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The Methylenetetrahydrofolate Reductase 677C→T Polymorphism as a Modulator of a B Vitamin Network with Major Effects on Homocysteine Metabolism

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Folates are carriers of one-carbon units and are metabolized by 5,10-methylenetetrahydrofolate reductase (MTHFR) and other enzymes that use riboflavin, cobalamin, or vitamin B6 as cofactors. These B vitamins are essential for the remethylation and transsulfuration of homocysteine, which is an important intermediate in one-carbon metabolism. We studied the *MTHFR* 677C→T polymorphism and B vitamins as modulators of one-carbon metabolism in 10,601 adults from the Norwegian Colorectal Cancer Prevention (NORCCAP) cohort, using plasma total homocysteine (tHcy) as the main outcome measure. Mean concentrations of plasma tHcy were 10.4 μmol/liter, 10.9 μmol/liter, and 13.3 μmol/liter in subjects with the CC (51%), CT (41%), and TT (8%) genotypes, respectively. The *MTHFR* 677C→T polymorphism, folate, riboflavin, cobalamin, and vitamin B6 were independent predictors of tHcy in multivariate models ($P < .001$), and genotype effects were strongest when B vitamins were low ($P \leq .006$). Conversely, the *MTHFR* polymorphism influenced B vitamin effects, which were strongest in the TT group, in which the estimated tHcy difference between subjects with vitamin concentrations in the lowest compared with the highest quartile was 5.4 μmol/liter for folate, 4.1 μmol/liter for riboflavin, 3.2 μmol/liter for cobalamin, and 2.1 μmol/liter for vitamin B6. Furthermore, interactions between B vitamins were observed, and B vitamins were more strongly related to plasma tHcy when concentrations of other B vitamins were low. The study provides comprehensive data on the MTHFR–B vitamin network, which has major effects on the transfer of one-carbon units. Individuals with the TT genotype were particularly sensitive to the status of several B vitamins and might be candidates for personalized nutritional recommendations.

The flavoenzyme 5,10-methylenetetrahydrofolate reductase (MTHFR [MIM 236250]) regulates the flow of folates between the production of nucleotides and the supply of methyl groups for methionine synthesis[1,2] and has major effects on the distribution of intracellular folates.[3] The MTHFR substrate is 5,10-methylenetetrahydrofolate, which is formed from serine and tetrahydrofolate by vitamin B6–dependent serine hydroxymethyltransferase.[2,4] The product of the MTHFR reaction is 5-methyltetrahydrofolate, which is the methyl donor in the conversion of homocysteine to methionine catalyzed by cobalamin-dependent methionine synthase.[2,4] Methionine may be incorporated into proteins or may serve as the precursor of S-adenosylmethionine, a universal methyl group donor, which is converted to homocysteine after demethylation.[2,5] Homocysteine is metabolized through two vitamin B–dependent pathways and may be either remethylated and recycled as methionine or removed from the remethylation cycle by undergoing irreversible B6-dependent transsulfuration to form cysteine.[5] This makes homocysteine a key intermediate in one-carbon metabolism and explains why B vitamins involved in the transfer of one-carbon units are related to plasma concentrations of total homocysteine (tHcy).[6]

The 677C→T transition in the *MTHFR* gene (dbSNP accession number *rs1801133*) results in an Ala222Val substitution in the polypeptide chain,[7] which is associated with a thermolabile[8] enzyme. MTHFR in lymphocytes from subjects with the TT genotype has ~30% of the catalytic activity of the wild type, whereas the CT genotype has 65% catalytic activity.[7] Lower catalytic activity is associated with a redistribution of one-carbon substituted folates away from 5-methyltetrahydrofolate toward more oxidized forms, which may be used for DNA synthesis and repair.[3,4] The *MTHFR* polymorphism is associated with clinical endpoints; an increased risk of cardiovascular disease[9,10] and neural tube defects (MIM 601634)[11,12] and a lower risk of colorectal cancer (MIM 114500)[13–15] are found in subjects with the variant compared with the wild-type enzyme. The *MTHFR* 677C→T polymorphism is the single most important genetic determinant of plasma tHcy.[16,17] Much of the interest in this polymorphism stems from its association with moderate hyperhomocysteinemia (MIM 603174), which is a risk factor for occlusive arterial disease,[18] cognitive decline,[19] and osteoporosis.[20,21] It is still not clear whether these conditions are caused by homocysteine toxicity[22] or if an elevated concentration of plasma tHcy is mainly an epiphenomenon.[23–25]

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Table 1. Characteristics of the Study Population

Characteristic	All		*MTHFR* 677C→T Genotype (Median [1st–99th Percentile])			
	Mean	Median (1st–99th Percentile)	CC (n = 5,452)	CT (n = 4,299)	TT (n = 850)	P for trend
Age, years	56	55 (50–64)	55 (50–64)	55 (50–64)	55 (50–64)	.11
Plasma tHcy, μmol/liter	10.8	10.2 (5.8–24.2)	9.9 (5.7–19.2)	10.4 (5.9–22.8)	11.2 (5.7–43.3)	<.001
Serum folate, nmol/liter	17.1	13.7 (4.7–59.8)	14.5 (5.2–60.8)	13.4 (4.8–59.9)	10.5 (3.5–50.1)	<.001
Plasma riboflavin, nmol/liter	18.1	10.4 (3.0–135)	10.4 (3.0–150)	10.4 (2.8–135)	10.7 (2.8–119)	.06
Serum cobalamin, pmol/liter	331	307 (128–732)	308 (127–734)	308 (130–730)	300 (113–766)	.19
Plasma vitamin B6, nmol/liter	62.8	48.0 (12.7–295)	47.9 (13.3–280)	49.0 (12.6–336)	44.4 (11.9–286)	.34
Plasma creatinine, μmol/liter	70	69 (44–105)	69 (44–106)	70 (45–104)	66 (45–101)	.01

Most published work on B vitamins and homocysteine has focused on folate and cobalamin in smaller studies, which do not allow a comprehensive investigation of the various components of the metabolic network related to one-carbon metabolism. The present study included 10,601 middle-aged or elderly men and women from a population-based cohort, and our aim was to assess the *MTHFR* polymorphism and B vitamins as modulators of one-carbon metabolism, with use of plasma tHcy as the main outcome measure.

Subjects and Methods

Subjects and Study Design

The Norwegian Colorectal Cancer Prevention (NORCCAP) cohort was established to study the utility of sigmoidoscopy and occult blood testing as screening modalities for early detection of colorectal cancer in men and women aged 50–64 years.[26] Study subjects were randomly selected from the population registries in the city of Oslo and the county of Telemark and were included from 1999 to 2001. The study was approved by the Regional Ethics Committee and the Data Inspectorate. The procedures followed were in accordance with the Helsinki Declaration, and written informed consent was obtained from all participants.

Biochemical Analyses

Whole blood collected into EDTA tubes was immediately put on ice and was centrifuged at 11,000 *g* for 10 min to obtain plasma. Blood samples collected into serum tubes without additives were allowed to clot at room temperature and were centrifuged within 1 h. Plasma and serum samples were stored at −80°C until analysis. tHcy was analyzed in plasma by gas chromatography–mass spectrometry,[27] whereas riboflavin, vitamin B6 (pyridoxal-5′-phosphate), and creatinine were analyzed in plasma by liquid chromatography–tandem mass spectrometry.[28] Folate[29] and cobalamin[30] were determined in serum by microbiological methods. *MTHFR* 677C→T genotyping was performed by real-time PCR with 5V exonuclease (Taqman) probes.[31]

Statistical Methods

Means and medians with percentiles were used for descriptive statistics. Correlation analyses were performed using partial Spearman correlation coefficients adjusted for sex, age, and study center. The χ^2 test was used to compare proportions.

We used simple linear regression models and models with multiple adjustments to identify predictors of tHcy. Independent variables were represented in the model as indicator variables denoting membership in two or more categories for sex, age, the *MTHFR* 677C→T polymorphism, folate, riboflavin, cobalamin, vitamin B6, and creatinine. Thus, the regression coefficients estimated the difference in mean tHcy between the chosen reference category and the other categories for each factor. Concentrations of tHcy across categories of each factor were tested for linear trend.

We investigated the possible interaction between *MTHFR* 677C→T genotype and other tHcy predictors, by including product terms between genotype and each predictor in a multiple linear regression model with tHcy as the dependent variable, retaining the main effects of all variables in the model. Furthermore, the data were presented according to *MTHFR* genotype and B vitamin concentrations, and the regression analyses were performed separately for the CC, CT, and TT genotypes, at concentrations of folate, riboflavin, cobalamin, and vitamin B6 below and above the median. All statistical analyses were performed by SPSS version 11.0. Tests were two-tailed, and P values <.05 were considered statistically significant.

Results

Population Characteristics and Blood Indices

The study population (n = 10,601, 49% male) was predominantly white (>98%) and had a mean age of 56 years (table 1). *MTHFR* 677C→T genotype frequencies were 50.3% (CC), 41.3% (CT), and 8.3% (TT) for men and 52.5% (CC), 39.8% (CT), and 7.7% (TT) for women. The genotype distribution was in Hardy-Weinberg equilibrium for each sex and for the whole study group ($P \geq .69$). Mean concentrations of metabolites and vitamins measured for all genotypes combined were 10.8 μmol/liter for plasma tHcy, 17.1 nmol/liter for serum folate, 18.1 nmol/liter for plasma riboflavin, 331 pmol/liter for serum cobalamin, 62.8 nmol/liter for plasma vitamin B6, and 70 μmol/liter for plasma creatinine (table 1). Plasma tHcy was higher in subjects with the T allele as compared with the wild type. In subjects with plasma tHcy ≥20 μmol/liter (n = 209), the prevalence of the TT genotype was 48% (data not shown). Serum folate decreased according to the number of T alleles. Concentrations of riboflavin, cobalamin, and vitamin B6 were independent of genotype (table 1).

Correlations

Simple relationships between variables were calculated as nonparametric Spearman correlation coefficients, which were adjusted for sex, age, and study center. Concentrations of several B vitamins were positively related (table 2). The riboflavin-folate and the vitamin B6–folate relationships were modified by *MTHFR* genotype. Plasma tHcy was inversely related to all B vitamins, and the tHcy–B vitamin relationships were modified by the *MTHFR* polymorphism and were strongest in subjects with the TT genotype, particularly for folate and riboflavin (table 2). A positive relationship ($r = 0.21$) was observed between tHcy and creatinine.

Table 2. Spearman Correlation Coefficients

Variable and Genotype	tHcy	Folate	Riboflavin	Cobalamin
Folate:	−.44			
CC	−.38			
CT	−.45			
TT	−.58			
Riboflavin:	−.18	.26		
CC	−.15	.23		
CT	−.19	.28		
TT	−.38	.35		
Cobalamin:	−.24	.16	.20	
CC	−.23	.14	.19	
CT	−.25	.19	.22	
TT	−.28	.16	.17	
Vitamin B6:	−.24	.39	.35	.18
CC	−.20	.35	.34	.16
CT	−.24	.41	.36	.20
TT	−.38	.47	.43	.17

NOTE.—Nonparametric Spearman correlation coefficients adjusted for sex, age, and study center are shown for the entire population ($n = 10{,}570$) and separately for the *MTHFR* 677CC ($n = 5{,}433$), 677CT ($n = 4{,}282$), and 677TT ($n = 843$) genotypes. All correlations were highly significant ($P < .001$).

The MTHFR *677C→T Polymorphism and B Vitamins as tHcy Predictors*

The *MTHFR* polymorphism, folate, riboflavin, cobalamin, vitamin B6, and creatinine were independently related to plasma tHcy in regression models adjusted for sex, age, and study center ($P < .001$; data not shown) and in models with multiple adjustments ($P < .001$) (table 3).

The estimated difference in mean plasma tHcy between subjects with the TT genotype compared with the CC genotype was 2.4 μmol/liter (table 3). Folate was a strong tHcy predictor, and tHcy was 3.0 μmol/liter higher in subjects in the lowest compared with the highest quartile of folate concentrations (table 3). *MTHFR* genotype strongly modified folate effects, and, in TT subjects, plasma tHcy was 5.4 μmol/liter higher in the lowest compared with the highest folate quartile.

Riboflavin was only a weak tHcy predictor in subjects with the CC and CT genotypes, but, in the TT group, riboflavin was the second strongest tHcy predictor, and the difference between extreme riboflavin quartiles was 4.1 μmol/liter of plasma tHcy (table 3).

The cobalamin-tHcy and vitamin B6–tHcy relationships were similarly but less strongly related to genotype. The tHcy difference between extreme vitamin quartiles in subjects with the TT genotype was 3.2 μmol/liter for cobalamin and 2.1 μmol/liter for vitamin B6 (table 3). Sex, but not age or creatinine, interacted with genotype.

Modification of the MTHFR *Genotype–tHcy Relationship by B Vitamins*

Predictors of plasma tHcy were studied in subjects with the CC, CT, and TT genotypes at folate concentrations below and above the median, with the use of regression models with multiple adjustments (fig. 1). There was an inverse relationship between riboflavin and plasma tHcy, which was modified by *MTHFR* genotype, both at low and high folate concentrations (fig. 1). The relationship was strongest at low folate levels, however, and, for subjects in the TT group, the estimated tHcy difference between extreme riboflavin quartiles was 5.0 μmol/liter, whereas the corresponding difference was 1.8 μmol/liter at high folate levels. At low folate levels, cobalamin was also strongly and inversely related to tHcy (fig. 1). At high folate concentrations, this relationship was dramatically weakened, particularly in the TT group. Vitamin B6 was related to tHcy both at low and high folate concentrations, but at high folate levels no genotype effect was observed (fig. 1).

MTHFR genotype and B vitamin effects were similarly studied at concentrations of plasma riboflavin below and above the median (fig. 2). Folate was robustly related to plasma tHcy, both at low and high riboflavin concentrations, but high riboflavin levels weakened the folate-tHcy relationship in subjects with the TT genotype. Cobalamin was related to plasma tHcy at low and high riboflavin levels, but the relationship was much weaker and was not significantly modified by genotype when riboflavin levels were high (fig. 2). Vitamin B6 was moderately related to plasma tHcy at low and high riboflavin levels, particularly in subjects with the variant enzyme, but the genotype dependency of the vitamin B6–tHcy relationship was weaker when riboflavin levels were high.

Concentrations of cobalamin below and above the median had a similar but less pronounced effect on the folate-tHcy, riboflavin-tHcy, and vitamin B6–tHcy relationships in subjects with different *MTHFR* genotypes (fig. 3). High levels of cobalamin weakened the genotype dependency of the B vitamin–tHcy associations. Vitamin B6 had a similar impact on the effects of other B vitamins and the *MTHFR* genotype (fig. 4). Folate, riboflavin, and cobalamin were determinants of plasma tHcy both at low and high levels of vitamin B6, but high levels of vitamin B6 attenuated the relationships between the other B vitamins and

Table 3. Determinants of Plasma tHcy according to *MTHFR* 677C→T Genotype

Variable	All		*MTHFR* Genotype						
			CC (n = 5,452)		CT (n = 4,299)		TT (n = 850)		
	Estimated tHcy Difference[a]	P for trend	Estimated tHcy Difference[a]	P for trend	Estimated tHcy Difference[a]	P for trend	Estimated tHcy Difference[a]	P for trend	Interaction Term (P)[b]
Sex (vs. female; n = 5,386):									
Male (n = 5,213)	.4 (.2–.5)	<.001	.3 (.1–.5)	<.001	.4 (.2–.6)	<.001	1.0 (−.1–2.0)	.13	.04
Age, years (vs. 50–53; n = 2,540):									
54–55 (n = 3,052)	.5 (.3–.7)		.5 (.4–.7)		.4 (.1–.6)		1.0 (−.2–2.2)		
56–59 (n = 2,637)	.7 (.5–.9)	<.001	.7 (.5–.9)	<.001	.7 (.4–1.0)	<.001	.5 (−.7–1.7)	.16	.34
60–64 (n = 2,370)	1.2 (1.0–1.3)		1.2 (1.0–1.4)		1.0 (.8–1.3)		1.5 (.2–2.7)		
Serum folate, nmol/liter (vs. 20.2–151; n = 2,647):									
13.7–20.2 (n = 2,647)	.8 (.6–1.0)		.8 (.6–1.0)		.9 (.6–1.1)		1.3 (−.2–2.9)		
10.1–13.7 (n = 2,646)	1.2 (1.0–1.4)	<.001	1.3 (1.1–1.5)	< .001	1.3 (1.0–1.6)	<.001	1.1 (−.4–2.7)	<.001	<.001
1.5–10.1 (n = 2,651)	3.0 (2.8–3.2)		2.3 (2.1–2.5)		3.0 (2.7–3.3)		5.4 (4.0–6.8)		
Plasma riboflavin, nmol/liter (vs. 18.1–596; n = 2,644):									
10.4–18.1 (n = 2,656)	.1 (−.1–.3)		.1 (−.1–.3)		.0 (−.3–.3)		−.1 (−1.4–1.1)		
6.8–10.4 (n = 2,649)	.2 (.0–.4)	<.001	.1 (−.1–.3)	< .001	.2 (−.1–.5)	<.001	1.0 (−.2–2.3)	<.001	<.001
.9–6.8 (n = 2,652)	.8 (.6–1.0)		.4 (.2–.6)		.7 (.4–.9)		4.1 (2.7–5.5)		
Serum cobalamin, pmol/liter (vs. 380–6,500; n = 2,644):									
307–380 (n = 2,650)	.3 (.1–.5)		.2 (.0–.4)		.5 (.2–.7)		.6 (−.7–1.8)		
245–307 (n = 2,646)	.5 (.3–.7)	<.001	.4 (.2–.5)	<.001	.6 (.3–.8)	<.001	1.4 (.1–2.6)	<.001	<.001
34–245 (n = 2,645)	1.5 (1.3–1.6)		1.2 (1.0–1.4)		1.5 (1.3–1.8)		3.2 (2.0–4.5)		
Plasma vitamin B6, nmol/liter (vs. 73.2–1,093; n = 2,651):									
48.0–73.1 (n = 2,655)	.1 (−.1–.3)		.2 (.0–.4)		.1 (−.2–.4)		−.5 (−1.9–.8)		
32.6–47.9 (n = 2,642)	.1 (−.1–.3)	<.001	.2 (.0–.4)	<.001	.0 (−.3–.3)	<.01	−.4 (−1.8–1.0)	.01	.006
4.3–32.6 (n = 2,653)	.7 (.5–.9)		.5 (.3–.8)		.5 (.2–.8)		2.1 (.6–3.7)		
Serum creatinine, μmol/liter (vs. 30–61; n = 2,650):									
61–69 (n = 2,646)	.3 (.1–.5)		.5 (.3–.7)		.1 (−.1–.4)		.4 (−.8–1.5)		
69–78 (n = 2,659)	.8 (.7–1.0)	<.001	1.0 (.8–1.2)	<.001	.5 (.2–.8)	<.001	1.5 (.2–2.9)	.003	.29
78–571 (n = 2,645)	1.7 (1.5–1.9)		1.8 (1.6–2.0)		1.5 (1.2–1.8)		2.5 (1.0–4.0)		
MTHFR 667C→T genotype (vs. CC; n = 5,254):									
CT (n = 4,299)	.4 (.3–.5)	<.001							
TT (n = 850)	2.4 (2.2–2.6)								

NOTE.—Data were analyzed by multiple regression with tHcy as the dependent variable. All variables in the table and study center were included in the model.

[a] Values are given as means (95% CIs), in μmol/liter.

[b] P for the product term between *MTHFR* genotype and the various tHcy predictors.

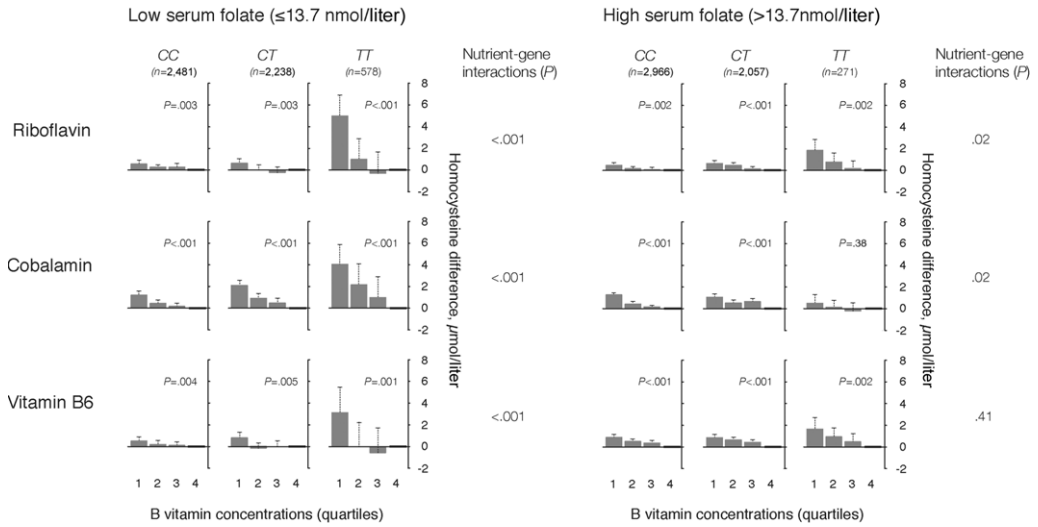

Figure 1. B vitamins as determinants of plasma tHcy according to folate concentrations and *MTHFR* 677C→T genotype. The population was stratified according to levels of serum folate (below and above the median) and *MTHFR* 677C→T genotype. The riboflavin-tHcy, cobalamin-tHcy, and vitamin B6–tHcy relationships were then studied in a regression model, which included these vitamins in addition to sex, age, creatinine, and study center. Means with upper limits of 95% CIs and *P* for trend across quartiles are shown in each panel. Nutrient-gene interaction terms were calculated as the product between *MTHFR* genotype and the various B vitamins.

tHcy, and the cobalamin-tHcy relationship showed no genotype dependency at high vitamin B6 levels.

Discussion

We studied the *MTHFR* 677C→T polymorphism and several B vitamins that are involved in one-carbon metabolism in a large population-based cohort (n = 10,601), using plasma tHcy as the main outcome measure. The *MTHFR* polymorphism, folate, riboflavin, cobalamin, and vitamin B6 were independently related to plasma tHcy. The *MTHFR* polymorphism had the strongest impact when B vitamin levels were low. Conversely, *MTHFR* genotype modified B vitamin–tHcy relationships, which were strongest in subjects with the T allele, particularly for folate and riboflavin. Finally, interactions between B vitamins were observed, and B vitamin–tHcy relationships were stronger when concentrations of other B vitamins were low.

Study Design

The large study population allowed us to investigate subgroups and to obtain precise estimates of *MTHFR* 677C→T polymorphism and B vitamin effects. The homogeneity of the study population with respect to age and ethnicity reduced the potential for confounding from these variables. B vitamin status was determined in blood. The relationship between the estimated intake of folate and vitamin B6 and blood vitamin concentrations is relatively strong, with an adjusted correlation coefficient in the range 0.5–0.6.[32,33] Circulating vitamin B12 shows a somewhat weaker relationship to intake data, which may reflect impaired absorption in the elderly.[32]

The distribution of *MTHFR* genotypes may be assumed to be unrelated to diet and lifestyle factors. Therefore, the investigation of phenotypic effects of the *MTHFR* polymorphism provides a means for reducing the effect of confounders that may distort interpretations of conventional observational studies.[34,35] A similar approach was used for the investigation of B vitamin effects. Although B vitamin status may be related to lifestyle,[36] the problem of confounding may be reduced by assessing B vitamin effects across subgroups defined by genotype.

MTHFR *677C→T Genotype and Blood Indices*

The T allele frequency was ~0.3, which accords well with previous reports from population-based studies in northern Europe.[37] Concentrations of plasma tHcy and B vitamins were comparable to those reported in other studies.[28,38,39] The concentration of plasma tHcy increased, and folate decreased, according to the number of T alleles, as has been shown by others.[40]

B Vitamin Interrelationships

Spearman correlation studies showed that B vitamins were positively related to each other, as has been shown elsewhere.[38] Such a positive relationship may be caused by common dietary sources for B vitamins[41] or may be explained by the fact that the metabolism of several B vitamins depends on other B vitamins.[1,42,43] A novel finding is that the riboflavin-folate relationship was *MTHFR* genotype–dependent. The variant enzyme associated with

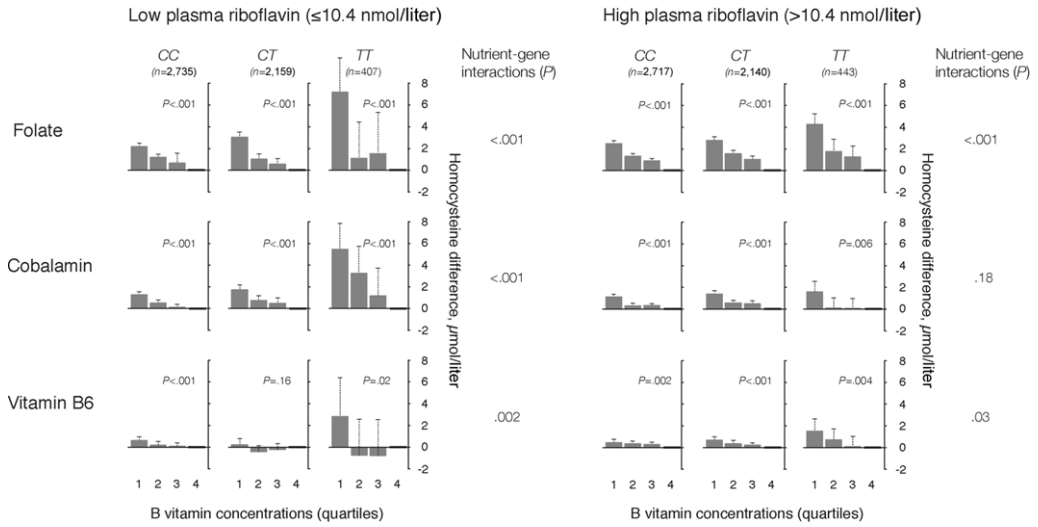

Figure 2. B vitamins as determinants of plasma tHcy according to riboflavin concentrations and *MTHFR* 677C→T genotype. The population was stratified according to levels of plasma riboflavin (below and above the median) and *MTHFR* 677C→T genotype. The folate-tHcy, cobalamin-tHcy, and vitamin B6–tHcy relationships were then studied in a regression model, which included these vitamins in addition to sex, age, creatinine, and study center. Means with upper limits of 95% CIs and *P* for trend across quartiles are shown in each panel. Nutrient-gene interaction terms were calculated as the product between *MTHFR* genotype and the various B vitamins.

the 677C→T transition of the *MTHFR* gene has lower affinity for its flavin cofactor than the wild-type enzyme.[44,45] Thus, the variant enzyme might depend on higher concentrations of riboflavin for sufficient catalytic activity. An inadequate riboflavin status might therefore reduce the formation of 5-methyltetrahydrofolate, the prevailing folate species in serum.[11]

MTHFR *Genotype and B Vitamins as Modulators of One-Carbon Metabolism*

The *MTHFR* 677C→T polymorphism was an independent predictor of plasma tHcy. This association was modified by several B vitamins, particularly folate and riboflavin, and the impact of the T allele was strongest when B vitamins were low. The folate-*MTHFR*[40] and riboflavin-*MTHFR*[38] relationships provide a paradigm of nutrient-gene interactions,[46] which may be explained by the role of riboflavin as cofactor for MTHFR,[1,45] whereas folate serves as a substrate for MTHFR and stabilizes the enzyme.[45]

Other B vitamins displayed similar but somewhat weaker interactions with the *MTHFR* polymorphism than folate and riboflavin. A genotype dependency of the cobalamin-tHcy relationship has been reported in one study.[47] Cobalamin serves as the cofactor for methionine synthase, which uses the product of the MTHFR reaction, 5-methyltetrahydrofolate, as a cosubstrate. Vitamin B6 is the cofactor for serine hydroxymethyltransferase, which forms 5,10-methylenetetrahydrofolate, the MTHFR substrate,[2,4,5] and is also involved in homocysteine transsulfuration.[5] Thus, cobalamin and vitamin B6 might be expected to have an impact on the metabolic network of which MTHFR is a part.

B vitamins not only modify the phenotypic expression of the *MTHFR* 677C→T polymorphism but are tHcy predictors in their own right. This has consistently been shown in several studies for folate[39,48] and cobalamin,[39,49] whereas the highly *MTHFR* genotype–dependent riboflavin-tHcy relationship was demonstrated more recently.[38,50]

Several studies show that vitamin B6 is a strong determinant of plasma tHcy in human subjects after methionine loading.[5,51] However, data on the vitamin B6–tHcy relationship in subjects who have not undergone methionine loading are less consistent; some,[52] but not all,[53,54] studies report that vitamin B6 is an independent determinant of plasma tHcy. Our study shows that the vitamin B6–tHcy relationship is largely driven by the TT group, which comprises only 10% of most populations of Asian or European descent,[37] and this may explain why a relationship between vitamin B6 and plasma tHcy has not been observed in smaller studies. In addition, interactions between vitamin B6 and other B vitamins might explain apparent inconsistencies between studies on vitamin B6 and tHcy, because B vitamin status may vary between study populations.

Implications

At present, plasma tHcy is an established marker of folate and cobalamin status and may be used for the diagnosis and follow-up of deficiency states.[39,48,49] Our data indicate that elevated tHcy may also reflect impaired riboflavin or vitamin B6 status, particularly in individuals with the TT genotype. Because a large fraction of hyperhomocysteinemic subjects have the TT genotype,[55] deficiencies of

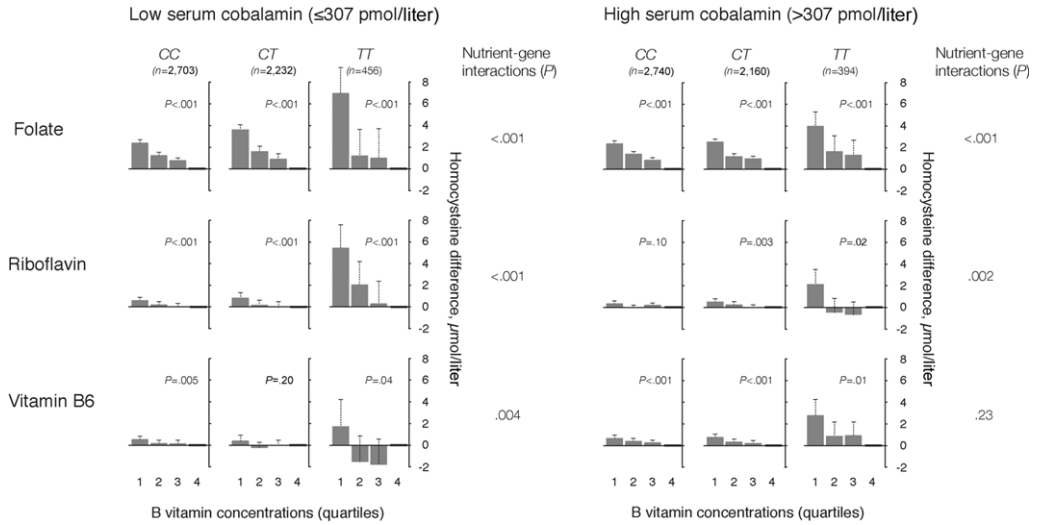

Figure 3. B vitamins as determinants of plasma tHcy according to cobalamin concentrations and *MTHFR* 677C→T genotype. The population was stratified according to levels of serum cobalamin (below and above the median) and *MTHFR* 677C→T genotype. The folate-tHcy, riboflavin-tHcy, and vitamin B6–tHcy relationships were then studied in a regression model, which included these vitamins in addition to sex, creatinine, and study center. Means with upper limits of 95% CIs and *P* for trend across quartiles are shown in each panel. Nutrient-gene interaction terms were calculated as the product between *MTHFR* genotype and the various B vitamins.

these vitamins may be an important cause of moderate hyperhomocysteinemia.

Our results indicate the existence of a functional metabolic network including the MTHFR enzyme and several B vitamins, which suggests that physiological and balanced regimes may be needed for the treatment of B vitamin deficiencies and for B vitamin intervention trials. In pernicious anemia, folate treatment may effectively reduce plasma tHcy even though underlying metabolic derangements may progress if cobalamin is not also given.[56] B vitamin intervention trials designed to assess the impact of plasma tHcy–lowering treatment on cardiovascular disease risk have generally been negative.[25,57] Most studies have included only folic acid and vitamin B6, however, and more balanced regimens including several B vitamins at physiological doses may be needed to optimize such trials.[58]

We found that the *MTHFR* 677C→T polymorphism and B vitamins were interactive modulators of the metabolism of homocysteine, which is an important intermediate in the transfer of one-carbon units. Diseases that might be caused by derangements of one-carbon metabolism might similarly depend on *MTHFR* genotype and B vitamins. Several studies support this idea, and low blood concentrations of folate strengthen the association between the *MTHFR* polymorphism and the risk of cardiovascular disease.[10] The *MTHFR* polymorphism is apparently a weaker predictor of coronary heart disease in American than in European populations,[10,59] and this might be explained by mandatory B vitamin fortification of food items in the United States,[60] which predictably increases the dietary intake of riboflavin and folate.

Blood concentrations of folate also modulate the impact of the *MTHFR* polymorphism, with respect to the risk of colorectal cancer.[13,15] The relationship between *MTHFR* genotype and colorectal cancer may also be modified by cobalamin[15] and vitamin B6.[15,61] Several studies report an interaction between the *MTHFR* polymorphism and folate as risk factors of colorectal adenoma.[62,63] Moreover, interactions between *MTHFR* genotype and B vitamins have been observed in studies on intermediary endpoints that may be related to the development of cancer. Some studies show that the *MTHFR* 677C→T polymorphism and folate status are interactive determinants of DNA methylation in human leukocytes[64–66] and of the incorporation of one-carbon units into monocyte DNA.[67]

Conclusions

The present study provides comprehensive metabolic data from a population-based cohort and has sufficient statistical power to give a detailed record of the relationship between MTHFR and several B vitamins, which form a network with major effects on the transfer of one-carbon units. B vitamins strongly modified genotype effects and vice versa. Individuals with the TT genotype were particularly sensitive to the status of several B vitamins and might be candidates for personalized nutritional recommendations. Our results corroborate and extend observations from in vitro studies and shed light on published data on the *MTHFR* polymorphism and B vitamins as determinants of plasma tHcy and chronic diseases. Awareness of the cooperativity between B vitamins involved in one-carbon metabolism should provide guidance on the

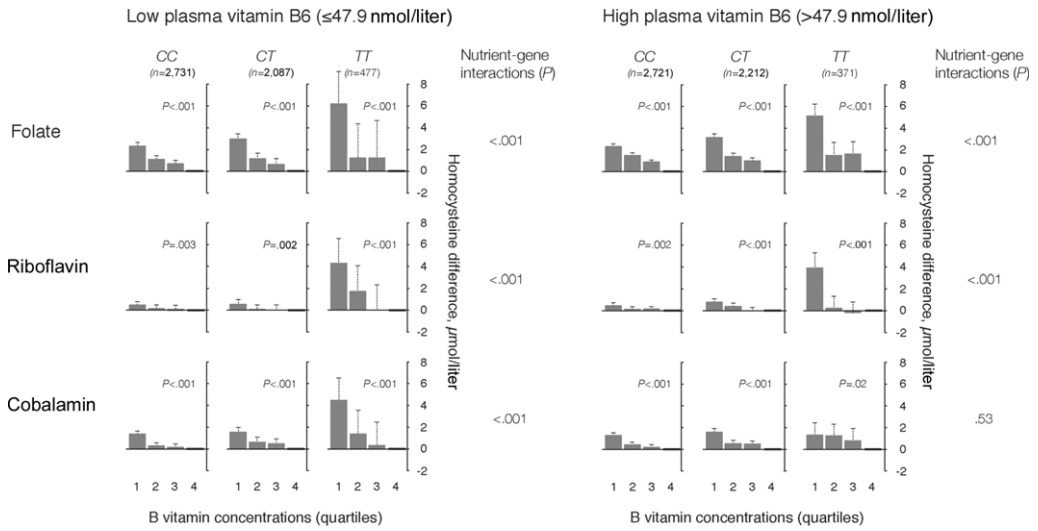

Figure 4. B vitamins as determinants of plasma tHcy according to vitamin B6 concentrations and *MTHFR* 677C→T genotype. The population was stratified according to levels of plasma vitamin B6 (below and above the median) and *MTHFR* 677C→T genotype. The folate-tHcy, riboflavin-tHcy, and cobalamin-tHcy relationships were then studied in a regression model, which included these vitamins in addition to sex, age, creatinine, and study center. Means with upper limits of 95% CIs and *P* for trend across quartiles are shown in each panel. Nutrient-gene interaction terms were calculated as the product between *MTHFR* genotype and the various B vitamins.

design of B vitamin regimens to be used in future intervention trials.

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Web Resources

The URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ (for *MTHFR* 677C→T)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MTHFR, neural tube defects, colorectal cancer, and hyperhomocysteinemia)

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