The C677T mutation in the methylenetetrahydrofolate reductase gene predisposes to hyperhomocysteinemia in children with familial hypercholesterolemia treated with cholestyramine

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In children with familial hypercholesterolemia, heterozygosity and homozygosity for the C677T mutation in the methylenetetrahydrofolate reductase gene was associated with low serum folate and increased susceptibility to elevation of plasma total homocysteine during cholestyramine treatment. Because of the independent relationship between elevated plasma total homocysteine and cardiovascular disease, folate supplementation may be prudent in these children. (J Pediatr 1998;132:365-8)

Treatment options are limited for children with severely elevated concentrations of low-density lipoprotein cholesterol because of heterozygous familial hypercholesterolemia. Low-density lipoprotein cholesterol concentrations that remain elevated after an adequate trial of diet may be lowered with bile acid–binding resins, including colestipol and cholestyramine.[1]

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In a randomized study of cholestyramine in children with FH, we recently reported that the mean concentration of plasma total homocysteine increased in the cholestyramine group.[2] Even moderately elevated tHcy is an independent risk factor for occlusive vascular disease.[3] In adults, a 5 μmol/L increment in tHcy may increase the risk of coronary artery disease by as much as an increase in cholesterol of 0.5 mmol/L. Thus this influence of cholestyramine on tHcy may counteract its beneficial effects related to reduction of the cholesterol level.

Elevated tHcy is usually due to deficiencies of nutritional coenzymes required for homocysteine metabolism, including folate, vitamin B_{12}, and vitamin B_6. In addition, genetic defects in the enzymes required for homocysteine metabolism cause elevated tHcy and may interact with nutritional deficits. Notably, homozygosity for an alanine-to-valine (C677T) missense mutation in the gene coding for methylenetetrahydrofolate reductase is common in the general population and leads to a thermolabile enzyme.[4] Individuals with this enzyme variant often have elevated tHcy levels, particularly under conditions of low dietary folate intake.[5]

FH	Familial hypercholesterolemia
MTHFR	Methylenetetrahydrofolate reductase
tHcy	Plasma total homocysteine

Cholestyramine and other resins may increase tHcy by inhibiting the absorption of folate.[6] In this study we investigated whether the elevation of tHcy observed during cholestyramine treatment was related to the MTHFR genotype in children with FH.

SUBJECTS AND METHODS

Subjects were 96 boys and girls 6 to 11 years old with FH who participated in a 1-year open study of diet equivalent to the American Heart Association Step 1

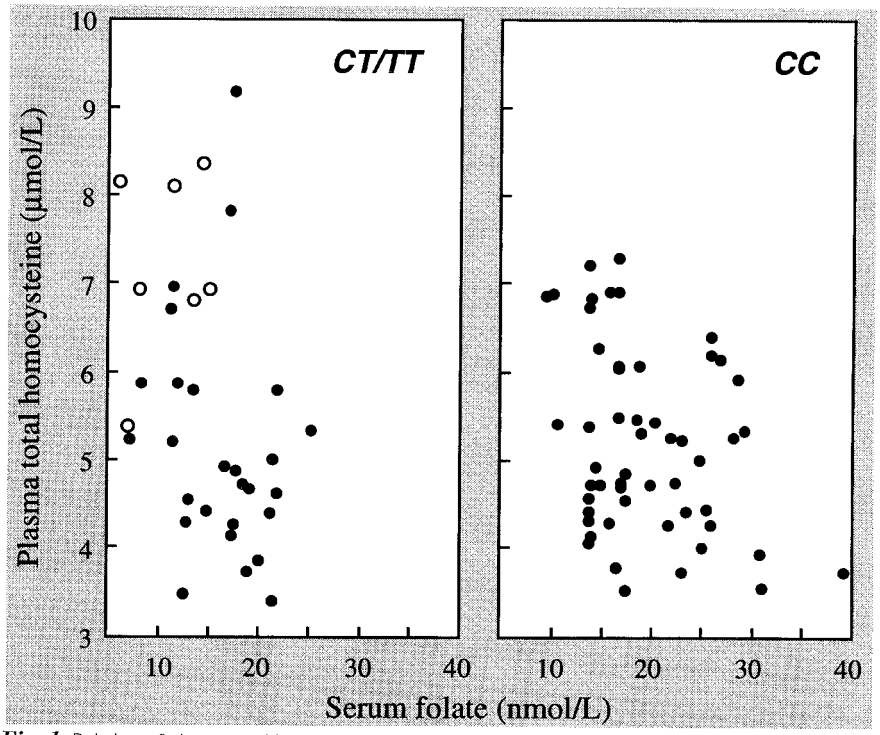

Fig. 1. Relation of plasma total homocysteine to serum folate in children with normal genotype (CC) and in children who were heterozygotes (CT) or homozygous (TT) for the C677T mutation in the methylenetetrahydrofolate gene. TT subjects are indicated by open symbols.

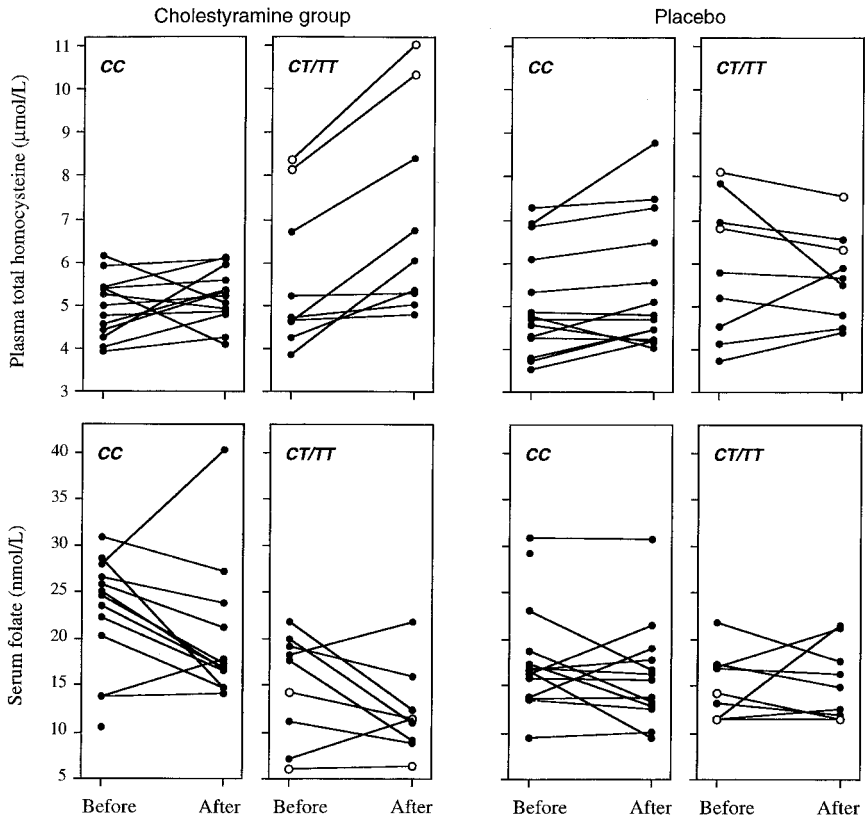

Fig. 2. Individual changes in plasma total homocysteine and serum folate in the cholestyramine and placebo groups according to methylenetetrahydrofolate reductase genotype. *CC*, Without the C677T mutation; *CT*, heterozygous; *TT*, homozygous for the C677T mutation. TT subjects are indicated by open symbols.

diet as described previously.[2] After the end of this phase, 72 were randomized to 8 gm of cholestyramine or to matching placebo granules for 1 year. The children were all of Norwegian descent and were prepubertal at inclusion. Twenty-two of 36 in the cholestyramine group and 26 of 36 in the placebo group completed 1 year. Dropouts were usually due to unpalatability of the drug.[2]

For analysis of tHcy, ethylenediamine tetraacetic acid blood samples were immediately placed on ice, and plasma was separated within 30 minutes, and then stored at –20° C. Plasma tHcy was measured using a modification of a fully automated assay.[7] DNA was extracted from blood mononuclear cells and then genotyped by a procedure including polymerase chain reaction amplification, restriction cleavage, and analysis of the DNA fragments by capillary electrophoresis and laser-induced fluorescence detection.[4,8] Serum folate concentration was measured by radioassay with a commercial kit.

Blood collected at baseline was available for determination of plasma tHcy, serum folate, and MTFHR status in 96, 93, and 92 subjects, respectively. Of those 48 subjects who completed 1-year follow-up, we missed data on tHcy, folate, and MTHFR status from 2, 2, and 1 subjects, respectively, all in the placebo group.

Because the distribution of tHcy and serum folate was skewed, logarithmic transformation was used to normalize their distributions. Spearman's ρ was calculated to examine the correlation between tHcy and serum folate. We used analysis of variance to test for differences between genotypes. Among subjects who took cholestyramine for 1 year, Fisher's Exact Test was used to compare the proportion of subjects with thermolabile MTHFR who experienced or did not experience a rise in tHcy concentration to more than 10 μmol/L.

RESULTS

For the total group (n = 96), the mean, median, and 90th percentile concentrations of tHcy were 5.3, 5.0, and 6.9 μmol/L, respectively, with a range of 2.7 to 9.2 μmol/L. Thus none of the subjects initially had a tHcy concentration greater

Table. tHcy and folate concentrations (mean ± standard deviation) in children with FH according to MTHFR genotype

	MTHFR genotype		
	Normal (CC) (n = 56)	Heterozygote (CT) (n = 29)	Thermolabile (TT) (n = 7)
Male/female	32/24	18/11	5/2
Age (yr)	8.0 ± 1.3	8.1 ± 1.4	7.4 ± 1.6
Total cholesterol (mmol/L)	8.8 ± 1.4	8.4 ± 1.6	9.0 ± 1.5
Plasma tHcy (μmol/L)	5.2 ± 1.1	5.2 ± 1.3	7.2 ± 1.1*
Serum folate (nmol/L)†	19.5 ± 6.4	16.3 ± 4.6	10.8 ± 3.7‡

*p = 0.0004 by analysis of variance.
†n = 55, 27, and 7 in the normal, heterozygote, and thermolabile groups, respectively.
‡p = 0.0001 by analysis of variance.

than 10 μmol/L. Plasma tHcy concentration was similar in the 72 subjects who were randomized and the 24 who were excluded from the study (5.3 ± 1.3 μmol/L vs 5.4 ± 1.1 μmol/L) and in the 48 subjects who completed the study versus the 24 subjects who dropped out (5.2 ± 1.4 μmol/L vs 5.3 ± 1.3 μmol/L). Likewise, the distribution of normal CC, heterozygous CT, and homozygous mutant TT genotypes was similar in subjects who were randomized (60%, 31%, and 9%) or excluded (64%, 32%, and 4%) and in those who completed (62%, 30%, and 8%) or dropped out (56%, 35%, and 9%) of the study.

The distribution of age, gender, and serum total cholesterol was similar according to MTHFR genotype (Table). Mean tHcy concentration was the same in subjects with the CC and CT genotypes, but was higher in subjects with the TT genotype, who also had lower serum folate concentration (Table and Fig. 1). The relationship between tHcy and serum folate tended to be more marked in the CT and TT groups combined (Spearman's ρ = –0.45; p = 0.01; Fig. 1) than in the CC normal genotype group (Spearman's ρ = 0.28; p = 0.04).

During cholestyramine treatment, tHcy concentration increased in subjects with the C677T mutation in one or both alleles (CT/TT genotypes) but not in subjects with the CC genotype (Fig. 2). In contrast, we observed a reduction in serum folate in most subjects regardless of genotype. In the placebo group, tHcy and folate concentrations showed no consistent changes, including the two subjects with the TT genotype (Fig. 2). Changes in tHcy were not related to compliance or to changes in cholesterol concentration (data not shown).

In 2 of 22 subjects, tHcy concentration increased to > 10 μmol/L after 1 year of cholestyramine and both had a low serum folate level.[2] Their tHcy concentrations rose from 8.2 to 10.3 μmol/L and from 8.4 to 11.0 μmol/L, respectively. Both were homozygous (TT) for the thermolabile variant, whereas none of the remaining 20 subjects had this genotype (p = 0.02 by Fisher's Exact Test).

For the subjects included in this study, the correlation between energy-adjusted dietary folate and serum folate was significant (Spearman's ρ = 0.24; p = 0.03). None of the subjects were prescribed folic acid supplements, but two in the placebo group took multivitamins that contain a low dose of folic acid (100 μg).

DISCUSSION

Individuals with the C677T mutation in one or both alleles of the MTHFR gene may be susceptible to increased tHcy concentration during treatment with cholestyramine. This post hoc analysis is consistent with published data indicating that MTHFR thermolability may predispose to elevated tHcy levels under conditions of negative folate homeostasis.[6] Moreover, there appeared to be a steeper negative correlation between plasma tHcy and serum folate in subjects with the mutation than in normal subjects, which concurs with data from a recent Norwegian study of an adult population.[9] These data suggest an interaction between genotype and folate status. Thus, if folate availability decreases during cholestyramine therapy, the presence of the CT and TT genotypes may lead to increased tHcy, whereas the drug-induced reduction in serum folate level seems less deleterious for subjects with the normal CC genotype.

This study was conducted in children with FH, a small subgroup of the general population. The findings should therefore be confirmed in other populations as well. However, the mean tHcy concentration among the prepubertal children in this study was similar to the mean concentration we found in children aged 8 to 12 years in a population-based study.[10] Moreover, the prevalence of the thermolabile MTHFR genotype was about 8%, which is also similar to the prevalence in the general Norwegian[9] and other Caucasian populations.[11] Thus the subjects investigated here did not appear to differ substantially from the general population with respect to the MTHFR genotype.

Recommended upper limits for tHcy concentration in children or adults have not been established because we lack data that show whether a reduction in the tHcy level leads to decreased cardiovascular risk. The mean tHcy concentration of children is about one half of the mean concentration previously reported for adults.[12] Our choice of 10 μmol/L tHcy is arbitrary because there is probably a linear relationship between tHcy concentration and cardiovascular risk.[13] Notably, the two subjects whose tHcy concentration increased to > 10 μmol/L also had the highest baseline tHcy concentrations in the study.

We and others have previously shown that folate concentration decreases during treatment with bile acid–binding resins.[6,14] Our data suggest that in subjects with the C677T mutation, the reduction in serum folate during cholestyramine treatment causes an elevation of tHcy. This effect of cholestyramine may

contribute to an increased cardiovascular risk in these children. Whether folate supplementation will decrease tHcy in the presence of thermolabile MTHFR remains to be shown. Thus definite recommendations in regard to which groups need supplementation must await further studies.

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