Phenotypic expression of the methylenetetrahydrofolate reductase 677C→T polymorphism and flavin cofactor availability in thyroid dysfunction1–3

Steinar Hustad, Bjørn G Nedrebø, Per Magne Ueland, Jørn Schneede, Stein Emil Vollset, Arve Ulvik, and Ernst A Lien

ABSTRACT

Background: The 5,10-methylenetetrahydrofolate reductase gene (MTHFR) 677C→T polymorphism modifies the risk of coronary artery disease and colon cancer and is related to plasma concentrations of total homocysteine (tHcy). Riboflavin status modulates the metabolic effect of the polymorphism, and thyroid hormones increase the synthesis of flavin cofactors.

Objective: The aim of the study was to investigate the phenotypic expression of the MTHFR 677C→T polymorphism in terms of plasma tHcy concentrations in patients with thyroid dysfunction.

Design: The study population consisted of 182 patients with hyperthyroidism. We studied plasma tHcy in relation to MTHFR genotype, riboflavin, and folate before and during 6 mo of treatment with antithyroid drugs.

Results: Before treatment, tHcy was higher in patients with the mutant enzyme than in those with the wild-type enzyme. A genotype effect was observed only at low riboflavin or folate concentrations (P ≤ 0.05). During treatment, concentrations of flavin cofactors in plasma decreased (P < 0.001), and tHcy increased (P < 0.001). The overall tHcy increase was greatest in patients with the T allele, particularly at low riboflavin concentrations (P = 0.004).


KEY WORDS Flavin adenine dinucleotide, flavin mononucleotide, Graves disease, polymorphism, genetics, riboflavin

INTRODUCTION

Riboflavin is a water-soluble vitamin and serves as the precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are cofactors for >150 reduction-oxidation enzymes. FMN is formed by the phosphorylation of riboflavin, and the largest fraction of FAD is subsequently adenylated to form FAD (1). Thyroid hormones increase the synthesis of both cofactors, mainly by enhancing the conversion of riboflavin to FMN, which is catalyzed by riboflavin kinase (2–4). Thyroid hormones also increase the hepatic activity of several enzymes, and animal experiments suggest that flavin cofactors may be mediators of these effects (2, 5, 6).

FAD-dependent 5,10-methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20) is an important folate-metabolizing enzyme and catalyzes the transformation of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (7), which serves as a methyl donor for the conversion of homocysteine to methionine, catalyzed by methionine synthase (8). In most tissues, this provides the sole pathway for homocysteine remethylation (8), and this explains why rare mutations causing severe MTHFR deficiency are associated with high plasma concentrations of total homocysteine (tHcy) and homocystinuria (9).

Mild MTHFR deficiency is observed in persons with the 677C→T transition in the MTHFR gene, which results in an alanine to valine substitution in the catalytic domain of the enzyme (10). The mutant enzyme is thermolabile (11), has lower affinity for FAD than does the wild-type enzyme, but is stabilized by the addition of folate or the flavin cofactor (12, 13). Enzyme activity in lymphocytes from persons with the CT and TT genotypes is ~30% and 65% of the wild-type activity, respectively (10). The T allele frequency is 0.3–0.4 in most populations of Asian or European descent (9). Several studies show that the MTHFR 677C→T polymorphism modifies the risk of certain diseases, eg, neural tube defects (14), coronary artery disease (15, 16), and colon cancer (17, 18). The variant enzyme is also related to moderate hyperhomocysteinemia, which is a risk factor for occlusive vascular disease (19, 20). Plasma tHcy is inversely related to riboflavin status in human subjects with the T allele but not in subjects with the CC genotype (21–23). This indicates that the metabolic effect of the polymorphism is modified by riboflavin.

The aim of the present study was to investigate the phenotypic expression of the MTHFR 677C→T polymorphism, in terms of plasma tHcy concentrations, in patients with thyroid dysfunction. FAD synthesis may increase in hyperthyroidism and be reduced by antithyroid treatment (2, 4). We hypothesized that this might affect MTHFR activity and folate metabolism and thus plasma tHcy (21, 23) in a genotype-specific manner. Our study population consisted of 182 patients with Graves disease, and we

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investigated the $\text{MTHFR} 677\text{C} \rightarrow \text{T}$ polymorphism, plasma tHcy, and B vitamins before and during treatment with antithyroid drugs.

**SUBJECTS AND METHODS**

**Study population**

Patients with Graves disease ($n = 182$) were recruited as outpatients at 4 hospitals in Norway (24). The diagnosis was based on clinical signs of hyperthyroidism, suppression of serum thyrotropin concentrations ($<0.05 \text{ mU/L}$), and increased serum concentrations of free thyroxine ($>20 \text{ pmol/L}$), and all patients had endocrine ophthalmopathy or thyrotropin receptor antibodies. Exclusion criteria were pregnancy, antithyroid treatment during the 12 mo before enrollment, drug allergy, ongoing immunosuppressive treatment, and noncompliance (24).

Patients were assessed clinically and biochemically before treatment and after 1.5, 3, and 6 mo of receiving antithyroid drugs. They were randomly assigned to 2 therapeutic regimens, either a low-dose titration regimen, whereby doses of antithyroid drugs were adjusted according to concentrations of free thyroxine, or a high-dose drug regimen, which included l-thyroxine (24). To prevent hypothyroidism, thyroxine supplementation was started when endogenous concentrations of free thyroxine were $<20 \text{ pmol/L}$. Carbimazole was the primary antithyroid drug and was used at an initial mean dosage of 29.5 mg/d, except for 6 patients who received propylthiouracil at a daily dose of 200–400 mg (24). For patients with data from follow-up ($n = 127$), there was no difference between the therapeutic regimens with respect to sex, age, tHcy, riboflavin, folate, cobalamin, creatinine, or $\text{MTHFR} 677\text{C} \rightarrow \text{T}$ genotype ($P \geq 0.2$).

The procedures followed were in accordance with the Helsinki Declaration, and the Regional Ethics Committee of the University of Bergen approved the study protocol. All participants gave written informed consent.

**Biochemical analyses**

Blood samples were collected into Venoject tubes with EDTA (Terumo Europe NV, Leuven, Belgium) after the subjects had fasted overnight. The samples were immediately put on ice, and plasma was obtained by centrifugation ($2000 \times g$, 10 min, 20 °C) within 30 min. Venoject plain silicon-coated tubes (Terumo Europe NV) were centrifuged ($2000 \times g$, 10 min, 20 °C) within 1 h after sampling to obtain serum. EDTA plasma and serum were stored at $-20^\circ\text{C}$ for $n = 1$ y and then at $-80^\circ\text{C}$ until analysis.

$\text{MTHFR} 677\text{C} \rightarrow \text{T}$ genotyping was performed in plasma by using a real-time polymerase chain reaction (25), and samples were analyzed in triplicate to confirm correct genotype assignment. Plasma tHcy was analyzed by using HPLC and fluorescence detection (26). Fluorimunoassays were used to measure serum thyrotropin, free thyroxine, and triiodothyronine (Wallac Oy, Turku, Finland), as well as thyroid receptor antibodies (DLD Diagnostika GmbH, Hamburg, Germany). Riboflavin, FMN, and FAD were analyzed in EDTA plasma by using capillary electrophoresis and laser-induced fluorescence detection with a modification (27) of the method described by Hustad et al (28). Serum folate was determined by using a $\text{Lactobacillus casei}$ microbiological assay (29), and serum cobalamin was determined by using a $\text{Lactobacillus leichmannii}$ microbiological assay (30). Serum creatinine was determined by using the alkaline picrate method for the CHEM 1 system (Technicon Instruments, Terrytown, NY).

**Statistical methods**

Means and medians with 10th and 90th percentiles were used for descriptive statistics, and bivariate correlations were determined by using Spearman coefficients. The chi-square test was used to compare proportions, whereas Student’s t test or univariate analysis of variance (ANOVA) was used to compare means of continuous variables.

We used multiple linear regression models to identify predictors of tHcy. Independent variables were represented in the models as indicator variables denoting membership in $\geq 2$ categories for sex, age, the $\text{MTHFR} 677\text{C} \rightarrow \text{T}$ polymorphism, riboflavin, folate, cobalamin, creatinine, and triiodothyronine. Thus, the regression coefficients estimated the difference in mean tHcy between the reference category and the other categories for each factor. Concentrations of tHcy across categories of each factor were tested for linear trend. Similar models were used to study the tHcy change during treatment in relation to various predictors.

We investigated the possible interaction between plasma riboflavin and $\text{MTHFR} 677\text{C} \rightarrow \text{T}$ genotype (coded as 0 for CC and 1 for CT or TT) by including a product term between the 2 variables in a multiple linear regression model in which tHcy was the dependent variable and riboflavin and genotype were retained as independent variables. Similarly, we investigated the interaction between serum folate and $\text{MTHFR}$ genotype.

Changes in concentrations of B vitamins, creatinine, and triiodothyronine during treatment were investigated by using ANOVA for repeated measurements. An ANOVA model, in which an unbalanced repeated-measure design was used to allow for missing observations, was applied to analyze tHcy data from all patients with follow-up ($n = 127$). The change in plasma tHcy over time was modeled as a quadratic polynomial containing a linear and a quadratic term because there was no significant contribution from a cubic term. Plasma riboflavin and $\text{MTHFR}$ genotype were included as covariates. The riboflavin-by-genotype-by-time interaction was analyzed in a model that included all two-way interactions. Finally, the data were stratified according to concentrations of riboflavin, and the genotype-by-time interaction was reanalyzed at riboflavin concentrations above and below the median to test whether the change in tHcy differed by genotype. Adjustment for age and sex did not influence the results, and therefore only unadjusted results are shown.

SPSS version 11.0 for Macintosh (SPSS Inc, Chicago) and the 5V module of BMDP (Statistical Solutions, Cork, Ireland) were used for statistical analyses. All tests were two-tailed, and $P$ values $<0.05$ were considered statistically significant.

**RESULTS**

**Patient characteristics and blood indexes**

The characteristics of the 182 patients with Graves disease who were included in the study are shown in Table 1. Data are given for sex, age, and $\text{MTHFR} 677\text{C} \rightarrow \text{T}$ genotype frequencies and for blood concentrations of tHcy, B vitamins, creatinine, and triiodothyronine. Plasma tHcy was higher in the TT genotype group (8.9 μmol/L) than in the CT (8.0 μmol/L) and CC (8.1 μmol/L) genotype groups and was the only variable that was related to $\text{MTHFR}$ genotype ($P = 0.01$, ANOVA).
Bivariate correlations are reported in Table 2. Plasma tHcy showed an inverse relation to riboflavin, folate, and cobalamin but was not correlated with triiodothyronine, FMN, or FAD. tHcy had a positive relation with serum creatinine. Riboflavin, folate, and cobalamin correlated significantly.

**Determinants of plasma tHcy**

Results of multiple regression analyses are reported in Table 3. Age, the MTHFR 677C→T polymorphism, riboflavin, folate, and cobalamin were independently related to plasma tHcy in the hyperthyroid phase. Serum folate was the strongest tHcy predictor, whereas the MTHFR polymorphism, riboflavin, and other variables showed a weaker relation to tHcy. In a model adjusted for sex and age, estimated mean tHcy concentrations were 0.3 and 3.2 μmol/L higher in the patients with the CC and TT genotypes, respectively, than in the patients with the CC genotype. The genotype-tHcy relation was modified by riboflavin and folate (Table 4) but not by sex, age, cobalamin, or creatinine ($P \geq 0.2$).

**Effects of antithyroid treatment**

The change in plasma tHcy during antithyroid treatment was studied in relation to the MTHFR 677C→T polymorphism and riboflavin in patients with complete series of blood samples ($n = 80$). After 1.5 mo of treatment, 74% of the patients attained biochemical euthyroidism (serum free thyroxine concentration <20 pmol/L), and after 6 mo, all the patients were euthyroid. None of the patients developed hypothyroidism during treatment.

As shown in Figure 1, plasma concentrations of FMN and FAD decreased consistently during treatment, and FMN decreased significantly more than did its precursor, riboflavin. Concentrations of other analytes before and during treatment are shown in Table 5. Concentrations of B vitamins and triiodothyronine decreased significantly, whereas creatinine concentrations increased significantly.

Changes in plasma tHcy concentration during treatment are depicted in Figure 2. Normalization of thyroid function was accompanied by an increase in tHcy, which occurred mainly during the first 1.5 mo. This initial tHcy increase was predicted by MTHFR genotype in a regression model adjusted for sex and age. The tHcy change was related to pretreatment concentrations of riboflavin (Figure 2) but not of folate ($P = 0.2$). An effect of genotype was observed when riboflavin was in the lowest tertile, but this effect was not observed at higher riboflavin concentrations (Figure 2).

Finally, the change in plasma tHcy concentration during 6 mo of antithyroid treatment was analyzed by using ANOVA for repeated measurements, which permitted the inclusion of all patients with data from follow-up ($n = 127$). As shown in Table 6, tHcy concentrations increased significantly, and the change was strongest during the first phase of treatment. At low riboflavin concentrations, the increase in tHcy concentration was MTHFR genotype dependent (as indicated by a significant MTHFR genotype-by-time interaction), and the effect was strongest in the patients with the TT genotype. At higher riboflavin concentrations, the increase in tHcy concentration was not related to genotype.

**DISCUSSION**

The aim of the present study was to investigate the phenotypic expression of the MTHFR 677C→T polymorphism, in terms of plasma tHcy concentrations, in relation to thyroid and riboflavin status. We studied patients with hyperthyroidism before and during antithyroid treatment. At baseline, tHcy concentrations...
TABLE 4
Determinants of total homocysteine (tHcy) in the hyperthyroid phase

<table>
<thead>
<tr>
<th>tHcy determinant</th>
<th>Simple adjustment</th>
<th>Multiple adjustments</th>
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<tbody>
<tr>
<td></td>
<td>Estimated tHcy difference</td>
<td>P for trend</td>
</tr>
<tr>
<td></td>
<td>µmol/L</td>
<td></td>
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<tr>
<td>Sex [compared with female (n = 157)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n = 25)</td>
<td>1.1 (−0.1, 2.3)</td>
<td>0.08</td>
</tr>
<tr>
<td>Age [y; compared with &lt;33 (n = 45)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34–43 (n = 47)</td>
<td>−0.4 (−1.4, 0.6)</td>
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<tr>
<td>44–50 (n = 45)</td>
<td>0.2 (−1.0, 1.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>&gt;51 (n = 45)</td>
<td>1.4 (0.3, 2.6)</td>
<td></td>
</tr>
<tr>
<td>MTHFR 677C→T genotype [compared with CC (n = 100)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (n = 67)</td>
<td>0.3 (−0.5, 1.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>TT (n = 15)</td>
<td>3.2 (1.7, 4.6)</td>
<td></td>
</tr>
<tr>
<td>Plasma riboflavin [nmol/L; compared with &gt;13.7 (n = 44)]</td>
<td></td>
<td></td>
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<tr>
<td>9.9–13.4 (n = 44)</td>
<td>0.8 (−0.4, 1.9)</td>
<td></td>
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<tr>
<td>6.6–9.8 (n = 47)</td>
<td>0.9 (−0.2, 2.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>&lt;6.5 (n = 47)</td>
<td>1.8 (0.7, 2.9)</td>
<td></td>
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<tr>
<td>Serum folate [nmol/L; compared with &gt;15.8 (n = 43)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.6–15.7 (n = 44)</td>
<td>0.7 (−0.3, 1.7)</td>
<td></td>
</tr>
<tr>
<td>8.1–10.5 (n = 46)</td>
<td>1.4 (0.4, 2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;8.0 (n = 46)</td>
<td>3.7 (2.7, 4.7)</td>
<td></td>
</tr>
<tr>
<td>Serum cobalamin [nmol/L; compared with &gt;512 (n = 40)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>401–502 (n = 41)</td>
<td>0.7 (−0.5, 1.9)</td>
<td></td>
</tr>
<tr>
<td>311–397 (n = 41)</td>
<td>1.8 (0.6, 3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;309 (n = 39)</td>
<td>2.4 (1.2, 3.6)</td>
<td></td>
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<tr>
<td>Serum creatinine [µmol/L; compared with &lt;52 (n = 42)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52–58 (n = 46)</td>
<td>0.6 (−0.6, 1.7)</td>
<td></td>
</tr>
<tr>
<td>59–65 (n = 45)</td>
<td>1.2 (0.0, 2.3)</td>
<td>0.09</td>
</tr>
<tr>
<td>&gt;66 (n = 39)</td>
<td>0.9 (−0.4, 2.2)</td>
<td></td>
</tr>
</tbody>
</table>

1 Data were analyzed by using multiple regression with tHcy as the dependent variable. MTHFR, 5,10-methylenetetrahydrofolate reductase.
2 Adjusted for sex and age.
3 Adjusted for sex, age, plasma riboflavin, serum folate, serum cobalamin, serum creatinine, serum triiodothyronine, and MTHFR 677C→T genotype.
4 P; 95% CI in parentheses.

were higher in the patients with the variant enzyme than in those with the wild-type enzyme, and riboflavin and folate modified the genotype effect. During treatment, concentrations of flavin cofactors in plasma decreased, and plasma tHcy increased. The increase in tHcy was genotype dependent in the patients with low riboflavin concentrations. This is the largest study published to date on the MTHFR 677C→T polymorphism in patients with thyroid dysfunction.

TABLE 4
Modification of the riboflavin–total homocysteine (tHcy) and the folate-tHcy relations by 5,10-methylenetetrahydrofolate reductase (MTHFR) genotype

<table>
<thead>
<tr>
<th>CC genotype (n = 100)</th>
<th>CT or TT genotype (n = 82)</th>
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<tbody>
<tr>
<td></td>
<td>Estimated tHcy difference</td>
</tr>
<tr>
<td></td>
<td>µmol/L</td>
</tr>
<tr>
<td>Plasma riboflavin (nmol/L; compared with &gt;13.7)</td>
<td></td>
</tr>
<tr>
<td>9.9–13.4</td>
<td>−0.4 (−1.8, 0.9)</td>
</tr>
<tr>
<td>6.6–9.8</td>
<td>0.0 (−1.3, 1.2)</td>
</tr>
<tr>
<td>&lt;6.5</td>
<td>0.3 (−1.0, 1.7)</td>
</tr>
<tr>
<td>Serum folate (nmol/L; compared with &gt;15.8)</td>
<td></td>
</tr>
<tr>
<td>10.6–15.7</td>
<td>1.2 (0.0, 2.4)</td>
</tr>
<tr>
<td>8.1–10.5</td>
<td>1.2 (0.1, 2.3)</td>
</tr>
<tr>
<td>&lt;8.0</td>
<td>2.5 (1.3, 3.7)</td>
</tr>
</tbody>
</table>

1 Data were analyzed by using multiple regression with tHcy as the dependent variable and riboflavin or folate as the independent variable.
2 P; 95% CI in parentheses.
3 Riboflavin-by-genotype and folate-by-genotype interaction models.
4 Data were analyzed by using multiple regression with tHcy as the dependent variable and riboflavin, MTHFR genotype (CC = 0 and CT or TT = 1), and a riboflavin–MTHFR genotype product term as the independent variables. The P value applies to the product term.
5 tHcy is the dependent variable, and folate, MTHFR genotype, and a folate-genotype product term are the independent variables. The P value applies to the product term.
The investigation of phenotypic effects of genetic polymorphisms provides a means for eliminating the effect of confounders related to lifestyle and for assessing the effect of factors that interact with genetic variants (31). We studied the MTHFR polymorphism in relation to plasma riboflavin because riboflavin modifies the effect of the polymorphism (21, 23). Riboflavin status is itself modified by hormones (3, 4, 32), and the longitudinal design of the study allowed us to investigate the polymorphism under conditions of variable thyroid status.

We found higher plasma concentrations of FMN in patients with hyperthyroidism than in previous studies of euthyroid subjects, whereas riboflavin concentrations were similar (21, 27). Moreover, normalization of thyroid function was associated with a consistent decrease in plasma concentrations of flavin cofactors, particularly FMN, which decreased significantly more than did its precursor, riboflavin. Antithyroid treatment of hyperthyroid patients may also be associated with reduced concentrations of FMN and FAD in tissues. This idea gains support from animal experiments, which showed that the activity of riboflavin kinase is higher in the liver of hyperthyroid rats (2, 4) and lower in the liver of hypothyroid rats (2, 5) than in the liver of control rats. Concentrations of FMN (3) and FAD (2, 3) do not increase above the normal in the liver of hypothyroid rats (2, 5) than in the liver of control rats. Furthermore, thyroid hormone deficiency is associated with low hepatic concentrations of FMN (3, 5) and FAD (2, 3, 5).

Reduced availability of flavin cofactors during treatment of patients with hyperthyroidism might influence the activity of flavoenzymes, including MTHFR. Hepatic (35–38) but not renal (35, 37) MTHFR activity is higher in hyperthyroid rats than in control rats, and enzyme activity is reduced by thyroidectomy (36, 37) or by giving antithyroid drugs to euthyroid animals (37–39). The combined effect of thyroid hormones and riboflavin status on MTHFR has not been investigated previously. However, the observation that thyroid hormone deficiency (36–40) and riboflavin deficiency (41–43) have similar effects on MTHFR activity (36–38, 41, 43), folate metabolism (36, 38–43), and some folate-dependent metabolic pathways (36–42) supports the idea that riboflavin may be a mediator of thyroid hormone effects.

We found that hyperthyroid patients had low tHcy concentrations, which increased during antithyroid treatment, and this is in accordance with the results of previous studies (44–46). The MTHFR 677C→T polymorphism modified plasma tHcy concentrations, which were higher in the patients with the variant enzyme than in those with the wild-type enzyme. The genotype effect was strongest at low concentrations of riboflavin or folate. Similar genotype-tHcy relations and modifications of the genotype effect by riboflavin and folate have been observed in healthy subjects (9, 19). The absence of a genotype effect in some studies (9, 44) may be related to high intakes of riboflavin or folate (9).

The increase in tHcy during antithyroid treatment was predicted by MTHFR genotype, and the strongest response was observed in patients with the T allele and low plasma riboflavin concentrations. The model presented in Figure 3 depicts the relation between the phenotypic expression of the MTHFR 677C→T polymorphism, riboflavin, and thyroid hormones. Low riboflavin concentrations may be associated with low intracellular concentrations of flavin cofactors (27), which could decrease further during treatment (2–4). A decrease in tissue

![Image](https://academic.oup.com/ajcn/article-abstract/80/4/1050/4690360/mediation.png)
FAD concentrations might reduce MTHFR activity (41, 43), particularly in the presence of the variant enzyme, which is characterized by a low affinity for FAD (12, 13). This might explain the increase in tHcy during antithyroid treatment in the patients with the T allele and low riboflavin concentrations.

**FIGURE 2.** Mean (±SEM) changes in plasma total homocysteine (tHcy) concentration from baseline (0 mo) during treatment according to 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T genotype and pretreatment plasma riboflavin concentration. Only patients with complete series of blood samples (n = 80) were included. During treatment, there was a significant overall increase in tHcy (P < 0.001, ANOVA), which was most pronounced during the initial 1.5 mo. This initial increase in tHcy was predicted by MTHFR genotype (CC = 0, CT = 1, and TT = 2) in a regression model adjusted for sex and age (P = 0.047) and was inversely related to pretreatment riboflavin concentrations (P = 0.04). The tHcy response was related to MTHFR genotype when plasma riboflavin was in the lowest tertile (upper panels; P = 0.02) but not when riboflavin concentrations were higher (middle and lower panels; P = 0.6).

**TABLE 6**
Change in total homocysteine (tHcy) during antithyroid treatment according to 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T genotype and riboflavin concentration

<table>
<thead>
<tr>
<th>Plasma riboflavin</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Genotype-by-time interaction, P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Linear</td>
<td>Quadratic</td>
<td>Linear</td>
</tr>
<tr>
<td>≤9.8 nmol/L</td>
<td>36</td>
<td>0.23 (&lt;0.0001)</td>
<td>−0.007 (&lt;0.0001)</td>
<td>18</td>
</tr>
<tr>
<td>&gt;9.8 nmol/L</td>
<td>33</td>
<td>0.08 (0.03)</td>
<td>−0.001 (0.3)</td>
<td>27</td>
</tr>
</tbody>
</table>

1 ANOVA for repeated measurements was used to analyze data from 127 patients (80 patients with complete data) who were monitored during 6 mo of antithyroid treatment. There was a significant riboflavin-by-genotype-by-time interaction [P = 0.0015 (linear) and 0.0048 (quadratic)] and a significant genotype-by-time interaction [P = 0.033 (linear) and 0.035 (quadratic)]. Data were stratified according to baseline riboflavin concentrations (above or below the median), and the genotype-by-time interaction was reanalyzed at each concentration.

2 Estimated average change; P value in parentheses.

3 Wald test.

**FIGURE 3.** The relation between the phenotypic expression of the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism, riboflavin, and thyroid hormones. The model integrates data from experimental and human genetic studies and explains how thyroid hormones modify the metabolic effect of the MTHFR polymorphism in terms of plasma total homocysteine (tHcy) concentrations. Thyroid hormones increase the activity of enzymes involved in riboflavin metabolism, particularly riboflavin kinase (RK), and thereby augment the synthesis of flavin adenine dinucleotide (FAD), a cofactor for MTHFR. The thermolabile enzyme associated with the 677C→T transition of the MTHFR gene has an alanine (Ala)-to-valine (Val) substitution and a higher FAD dissociation rate than does the wild-type enzyme, which results in lower cofactor binding. MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate (CH$_2$-THF) to 5-methyltetrahydrofolate (CH$_3$-THF), which is required for the remethylation of homocysteine (Hcy) to methionine (Met), and low MTHFR activity is associated with high plasma tHcy concentrations. T$_3$, triiodothyronine; T$_4$, thyroxine; FMN, flavin mononucleotide; FS, FAD synthase.

FAD concentrations might reduce MTHFR activity (41, 43), particularly in the presence of the variant enzyme, which is characterized by a low affinity for FAD (12, 13). This might explain the increase in tHcy during antithyroid treatment in the patients with the T allele and low riboflavin concentrations.
In conclusion, the present study indicates that thyroid status affects the phenotypic expression of the MTHFR 677C→T polymorphism by modifying FAD synthesis. This mechanism could partly explain how thyroid dysfunction affects the metabolism of folates and homocysteine.

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SH participated in data interpretation and wrote the initial draft of the manuscript. BGN was responsible for the study design, recruited patients, and wrote clinical details. PMU and JS participated in the writing process.

SEV gave advice on statistical methods and reviewed the manuscript. EAL contributed during all parts of the process. The authors had no financial conflicts of interest.

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