($\beta = 2.35$, $P < 0.04$). Similarly, there was little or no difference in regression coefficients for genotype associations estimated in models further adjusted for homocysteine.

Given that folate intake may modify associations with CVD risk for at least one of the polymorphisms under study, the role of folate was explored. In the HPFS, there was little or no difference in RBC folate between cases and controls (no other folate-related biomarker data were available). In regression models, RBC folate was not predictive of CHD, and including RBC folate in regression models did not change either the coefficient for the SNP or the coefficient for the gene-gene interaction. The occurrence of cases in the HPFS spanned January, 1998 when mandatory folate fortification of the U.S. food supply took effect; about one-half of the outcomes occurred prior to January, 1998. Stratifying regression models by date of case event did not meaningfully change model coefficients. No significant 2-way interactions (gene-nutrient) were observed between RBC folate and the *SHMT1* rs1979277 or *MTHFR* rs1801133 genotypes, and there were no significant 3-way interaction terms (gene-gene-nutrient; data not shown).

In the NHS, in addition to RBC folate, plasma concentrations of vitamin B-6, vitamin B-12, folate, and homocysteine were measured. Case accrual for NHS events ended in 1998; thus, all cases occurred prior to mandatory folate fortification in the US. There was little or no difference in folate-related biomarkers between cases and controls, with the exception of vitamin B-6 (the mean in controls was higher; $P = 0.08$). RBC folate, plasma folate, and plasma vitamin B-6 were not associated with CHD in

TABLE 2 The relation of *MTHFR* rs1801133 *677 C→T* genotype with CVD risk stratified by *SHMT1* rs1979277 *1420 C→T* genotype in the HPFS and NHS

	NHS[1]		HPFS[2]	
SHMT1 genotype	*CC/CT*	*TT*	*CC/CT*	*TT*
		OR (95% CI)		
MTHFR genotype				
MTHFR 677 C→T CT vs. *CC*	1.18 (0.8, 1.7)	4.34 (1.2, 16.2)	1.0 (0.7, 1.4)	0.46 (0.1, 1.7)
MTHFR 677 C→T TT vs. *CC*	1.25 (0.7, 2.2)	N/A[3]	0.68 (0.4, 1.2)	0.69 (0.2, 3.1)

[1] *n* = 227 cases. Unadjusted. No double homozygous variants among the cases.
[2] *n* = 245 cases. Unadjusted.
[3] N/A, not available.

models with or without adjusting for homocysteine, and adding biomarkers to regression models had little or no effect on coefficients for the SNP or the interaction. No 2-way gene-nutrient interactions involving any of the polymorphisms or biomarkers were identified, and there were no significant 3-way interactions (gene-gene-nutrient; data not shown).

Discussion

The published literature investigating the role of the *SHMT1* rs1979277 *1420 C→T* genotype in chronic disease risk focuses mainly on cancer risk, and the evidence for genotype associations with CVD is limited to 3 studies, underscoring the need for further population-level studies of *SHMT1* and CVD. Although no prior studies reported a direct association of the *SHMT1* rs1979277 SNP with CVD, a strong interaction between the *SHMT1* rs1979277 and *MTHFR* rs1801133 genotypes was identified, which is consistent with emerging findings in basic science studies of functional effects of the SNP. Thus, the association of the *MTHFR* rs1801133 *CT* and *TT* genotypes (vs. *CC*) with CVD risk are stronger in people with the *SHMT1* rs1979277 *TT* genotype (12).

Using data from nested case-control studies in the population-based, prospective NHS and HPFS cohorts, the gene-gene interaction hypothesis was tested. The NHS findings were consistent with associations previously reported in the Normative Aging Study (NAS): risk associated with the *MTHFR* rs1801133 *T* allele was stronger in women with the *SHMT1* rs1979277 *TT* genotype. The absence of double homozygote cases in the NHS precluded estimating the association of *MTHFR* rs1801133 *TT* genotype with CHD risk in women with the *SHMT1* rs1979277 *TT* genotype. The lack of double homozygote cases may indicate the selective loss of such individuals from the cohort, but this scenario is highly unlikely given observed association sizes. An alternative and more likely explanation is that observed genotype frequencies are a chance phenomenon. Although the fraction of all NHS cohort members who provided blood samples and the fraction who developed CHD and were included in this study should be random with respect to genotype, it is possible that a chance event led to the absence of the *MTHFR* rs1801133 *TT* / *SHMT1* rs1979277 *TT* double homozygote genotype class. Indeed, given the prevalence of the genotypes for each SNP in European-ancestry populations (expect 10.0% *SHMT1 TT* and 10.7% *MTHFR TT* in European-Ancestry populations), only 3 double homozygous individuals are expected in the 249 incident NHS cases under the null hypothesis of no association with CHD. The 3 cells that can be estimated from the NHS data are each consistent with past findings (12).

The findings in the HPFS are inconsistent with summary estimates from prior meta-analyses regarding the association of the *MTHFR* rs1801133 genotype on CVD risk. In the HPFS, the *MTHFR* rs1801133 *T* allele was associated with a decreased risk of CVD, thus opposite in direction to findings from prior meta-analyses (9–11). The HPFS findings are also inconsistent with the past report (12) and the NHS findings reported herein with regard to the gene-gene interaction between *MTHFR* rs1801133 and *SHMT1* rs1979277. It is unlikely that gender influences the findings given that the past report of the interaction was in the NAS, an all-male cohort. It is also unlikely that genotyping errors led to the paradoxical findings in the HPFS: an independent genotyping effort involving a larger sample from the NHS and HPFS case-control studies confirmed the same associations with CHD risk reported herein, based on imputed genotypes for *MTHFR* rs1801133 and *SHMT1* rs1979277; in this expanded subset, the imputed *MTHFR* rs1801133 *TT* genotype frequency was also 13.3% (data not shown). The differences in findings in the HPFS are also not likely to be due to differences in average folate or homocysteine levels. The mean folate and homocysteine levels in the cohorts are virtually identical, differing by $<10\%$ in all comparisons (data not shown). A possible explanation for the unexpected findings is random error.

A recent Institute of Medicine report on nutrigenomics states, "There is, today, an unprecedented opportunity to use foods and food components to aid in achieving the genetic potential of humans, improve the overall performance of humans, and reduce the risk for chronic disease" (35). Given that the evidence base needed to make nutrition recommendations tailored to individual genotype is far from complete, this study investigated the interaction of nutritional status with the genetic variants studied and carefully considered the gene-nutrient and gene-gene-nutrient interactions. No gene-nutrient or gene-gene-nutrient interactions involving *SHMT1* rs1979277 and/or *MTHFR* rs1801133 were evident in either the NHS or the HPFS. In the HPFS, blood markers of folate status were collected prior to the introduction of mandatory folate fortification, although approximately one-half of the case events occurred after fortification. In both the HPFS and the NHS, nutritional status was not assessed at a uniform time relative to disease occurrence in cases. Future work should carefully consider the timing of nutrient measurements and the role of folate fortification in the interpretation of findings.

While prior studies of the association of *MTHFR* rs1801133 *677 C→T* estimate the *TT* genotype (vs. *CC*) is associated with a 14–21% excess risk of CVD in the 10% of the population who have the *TT* genotype (9–11), our findings suggest that the risk associated with the *MTHFR* rs1801133 *677 C→T T* allele is limited to the subgroup with the *SHMT1* rs1979277 *1420 C→T TT* genotype, comprising ~4.5% of the population studied. Similarly, in a prior report (12), risk associated with the *MTHFR* rs1801133 *677 C→T CT* (vs. *CC*) genotype was limited to the subgroup with the *SHMT1* rs1979277 *1420 C→T TT* genotype, comprising ~5% of the population studied. Furthermore, CHD risk in men with the *MTHFR* rs1801133 *677 C→T TT* genotype was 10 times the risk of men with the rs1801133 *CC* genotype in the 1% of men who were also *SHMT1* rs1979277 *1420 C→T TT* genotype (12). A prior report from the NHS found only moderate increases in CHD risk associated with *MTHFR 677 C→T* (rs1801133) variant genotypes (33), but the authors did not consider the *MTHFR* rs1801133 – *SHMT1* rs1979277 interaction investigated herein. Given that specific genotype and nutrition combinations may be required to confer risk, researchers wishing to further clarify the role of folate-related genes in chronic disease will need to consider the possibility that risk may be stronger in population subgroups.

The effect of the *SHMT1* rs1979277 and *MTHFR* rs1801133 polymorphisms on protein structure and function supports the population-level interaction observed in the NAS and NHS cohorts. Woeller et al. (6) established that the *SHMT1* rs1979277 polymorphism, which results in an L474F amino acid change, weakens the interaction between the UBC9 SUMO conjugating enzyme and SHMT1 and prevents the addition of small ubiquitin-like modifiers (SUMOylation) in vitro (6), inhibiting nuclear transport (7) and thereby resulting in the accumulation of the variant SHMT1 protein in the cytoplasm. An increase in cytoplasmic levels of the SHMT1 protein impairs homocysteine remethylation and binds the same substrate as MTHFR,

5-methylTHF (3); therefore, an accumulation of SHMT1 in the cytoplasm is hypothesized to exacerbate the reduced enzyme activity of the variant A222V MTHFR protein (6). This proposed biological mechanism for the gene-gene interaction observed in the NAS and NHS suggests a pathogenic effect of the polymorphisms mediated by direct effects of homocysteine and/or effects on cellular methylation potential. Neither study demonstrated that the gene-gene interaction was mediated by homocysteine, but homocysteine may be an incomplete marker of cellular methylation potential or a single homocysteine measurement may not be an adequate reflection of either long-term homocysteine, homocysteine at the time of the event, or rate of change in homocysteine, depending on which aspect is most informative of risk. Additional biomarker data, including longitudinal measurements of homocysteine beginning prior to the event (36) and/or measures of other remethylation pathway biomarkers, such as *S*-adenosylhomocysteine (37), may be more informative.

Although cells contain a second serine hydroxymethyltransferase, encoded by the *SHMT2* gene, which exhibits functional redundancy with the SHMT1 enzyme under investigation here (7), evidence suggests the functional redundancy is incomplete. The *SHMT2* gene encodes 2 transcripts, an SHMT2 protein that localizes only to the mitochondria, and an SHMT2α protein that, lacking a mitochondrial targeting sequence, localizes to the cytoplasm and nucleus (7). While the SHMT2α protein provides some functional redundancy to the SHMT1 protein, studies of SHMT1$^{-/-}$ mice confirm persistent aberrations in folate-related metabolism (7,38), arguing against complete compensation for loss of SHMT1 function. Thus, there is some justification for the penetrance of the *SHMT1* rs1979277 SNP despite expression of SHMT2 protein.

This study directly addresses an important gap in the literature by investigating the relation of the *SHMT1* rs1979277 *TT* genotype to heart disease and takes advantage of existing data from 2 nested case-control studies conducted in large epidemiologic prospective cohort studies. A weakness was the inability to test the *MTHFR TT* / *SHMT1 TT* strata of the gene-gene interaction in the NHS cohort because of the complete absence of double homozygote cases. However, the *MTHFR-SHMT1* interaction that was detected agreed in direction and magnitude with a previous report (12). The study reported herein had a well-defined hypothesis linked to findings in basic science and focused on only 2 genetic variants, the associated gene-gene and gene-nutrient interactions and mediation by a folate-related biomarker.

In summary, in the NHS, the association of the *MTHFR* rs1801133 genotype was modified by *SHMT1* rs1979277 genotype and associations were consistent in magnitude and direction with previously published findings. The biological plausibility for an interaction between *MTHFR* rs1801133 and *SHMT1* rs1979277 is strong, yet a review of published literature identified only one prior publication investigating the association of the *MTHFR* rs1801133 / *SHMT1* rs1979277 interaction in relation to CVD. Further studies are warranted, but future work must consider folate fortification and the range of folate nutrition in the population as well as the timing and collection of data on dietary intake, nutrition status, and folate pathway biomarkers, and include a more complete evaluation of genetic variation across the network of folate-related genes.

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