# Association Between Plasma Total Homocysteine and Parental History of Cardiovascular Disease in Children With Familial Hypercholesterolemia

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***Background*** Recently, we reported a relation between plasma total homocysteine (tHcy) in children and cardiovascular disease (CVD) in their male relatives, suggesting that tHcy may partly explain the increased risk related to a family history of CVD. Because individuals with familial hyperlipidemias have an exceptionally high risk of premature CVD, we explored the relationship between tHcy and parental history of CVD in children with familial hypercholesterolemia (FH).

***Methods and Results*** Study subjects were 91 boys and 64 girls (age range, 7 to 17 years) with FH who were treated with a standard lipid-lowering diet at a tertiary care lipid clinic. We conducted a cross-sectional analysis of demographics, the diet, tHcy level, presence of the C677T mutation in the methylenetetrahydrofolate reductase gene (a common genetic cause of elevated tHcy) in children, and the prevalence of parental CVD. tHcy increased after puberty and was inversely related to parental educational level. Intakes of folate, vitamin C, and fruits and vegetables were inversely associated with tHcy, as were serum folate and vitamin $B_{12}$ (Spearman's $\rho$, $-0.2$ to $-0.4$; $P<.05$). tHcy was increased in children whose parent with FH had experienced CVD compared with children without parental CVD (median [interquartile range], 6.6 [5.3, 8.0] $\mu$mol/L versus 5.6 [4.7, 6.8] $\mu$mol/L; $P=.01$). This difference remained significant in multivariate regression analysis. Homozygosity for the C677T mutation was associated with a higher tHcy level and tended to be more frequent in the group with than in the group without a parental history of CVD (18% versus 8%; $P=.07$).

***Conclusions*** These findings suggest that a moderately elevated tHcy level may partly account for the contribution of the family history to risk of CVD in FH. Dietary recommendations for FH should include nutrients that affect homocysteine metabolism. **(*Circulation*. 1997;96:1803-1808.)**

***Key Words*** • genetics • homocysteine • risk factors • pediatrics • hypercholesterolemia

Children with heterozygous FH have an increased risk of premature CVD because of a mutation in the LDL receptor.[1] Current recommendations for treatment focus on the restriction of dietary saturated fat and cholesterol.[2] Youth who have an exceptionally high risk of disease, because of a greatly elevated serum cholesterol or high-risk family history, may be treated with bile acid–binding resins to lower serum LDL cholesterol levels.[3,4] A wealth of evidence supports these recommendations.[2] However, randomized trials proving the benefit of starting treatment in childhood rather than later in life are probably not feasible.[5]

The incidence and time of onset of clinical cardiovascular disease in patients with FH vary, depending on gender, cigarette smoking, LDL and HDL cholesterol levels, and other factors, including the family history of premature CVD.[1,6-9] The predictive effect of the family history may be due to both genetics and shared environmental exposures; however, the underlying biological mechanisms have not been completely elucidated.

Recent studies have shown that moderate elevation of plasma tHcy is an independent risk factor for CVD.[10,11] The risk is graded with apparently no threshold effect.[12,13] Elevated plasma tHcy, termed hyperhomocysteinemia, has been attributed to acquired and genetic factors.[14] The environmental factors include smoking,[15] coffee consumption,[16] and impaired folate or vitamin $B_{12}$ nutriture.[17] An important genetic factor is the C677T polymorphism of the methylenetetrahydrofolate reductase gene.[18] This is a common trait, present in its homozygous form in 5% to 10% of the general population. This enzyme variant is thermolabile with reduced catalytic activity, thereby impairing the formation of 5-methyltetrahydrofolate from 5,10-methylenetetrahydrofolate. Methyltetrahydrofolate is a methyl donor in the remethylation of homocysteine, and this explains why the enzyme variant predisposes to hyperhomocysteinemia, especially when folate status is impaired.[19-22]

To explore the hypothesis that tHcy level and the C677T mutation of methylenetetrahydrofolate reductase are associated with familial risk of premature CVD in FH, we examined these factors among children, in whom tHcy levels are less likely to be affected by established cardiovascular risk factors and by clinical or subclinical vascular lesions. Moreover, in heterozygous FH, death from CVD is rare before the age of 20 years. Thus, selection bias because of the death of severely affected individuals is avoided. We considered possible determinants of tHcy in the analyses, including demographics, developmental stage, and vitamin status.

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**Selected Abbreviations and Acronyms**

CVD = cardiovascular disease
FH = familial hypercholesterolemia
tHcy = total homocysteine

# Methods

## Setting

The patients attended a tertiary care ambulatory clinic for familial lipid disorders. Children with FH were identified by screening families with known FH or were referred by their primary care physician. The diagnosis of FH was made if the child had one or more total cholesterol levels of ≥6.8 mmol/L (260 mg/dL) and one or both parents and other relatives had serum cholesterol levels ≥7.8 mmol/L (300 mg/dL), consistent with autosomally dominant inheritance. The specific LDL receptor mutation causing FH[23,24] was identified in >80% of patients who met these criteria.

Patients visited the clinic at intervals of 3 to 18 months, depending on their level of risk and distance from the clinic. Visits involved a medical evaluation and a dietary counseling session. The diet was congruent with the National Cholesterol Education Program Step I diet including ≤30% of energy from fat, <10% from saturated fat, <100 mg cholesterol/4.2 MJ (1000 kcal), and adequate energy to support growth. The dietitian encouraged the youth and their families to eat starches, fruits, and vegetables.

## Subjects

From the medical records, we identified boys and girls aged 7 to 17 years with heterozygous FH registered at the clinic. Eligible subjects had started the cholesterol-lowering diet ≥18 months previously, were not mentally retarded, did not have hypertension or chronic disease, and were nonsmokers. In the case of siblings, one was chosen at random for this study. Because of travel distance or because they refused venipuncture, 9 eligible patients did not take part, leaving 155 subjects. The children and their parents gave informed consent, and the overall study was approved by the regional ethics committee.

The medical record of each subject was reviewed to obtain serum total and HDL cholesterol levels after the initial dietary instruction but not during use of cholesterol-lowering drugs. Height and weight were measured. Sexual maturation was scored from stages 1 to 5 according to Tanner.[25] Of 155 subjects, 8 were taking bile acid–binding resins regularly at the time of the study. The rest had stopped taking resins ≥4 weeks earlier.

## Dietary Assessment

Diet was assessed using a quantitative food frequency questionnaire[26] that was designed to reflect usual intake of 190 food items during the past year. At a clinic visit, children >12 years old were instructed on how to fill out the questionnaire. The questionnaire was completed at home, usually with the help of a parent. At a subsequent visit, usually within 3 months, the dietitian checked missing sections or double marks and made adjustments as guided by the child. The dietitian filled out the questionnaire for children ≤12 years old while interviewing the child and parent(s). All adjustments and interviews were done by the same dietitian. The completed questionnaire was read optically. Computation of daily intakes of nutrients and foods was done using a food database and software system developed at the Section for Dietary Research, University of Oslo. Micronutrient and food densities were calculated as amounts adjusted for energy intake (g/MJ or g/10 MJ). Validity of reported energy intake was good both for the dietitian-administered questionnaires and the self-administered questionnaires.[27]

The food frequency questionnaire included questions on demographics. Parental educational level was coded as 1 (≤ninth grade), 2 (completed secondary school), and 3 (completed university or professional training).

## History of CVD

The medical records of the subject and the parent with FH were reviewed for information on the occurrence of CVD. No subjects had experienced an event; all parents who had experienced cardiovascular events were <55 years old at the time of the event. The presence of CVD was based on a physician-diagnosed history of one or more of the following events: angina pectoris (n=19), myocardial infarction (n=14), sudden cardiac death (n=12), coronary artery bypass graft (n=11), percutaneous transluminal coronary angioplasty (n=3), and/or cerebral infarction (n=1). These events had occurred or started a mean of 7 (range, 2 to 16) years previously. Subjects whose parent had experienced one or more of these events composed the parental disease group (n=39).

## Laboratory Analyses

DNA was extracted from blood mononuclear cells, and polymerase chain reaction–based methods were used to identify the C677T mutation.[18] EDTA samples for measurement of tHcy were obtained after an overnight fast and were immediately placed on ice. The plasma was separated within 30 minutes and stored at −20°C. Plasma tHcy was determined using a modification of a fully automated assay based on precolumn derivatization followed by reversed-phase liquid chromatography.[28,29] The precision (between-day coefficient of variation) of the assay is ≈2%. tHcy measurement was missing for 7 subjects because of insufficient plasma. Serum folate and vitamin $B_{12}$ were measured by immunoassay using a commercial analyzer and kit (CIBA Corning ACS 180 Immunoassay Analyzer). The laboratory ranges for folate and vitamin $B_{12}$ levels were >5.7 nmol/L and 170 to 650 pmol/L, respectively.

## Statistical Analyses

We compared normally distributed variables in two groups using an unpaired $t$ test. The Mann-Whitney test or Kruskal-Wallis ANOVA was applied to compare skewed variables across two or more groups, respectively. Spearman rank correlation coefficients ($\rho$) were calculated to assess the relation of tHcy to other variables. Categoric variables were compared using $\chi^2$. Stepwise multiple regression analysis identified independent determinants of tHcy. Independent variables that were presented included age, pubertal stage, parental educational level, and parental CVD status. tHcy values were log-transformed before regression analysis because of a markedly skewed distribution. Because bile acid–binding resins elevate tHcy,[4] we analyzed data in Table 3 with and without subjects who were taking resins (n=8). The results were similar, but we chose to exclude the subjects taking resins. Two-tailed $P$ values of <.05 were considered statistically significant. Statistical analyses were performed using the StatView II package (Abacus Concepts) on a Macintosh computer.

# Results

The distribution of sex and age was similar in children whose parent had experienced a cardiovascular event (CVD+) and children whose parent had not experienced a cardiovascular event (CVD−) (Table 1). More than half of the subjects were in pubertal stage 1 (91 of 155). The remainder were about evenly distributed between pubertal stages 2 through 5. The two study groups were similar in regard to pubertal stage, body mass index, parental educational level, intake of macronutrients including fat (Table 1), intake of micronutrients (data not shown), blood lipid levels, and serum folate and vitamin $B_{12}$ (Table 2). Folate and vitamin $B_{12}$ levels were within the normal range of the laboratory,

**TABLE 1. Clinical Features and Diet of 155 Children and Adolescents With FH According to the Presence or Absence of Parental CVD (CVD+ or CVD−)**

| | CVD+ (n=39) | CVD− (n=116) |
|---|---|---|
| Sex, M/F | 23/16 | 68/48 |
| Age, y | 12.1±3 | 11.8±3 |
| Pubertal stage | 2.3±1 | 2.0±2 |
| Body mass index, kg/m$^2$ | 19.1±4 | 18.9±3 |
| Parental educational level, 1–3 | 1.7±1 | 1.8±1 |
| Macronutrient intake | | |
| Fat, E% | 26.2±4 | 26.3±4 |
| Saturated fat, E% | 9.5±2 | 9.6±2 |
| Polyunsaturated fat, E% | 5.2±1 | 5.2±1 |
| Monounsaturated fat, E% | 9.3±2 | 9.3±2 |
| Protein, E% | 15.1±2 | 15.2±2 |
| Carbohydrate, E% | 57.4±4 | 57.2±4 |

E indicates daily energy intake. Values are mean±SD.

with the exception of 1 subject who had a folate level of 5.2 nmol/L. Plasma tHcy was significantly higher in CVD+ than in CVD− subjects (Table 2 and Fig 1).

For the whole study group, the mean, geometric mean, and range of tHcy were 6.3, 6.0, and 3.3 to 33.0 μmol/L, respectively. There was no sex difference (data not shown). tHcy increased with age (Spearman's ρ, .45; $P$=.0001) and was significantly higher in pubertal than in prepubertal subjects (median [interquartile range], 6.6 [5.9, 8.6] μmol/L versus 5.2 [4.4, 6.4] μmol/L; $P$=.0001; Fig 2). tHcy was inversely related to mean parental educational level (Spearman's ρ, −0.22; $P$=.009). Intakes of vitamin C (Spearman's ρ, −0.27; $P$=.002), folate (Spearman's ρ, −0.23; $P$=.007), and fruits and vegetables (Spearman's ρ, −0.21; $P$=.01) were inversely correlated with tHcy, as were serum folate (Spearman's ρ, −0.40; $P$=.0001) and vitamin $B_{12}$ (Spearman's ρ, −0.30; $P$=.0009).

In stepwise multivariate regression analysis, pubertal stage, parental educational level (inverse relationship), and history of parental CVD remained significantly associated with tHcy level (Table 3). These variables accounted for 27% (95% confidence interval, 12% to 41%) of the variance in tHcy.

Of all subjects, 16 of 155 (10%) were homozygous for the C677T mutation. Homozygotes had a higher tHcy level than subjects with no mutation or heterozygotes (median [interquartile range], 7.2 [6.2, 8.2] μmol/L versus 5.7 [4.7, 6.8] μmol/L; $P$=.001) and tended to be more prevalent in the group with a history of parental CVD than in the group without parental CVD (18% versus 8%; $P$=.07; Table 4).

**TABLE 2. Lipids and Plasma tHcy Levels According to Study Group**

| | CVD+ (n=39) | CVD− (n=116) |
|---|---|---|
| Lipids, mmol/L | | |
| Total cholesterol | 8.3±1.7 | 8.5±1.4 |
| HDL cholesterol | 1.2±0.2 | 1.2±0.2 |
| Triglycerides | 0.9±0.4 | 0.9±0.5 |
| Plasma tHcy, μmol/L* | 6.6 (5.3, 8.0) | 5.6 (4.7, 6.8)† |
| Serum folate, nmol/L‡ | 15.2±6.1 | 16.3±5.6 |
| Serum vitamin $B_{12}$, pmol/L§ | 536±206 | 540±182 |

Values are presented as mean±SD except for plasma tHcy, for which median and interquartile range (25th percentile, 75 percentile) values are presented. To convert mmol/L to mg/dL, multiply by 38.7.
*n=37 and 111 in the CVD+ and CVD− groups, respectively.
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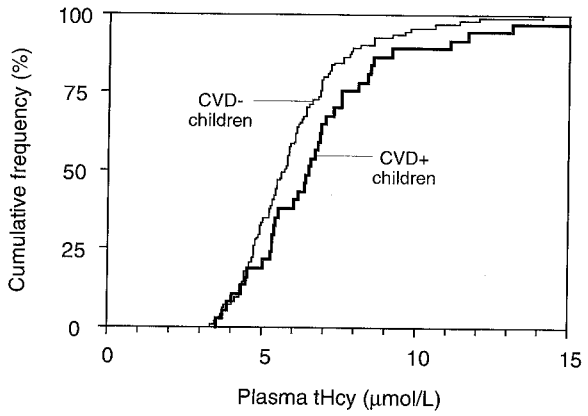


FIG 1. Cumulative frequency distribution of plasma tHcy levels in children with (CVD+) or without (CVD−) a parent with CVD

## Discussion

Our main finding is that tHcy level was higher in children with FH whose parent with FH had experienced CVD than in children without a parental history of CVD. The relation between increased plasma tHcy and parental CVD was independent of pubertal stage and parental educational level, both of which were associated with tHcy in multivariate analysis. tHcy level was inversely related to folate, vitamin C, and intake of fruits and vegetables. We also found a trend toward increased prevalence of homozygosity for the C677T mutation in the group with parental CVD.

Previous evidence suggests that tHcy is an independent risk factor for CVD in patients with hyperlipidemia. Glueck et al[30] showed that the prevalence of CVD was higher in hyperlipidemic patients with high compared with normal levels of tHcy. However, the identification of homocysteine as a causative factor in vascular lesions may be biased by the increase in tHcy that occurs secondary to the atherosclerotic process. Moreover, the diet and other risk factors may change after the onset of clinical disease.[10,15] These confounders are avoided by a study design that involves the offspring of parents with CVD. Thus, we recently showed that tHcy was elevated in a population-based sample of children who had a

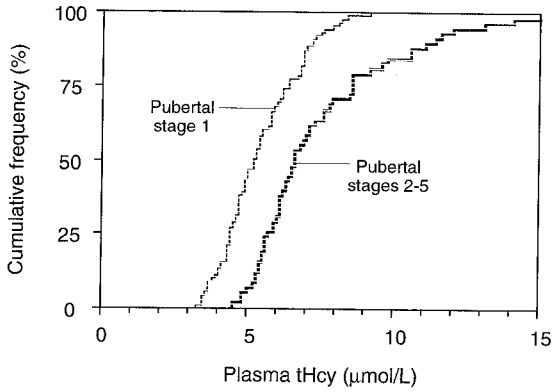


FIG 2. Cumulative frequency distribution of plasma tHcy levels according to pubertal stage

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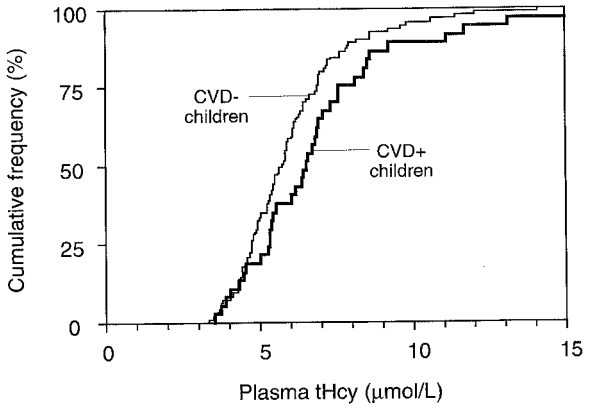


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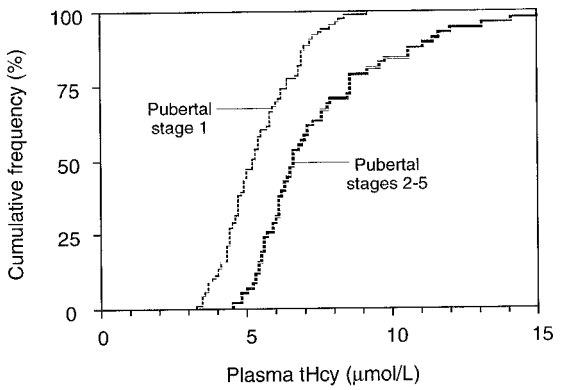


FIG 2. Cumulative frequency distribution of plasma tHcy levels according to pubertal stage

**TABLE 3. Determinants ($P<.05$) of Plasma tHcy in Stepwise Multiple Regression Analysis, Excluding Subjects Taking Resins**

| Dependent Variable | Independent Variables | Partial $R^2$ at Each Step |
|---|---|---|
| Plasma tHcy | Pubertal stage (1–5)* | .22 |
| | Parental educational level (1–3)† | .25 |
| | Parental CVD (0=no; 1=yes)* | .27 |

*Positive relation.
†Inverse relation.

male relative who died prematurely of CVD compared with children without familial CVD.[31] We used a similar design in the present study involving children with FH, who were nonsmokers and healthy, and whose tHcy levels were therefore unlikely to be biased by disease or risk factors. Consistent with these findings, we reported recently that plasma tHcy is related to increased carotid intima media thickness in children with FH.[32] Patients with FH probably have the same susceptibility to the established cardiovascular risk factors, including smoking, male sex, and hypertension, as the general population.[6,7] Moreover, some data suggest that LDL and homocysteine interact in the atherogenic process.[33] Thus, the present evidence that tHcy may also be a risk factor in FH is not surprising.

Our analyses cannot exclude the possibility that elevated tHcy is a marker of some other factor causally associated with increased prevalence of parental CVD. For example, low socioeconomic status is likely to be associated both with increased risk of CVD[34] and elevated tHcy.[31] Furthermore, parents with CVD are more likely to have smoked cigarettes, and their offspring may have higher tHcy levels because of passive smoking.[15] However, our study suggests that the contribution of the family history to the risk of premature CVD in FH[8,9] may be attributable in part to the effects of a shared environment and shared genes on tHcy level. Thus, we found a persistent inverse association between parental educational level and tHcy in the child, but we also observed a significant inverse association between educational level and intake of fruits and vegetables (data not shown). The contribution of shared genes to the predictive effect of the family history is suggested by our finding of a trend toward increased homozygosity for the C677T mutation of methylenetetrahydrofolate reductase in the group with parental CVD. This points to the C677T mutation as one candidate locus. Several[14,35-38] although not all[14,39] previous studies have suggested that this mutation is more prevalent in patients with premature vascular disease than in controls.

The normal values for tHcy in childhood and adolescence are lower than that of adults, but data are sparse.[40] In our prepubertal children without parental CVD, the median and 90th percentile levels were 5.2 and 7.0 μmol/L, respectively. These levels are similar to the median and 90th percentile levels we reported earlier among school children aged 8 to 12 years from middle to high socioeconomic classes.[31] In pubertal children, the median and 90th percentile levels were 6.5 and 10.6 μmol/L, respectively. These values may vary in groups whose dietary habits differ from our population.

The effect of puberty on tHcy level may be due to the influence of sex hormones, increased muscle mass, or both. The effect of muscle mass may be related to the large amount of homocysteine formed in conjunction with creatine-creatinine synthesis.[41] In line with this, a positive correlation between tHcy level and serum creatinine has been reported in adults[41] and children[31] and has been related to the sex difference in tHcy levels in adults.[41] We did not measure serum creatinine in the present study, but we found no difference in tHcy between the sexes, even though boys have larger muscle mass than girls. Further study involving a greater number of subjects in each pubertal stage is needed to establish the time point for the onset of the male-female differential in adults.

As expected, intake of micronutrients was associated with tHcy,[42] including vitamins C and folate. We also found that tHcy level was negatively correlated to intake of fruits and vegetables. The association between tHcy and intake of specific nutrients may be confounded by intercorrelations between nutrients and various food items. For example, fruits and vegetables are rich in both folate and vitamin C. However, although supplemental folate lowers tHcy,[43] high-dose vitamin C does not.[44] We did not correct for the diet or serum micronutrient levels in multivariate analysis because some micronutrients as folate are probably part of the causal pathway between elevated tHcy and CVD.[45]

The established relation between tHcy level and intake of vitamins and nutrients suggests that normal values of tHcy are influenced by dietary habits in our study population, who had been given expert nutritional counsel for their lipid disorder and had normal folate and vitamin $B_{12}$ levels. Furthermore, increasing the consumption of fruits and vegetables may decrease the risk of CVD by reducing tHcy but may also lead to increased intake of several putative cardioprotective factors other than those affecting tHcy metabolism.[46] Thus, recommending increased fruits and vegetables for children with FH is certainly reasonable. Recommenda-

**TABLE 4. Frequency of Methylenetetrahydrofolate Reductase Genotype and Plasma tHcy According to Study Group**

| | CVD+ | | CVD− | |
|---|---|---|---|---|
| Genotype | Genotype Frequency | tHcy (μmol/L) Median (Interquartile Range) | Genotype Frequency | tHcy (μmol/L) Median (Interquartile Range) |
| Ala/Ala | 17/39 (44%) | 6.3 (4.5, 7.0) | 64/116 (55%) | 5.5 (4.7, 6.5) |
| Val/Ala | 15/39 (38%) | 6.5 (5.3, 7.8) | 43/116 (37%) | 5.8 (4.7, 6.8) |
| Val/Val | 7/39 (18%)* | 7.5 (6.8, 10.8) | 9/116 (8%) | 6.9 (5.6, 7.7) |

*$P=.07$ compared with CVD−.

tion of folate supplementation should await the results of intervention studies in adults.

## Conclusions

Our data indicate that tHcy level may partly explain the independent risk associated with a family history of premature CVD in FH. tHcy level is inversely associated with intake of fruit, vegetables, and certain micronutrients, in particular, folate. The implications of these findings are twofold: We suggest that tHcy level should be determined in children with FH, as already suggested for adults with hyperlipidemia[30] or vascular disease.[47] Second, dietary recommendations should not only advise fat restriction but also consider nutrients associated with homocysteine metabolism.

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