

ARTICLE

Homocysteine levels – before and after methionine loading – in 51 Dutch families

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Elevated levels of homocysteine are a risk factor for vascular disease, thrombosis, neural tube defects and dementia. The 677C>T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene appears to be the most important single determinant of plasma homocysteine concentration. In the current study, we estimated heritability and fit a series of models of inheritance for both fasting and postmethionine-load homocysteine levels in the HOFAM-study (HOMocysteine in FAMILIES study), which included 306 participants from 51 pedigrees, ascertained through a hyperhomocysteinemic proband. The crude heritability was 21.6% for fasting and 67.5% for postloading homocysteine. After adjustment for MTHFR 677C>T genotype, heritability dropped to 5.2 and 63.9%, respectively. Segregation analysis revealed that a nongenetic model with equal transmission was the best fitting and most parsimonious model for fasting homocysteine levels, while a two-distribution, Mendelian model with residual familial correlation was best for postmethionine-load homocysteine levels. This study shows that postload homocysteine levels have a stronger genetic determination than do fasting homocysteine levels. The heritability of postload homocysteine levels were not strongly affected by adjustment for MTHFR 677C>T genotype, in contrast to fasting homocysteine levels. Further studies are needed to identify the genes responsible for the inheritance of postload homocysteine levels.

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Introduction

Homocysteine is an amino acid formed by demethylation of methionine. It can either be remethylated to methio-

nine in a folate- and vitamin B12-dependent pathway or trans-sulfurated to cysteine in a vitamin B6-dependent pathway. Elevated homocysteine levels (ie hyperhomocysteinemia) are a risk factor for vascular disease,^{1,2} venous thrombosis,^{2–4} neural tube defects⁵ and dementia.⁶

Plasma homocysteine levels are influenced by both genetic and lifestyle factors, including dietary intake of B-vitamins and renal function.⁷ In a recent study, Kluijtmans *et al*⁸ reported that the MTHFR 677TT genotype accounts for 9% of the variance of homocysteine in a population of

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young adults. Polymorphisms in genes of other homocysteine-related enzymes did not account for any substantial degree of variance. Although several studies demonstrated the heritability of hyperhomocysteinemia – both before and after methionine loading,^{9–13} only two studies investigated the question on how much of the variance in fasting homocysteine could be explained by genetic factors.^{14,15} The aim of this study was to assess the heritability of homocysteine (before and after methionine loading) and to assess for which part this heritability could be explained by the MTHFR 677TT genotype. Moreover, we investigated the most likely model of inheritance with segregation analysis.

Methods

Study population

The HOFAM-study (HOMocysteine in FAMilies study) is a family study of patients with vascular disease and hyperhomocysteinemia. The HOFAM study consisted of 51 pedigrees from which 306 persons participated. Probandes were ascertained through a register of hyperhomocysteinemic patients in the TweeSteden Hospital Tilburg. Data were collected on medical history, nutritional and lifestyle factors. In addition, plasma levels of homocysteine (before and after methionine loading), creatinine and folate were measured. The study was approved by the ethics committee of the TweeSteden Hospital. The mean number of family members was 6.0; 22 families had four or less individuals, 20 had 5–10 individuals, nine families had more than 10 individuals. In total, 19 pedigrees (37.3%) spanned two generations, 30 pedigrees (58.8%) spanned three generations, and two pedigrees (3.9%) spanned four generations.

Blood sampling

After overnight fasting, blood samples were collected before and 6 h after an oral methionine load (0.1 g L-methionine per kilogram bodyweight in 200 ml orange juice).¹⁶ Blood samples were obtained from the antecubital vein in 4.5 ml EDTA vacuum glass tubes and tubes for preparation of serum. These were used to determine homocysteine (EDTA), folate and vitamin B₁₂ concentrations (serum), vitamin B₆ (pyridoxal 5'-phosphate – PLP) (whole blood) and for detecting the MTHFR C677T mutation. The EDTA-samples for total homocysteine measurement were immediately placed on ice and centrifuged at 3500 g for 5 min within 2 h. The plasma was separated and stored at –20°C until analysis.

Total homocysteine concentrations were measured according to the method described by Araki and Sako.¹⁷ Folate and cobalamin concentrations were measured with the competitive protein binding assay on the Technicon Immuno 1 System (Technicon Instruments, Tarrytown, NY, USA). Determination of PLP was performed by HPLC

according to Ubbink *et al.*¹⁸ DNA was isolated from the buffy coat and mutation analysis was carried out by means of polymerase chain reaction (PCR) as described elsewhere.^{19,20} The primers generate a 198-bp fragment. The MTHFR C→T substitution at bp 677, creates a *HinfI* recognition sequence. If the mutation is present, *HinfI* digest the 198-bp fragment into a 175-bp and a 23-bp fragment. The fragments were analyzed by polyacrylamide gel electrophoresis.

Statistical analysis

Descriptive analysis and adjustment of homocysteine level General characteristics of this study population were analyzed using SAS. Both the pre- and the post-loading homocysteine levels were shown significantly positively skewed (coefficient of skewness = 4.5 and 1.8, respectively). After log transformation, both distributions were more closely approximated a normal distribution (coefficient of skewness = 0.73 and 0.55, respectively). Therefore, log-transformed homocysteine levels were used in all subsequent analysis.

Correlation analysis and multiple linear regression models were used to assess the relationship between log-transformed homocysteine level and age, sex, vitamin B intake, body mass index, smoking, alcohol drinking, participation in sports, serum level of creatinine, folate, vitamins B₆ and B₁₂. These covariates, identified as significant in the multiple regression analysis, were used to adjust homocysteine levels as possible confounders before analysis for familial correlation, heritability and segregation. Standardized residuals for log homocysteine level from the best fitting regression model were scaled by adding the original means to the residual values for pre-loading homocysteine (mean 2.5) and for post-loading homocysteine (mean 3.6).

Familial correlation and heritability Heritability and familial correlation were estimated by ASSOC and FCOR program, respectively, from the Statistical Analysis for Genetic Epidemiology Package (SAGE 4.5 2003). Both of unadjusted and adjusted log homocysteine levels were used in this analysis. MTHFR genotypes for the C677T variant were included as a covariate for ASSOC program and residual heritability was estimated.

Segregation analysis To investigate the role of possible genetic factors in determining the distribution of adjusted log-homocysteine levels, segregation analysis was conducted using the class D regressive model as implemented in the SEGREG program of the S.A.G.E (v4.5) package. We fit a series of genetic and nongenetic models of inheritance to the 51 families available here. Each model was compared to the most general model to compute likelihood ratio tests (LRT) and Akaike's information criteria scores were

computed to find the most parsimonious model of inheritability for these data.

Results

Study population

General characteristics of the study population are shown in Table 1. The mean age was 41.1 years for male and 42.5 years for female patients. The geometric means were 12.2 $\mu\text{mol/l}$ in both male and female patients for fasting homocysteine and 36.6 $\mu\text{mol/l}$ in male and 40.4 $\mu\text{mol/l}$ in female patients for postloading homocysteine. Creatinine concentrations were higher in male than in female patients. Vitamin B intake rates were 30.3% in male and 38.8% in female patients. Current smoking prevalence was higher in female (33.1%) than male (28.5%) patients, although more males were ex-smokers compared to female patients (33% *versus* 29%). Totally, 86% of males currently drank alcohol compared to 72% of female patients. There were no significant differences in distribution for other covariates examined between male and female except the history of alcohol drinking and participation in sports. When compared with the distribution of the family members, probands had significantly higher mean age, serum level of folate and vitamin B supplements intake for both of male and female.

Multiple regression models

In simple correlation analysis, the level of log prehomocysteine level was significantly correlated with vitamin B intake ($r = -0.15$), creatinine ($r = 0.14$), serum vitamin B₁₂ level ($r = -0.28$) and serum folate level ($r = -0.29$), all of which also had significant correlations among themselves. Age, serum creatinine, folate and serum vitamin B₁₂ level were significantly associated with the log-homocysteine level for both pre- and postloading homocysteine levels when multiple regression models were examined. Therefore, we considered age, sex, vitamin B intake history, serum levels of creatinine, folate and vitamin B₁₂ and other covariates including smoking, alcohol drinking as potential confounders and used them to adjust the pre- and postloading homocysteine levels before estimation familial correlations and before carrying out the segregation analysis. No adjustment of homocysteine was done in Model 1. Age, age² and age*sex were included in Model 2, and vitamin B intake history was added to adjust the homocysteine level in Model 3, and serum levels of smoking, alcohol drinking and others in Model 4. In Model 2, Age, age² and age*sex explained only 2% (R^2) of preloading log homocysteine level and 6% of post-loading level in regression analysis. When vitamin B intake was added as a covariate in regression (for Model 3), R^2 for pre- and postloading homocysteine level increased 5 and 7%, respectively. The R^2 for homocysteine level increased to

25% (preloading) and 16% (postloading) when all the confounders were included in regression model (Model 4).

Familial correlations and heritability

Even though correlations for preloading log-homocysteine were generally smaller (Table 2), parent-offspring and sib correlations were slightly greater than zero ($\rho_{\text{po}} = 0.15 \pm 0.08$, $\rho_{\text{sib}} = 0.12 \pm 0.08$ in Model 3) and spouse correlation was not ($\rho_{\text{spouse}} = 0.14 \pm 0.22$). Familial correlations including parent-offspring and sib correlations for post-loading log-homocysteine levels were all significantly greater than zero ($\rho_{\text{po}} = 0.30 \pm 0.09$, $\rho_{\text{sib}} = 0.31 \pm 0.10$ in Model 3) compared with spouse correlation ($\rho_{\text{spouse}} = -0.13 \pm 0.22$). These familial correlations suggest stronger genetic control for in postloading homocysteine compared to preloading homocysteine.

Association with MTHFR genotype (MTHFR C677T)

Preloading homocysteine The baseline heritability for log-homocysteine level was 21.6% in Model 1, but this residual h^2 dropped to only 5.2% once MTHFR genotype (C677T) was considered in the regression model as a covariate. The three genotypes at C677T had a small (but statistically significant) fixed effect under codominant model (LRT = 11.02, $P = 0.0009$). Fitting this same regression model with age and sex as covariates showed a similar modest but significant effect of genotype on fasting homocysteine (LRT = 9.53, $P = 0.0020$ for Model 2 with age and sex alone; LRT = 10.11, $P = 0.0015$ for Model 3 with age, sex, vitamin B intake; LRT = 11.6, $P = 0.0007$ for Model 4 with creatinine, vitamins B₆ and B₁₂ and folate, smoking, alcohol as additional covariates).

Table 3 also shows the mean of log-homocysteine levels for each C677T genotype. Log-homocysteine significantly increased with each T allele at C677T, except after adjustment for age, sex, vitamin intake, smoking, drinking, creatinine, folate, vitamin B₆, vitamin B₁₂ (Model 4). There were no significant differences among the means of log-homocysteine level according to genotype at the A1298C (data not shown).

Postloading homocysteine MTHFR genotype for C677T without any covariates had a significant effect on the postloading homocysteine levels (background: $h^2 = 67.5\%$, LRT = 4.27, $P = 0.0338$ for Model 1) (Table 2). With age and sex as covariates (Model 2), a fixed effect of MTHFR genotype approached statistical significance (LRT = 5.43, $P = 0.0198$); adding vitamin intake as covariate (LRT = 6.47, $P = 0.0110$, Model 3); or the full array of covariates (age, sex, creatinine, etc., Model 4) (LRT = 5.57, $P = 0.0110$) showed similar effects of genotype.

The estimated mean log-homocysteine increased with each T allele except when log-homocysteine level was adjusted for nine covariates (Table 3). Once again no

Table 1 General characteristics of the HOFAM study: 306 persons of 51 families

Variables	Male			P-value	Female			P-value
	Proband (n = 28) Mean (SD)	Family members (n = 117) Mean (SD)	Total (n = 145) Mean (SD)		Proband (n = 23) Mean (SD)	Family members (n = 138) Mean (SD)	Total (n = 161) Mean (SD)	
Age (years)	49.9 (12.5)	39.0 (15.2)	41.1 (15.3)	0.0006	49.2 (14.1)	41.3 (17.2)	42.5 (17.0)	0.0382
<i>Homocysteine (μmol/l)</i>								
Preloading	15.9 (15.9)	13.2 (5.1)	13.7 (8.3)	0.3909	11.1 (3.8)	12.8 (5.6)	12.6 (5.4)	0.0772
Postloading	41.9 (21.7)	38.4 (13.4)	39.0 (15.3)	0.4272	37.8 (12.0)	42.2 (18.1)	41.6 (17.4)	0.1420
<i>Log-homocysteine</i>								
Preloading	2.5 (0.6)	2.5 (0.3)	2.5 (0.4)	0.9712	2.4 (0.3)	2.5 (0.4)	2.5 (0.4)	0.1298
Postloading	3.6 (0.4)	3.6 (0.3)	3.6 (0.3)	0.4791	3.6 (0.3)	3.7 (0.4)	3.7 (0.4)	0.2890
Height (cm)	176.1 (6.0)	178.1 (7.4)	177.7 (7.2)	0.1915	165.5 (6.4)	164.8 (6.5)	164.9 (6.5)	0.6293
Weight (kg)	79.6 (13.8)	82.1 (12.7)	80.1 (13.6)	0.3691	72.6 (15.3)	70.9 (13.5)	71.1 (13.7)	0.5766
BMI (kg/m ²)	26.4 (3.7)	25.0 (3.7)	25.2 (3.7)	0.0660	26.5 (5.5)	26.0 (4.7)	26.1 (4.8)	0.6317
SBP (mmHg)	136.8 (20.2)	128.4 (15.6)	130.0 (16.8)	0.0173	133.9 (30.3)	128.1 (19.4)	128.9 (21.3)	0.3796
DBP (mmHg)	76.0 (79.9)	71.1 (12.3)	72.0 (12.0)	0.0521	70.1 (12.7)	69.7 (11.1)	69.7 (11.3)	0.8732
Cholesterol (mmol/l)	5.7 (1.4)	5.2 (1.1)	5.5 (1.2)	0.3766	5.6 (0.9)	5.5 (1.1)	5.5 (1.1)	0.6202
HDL-cholesterol (mmol/l)	1.3 (0.3)	1.4 (0.3)	1.3 (0.3)	0.0708	1.6 (0.4)	1.6 (0.3)	1.6 (0.4)	0.4801
Triglyceride (mmol/l)	2.2 (2.4)	3.2 (17.0)	3.0 (15.3)	0.5683	1.7 (2.0)	1.3 (0.7)	1.4 (1.0)	0.3422
Creatinine (μmol/l)	92.5 (26.8)	86.9 (15.1)	88.0 (18.0)	0.1400	73.5 (113.4)	73.6 (13.1)	73.6 (13.1)	0.9793
Vitamin B ₁₂ (pmol/l)	293.8 (274.7)	277.7 (92.9)	280.8 (145.4)	0.7629	281.9 (241.7)	256.2 (106.2)	260.0 (133.5)	0.6204
Folate (nmol/l)	33.0 (15.6)	15.4 (10.3)	18.8 (13.4)	<0.0001	40.9 (9.4)	12.0 (6.5)	16.1 (12.3)	<0.0001
Pyridoxal 5-phosphate (nmol/l)	150.1 (220.0)	101.6 (103.3)	111.0 (134.5)	0.2645	180.8 (125.5)	75.8 (31.9)	91.0 (103.1)	0.0527
	N (%)	N (%)	N (%)	P-value	N (%)	N (%)	N (%)	P-value
B-Vitamin supplements (yes)	18 (64.3)	26 (22.2)	44 (30.3)	<0.0001	11 (95.7)	24 (33.6)	46 (28.8)	<0.0001
<i>Smoking</i>								
Current smoker	6 (21.4)	35 (30.2)	41 (28.5)	0.0107	7 (30.4)	46 (33.6)	53 (33.1)	0.0569
Ex-smoker	16 (57.2)	32 (27.6)	48 (33.3)		9 (39.1)	38 (27.7)	47 (29.4)	
Never smoker	6 (21.4)	49 (42.2)	55 (38.2)		7 (30.4)	53 (38.7)	60 (37.5)	
<i>Alcohol drinking</i>								
Current drinker	23 (86.2)	101 (86.3)	124 (85.5)	0.0283	14 (60.8)	101 (73.7)	115 (71.8)	0.3367
Ex-drinker	3 (10.7)	1 (0.9)	4 (2.8)		1 (4.4)	1 (0.73)	2 (1.3)	
Never drinker	2 (7.1)	15 (12.8)	17 (11.7)		8 (34.8)	35 (25.6)	43 (26.9)	
Sports (yes)	12 (42.8)	60 (51.3)	72 (49.7)	0.4232	4 (17.4)	56 (40.9)	60 (37.5)	0.0313

Table 2 Familial correlation of homocysteine (pre- and postloading) with 51 families in HOFAM study

Relative pairs	No.	Model 1 ^a	Model 2 ^b	Model 3 ^c	Model 4 ^d
<i>Preloading log-homocysteine level</i>					
Spouse	22	0.206 (0.213)	0.127 (0.219)	0.138 (0.219)	0.112 (0.220)
Parents–offspring	200	0.080 (0.084)	0.126 (0.085)	0.151 (0.083)	0.056 (0.081)
Siblings	326	0.103 (0.077)	0.118 (0.080)	0.121 (0.081)	0.077 (0.071)
Heritability (%)		21.6 (14.3)	27.5 (14.8)	33.5 (15.5)	17.2 (15.6)
Heritability (%) ^e		5.2 (14.0)	11.9 (14.9)	18.8 (13.5)	1.0 (16.7)
<i>Postloading log-homocysteine level</i>					
Spouse	22	−0.068 (0.217)	−0.151 (0.213)	−0.127 (0.215)	−0.127 (0.218)
Parents–offspring	200	0.297 (0.097)	0.272 (0.091)	0.298 (0.091)	0.273 (0.090)
Siblings	326	0.374 (0.106)	0.300 (0.102)	0.309 (0.102)	0.265 (0.098)
Heritability		67.5 (14.6)	58.9 (12.6)	63.3 (12.6)	58.0 (12.8)
Heritability (%) ^e		63.9 (14.7)	54.2 (12.8)	58.4 (12.8)	54.1 (12.8)

^aModel 1: unadjusted.

^bModel 2: adjusted by age, age², sex, age*sex ($R^2 = 0.023$ (preloading), 0.057 (postloading)).

^cModel 3: adjusted by age, age², sex, age*sex, and B-vitamins supplements ($R^2 = 0.048$ (preloading), 0.073 (postloading)).

^dModel 4: adjusted by age, age², sex, age*sex, B-vitamins supplements, smoking, drinking, creatinin, folate, vitamins B₆ and B₁₂ ($R^2 = 0.252$ (preloading), 0.158 (postloading)).

^eAfter including MTHFR genotype as a covariate in the model.

Table 3 Homocysteine levels according to the MTHFR genotypes with 51 families in HOFAM study

Genotype	No.	Log-homocysteine (SD)	Geometric mean (μmol/l) (95% CI)	Adjusted log-homocysteine ^a (SD)	Geometric mean (μmol/l) (95% CI)
<i>Preloading homocysteine level by C677T</i>					
CC	133	2.41 (0.29)	11.1 (10.6–11.7)	2.41 (0.28)	11.1 (10.6–11.7)
CT	124	2.51 (0.34)	12.3 (11.6–13.1)	2.52 (0.33)	12.4 (11.7–13.2)
TT	44	2.67 (0.53)	14.4 (12.3–16.9)	2.69 (0.52)	14.7 (12.6–17.3)
P-value		0.0002		< 0.0001	
<i>Postloading homocysteine level by C677T</i>					
CC	133	3.56 (0.32)	35.2 (33.4–37.0)	3.52 (0.31)	33.8 (32.1–35.5)
CT	124	3.67 (0.37)	39.3 (36.6–42.1)	3.64 (0.35)	38.1 (35.9–40.4)
TT	44	3.73 (0.36)	41.7 (37.3–46.5)	3.71 (0.36)	40.9 (36.6–45.6)
P-value		0.007		0.0014	

Note: Five missing cases. ^aThe adjusted level by age, age², sex, age*sex, and B-vitamin supplements (Model 3).

significant differences among genotypic means for the A1298C variant were observed (data not shown).

Segregation analysis of homocysteine levels

Log-homocysteine levels adjusted for age, sex and vitamin B intake (Model 3) was used to fit a series of models using SEGREG program in the S.A.G.E. package. Familial correlations from Models 1, 2 and 3 showed consistent coefficients, especially in preloading homocysteine level.

The models fit to these data included: (1) four single distribution models with or without familial correlation but assuming no major gene effect (Models A–D); (2) six two distributions for Mendelian single gene effect with and without residual familial correlations (Models E–J); (3) three different distribution models with familial correlation, one of which allowed for Mendelian transmission

(Model K); an equal transmission model with no transmission of the underlying factor from parents to offspring (Model L); a modified Mendelian model with fixed transmission from two possible heterozygote AA and BB but estimated transmission from the heterozygote (Model M); (4) the general model, where all parameters were estimated from the data. Each model was compared to the general model using the likelihood ratio test to test specific hypotheses in a hierarchical manner.

Preloading homocysteine (Table 4) Single distribution models confirmed the familial correlations, seen in Table 2, but all single distributions regardless of familial correlation were strongly rejected compared to the general models (Model A–D), suggesting multiple underlying distributions exist in these data.

Table 4 Segregation analysis of adjusted homocysteine in 306 persons of 51 families (preloading)^a

Model	q_A	μ_{AA}	μ_{AB}	μ_{BB}	σ^2	τ_{AA}	τ_{AB}	τ_{BB}	ρ_{MF}	ρ_{PO}	ρ_{SS}	DF	$-2 \ln(L)$	AIC	$\chi^2 (P)$
<i>Single distribution</i>															
A. Single dis., no familial correlation	[100%]	2.50 ± 0.02	$= \mu_{AA}$	$= \mu_{AA}$	0.13 ± 0.01				[0]	[0]	[0]	9	234.73	238.73	78.31 (< 0.0001)
B. Single dis., $\rho_{PO} = \rho_{SS}$	[100%]	2.49 ± 0.02	$= \mu_{AA}$	$= \mu_{AA}$	0.13 ± 0.01				[0]	0.14 ± 0.05	$= \rho_{PO}$	8	226.73	232.73	70.31 (< 0.0001)
C. Single dis., father-child and mother-child correlation	[100%]	2.50 ± 0.03	$= \mu_{AA}$	$= \mu_{AA}$	0.13 ± 0.11				0.60 ± 0.13	$\rho_{FO} = 0.18 \pm 0.11$	0.17 ± 0.08	5	221.32	233.32	64.9 (< 0.0001)
D. Single dis., arbitrary familial correlation.	[100%]	2.50 ± 0.13	$= \mu_{AA}$	$= \mu_{AA}$	0.13 ± 0.01				0.58 ± 0.13	$\rho_{MO} = 0.11 \pm 0.09$ 0.13 ± 0.09	0.17 ± 0.08	6	221.72	231.72	65.3 (< 0.0001)
<i>Two distribution</i>															
E. Dominant, no familial correlation.	79.36 ± 4.37	2.46 ± 0.02	$= \mu_{AA}$	3.53 ± 0.12	0.08 ± 0.01	[1.0]	[0.5]	[0.0]	[0]	[0]	[0]	7	181.25	189.25	24.83 (0.0008)
F. Dominant, familial correlation	77.81 ± 4.37	2.45 ± 0.02	$= \mu_{AA}$	3.48 ± 0.10	0.08 ± 0.01	[1.0]	[0.5]	[0.0]	0.27 ± 0.23	0.04 ± 0.09	0.22 ± 0.09	4	173.47	187.47	17.05 (0.0019)
G. Dominant, no familial correlation, no transmission	79.57 ± 3.59	2.45 ± 0.02	$= \mu_{AA}$	3.55 ± 0.12	0.08 ± 0.01	q_A	q_A	q_A	[0]	[0]	[0]	7	180.21	188.21	23.79 (0.0012)
H. Recessive, no familial correlation	20.64 ± 4.37	3.53 ± 0.12	$= \mu_{BB}$	2.46 ± 0.02	0.08 ± 0.01	[1.0]	[0.5]	[0.0]	[0]	[0]	[0]	7	181.25	189.25	24.83 (0.0008)
I. Recessive, familial correlation	22.19 ± 4.37	3.48 ± 0.10	$= \mu_{BB}$	2.45 ± 0.02	0.08 ± 0.01	[1.0]	[0.5]	[0.0]	0.27 ± 0.23	0.04 ± 0.09	0.23 ± 0.09	4	173.47	187.47	17.05 (0.0019)
J. Recessive, no familial correlation, no transmissions	97.89 ± 0.75	2.45 ± 0.02	$= \mu_{BB}$	3.55 ± 0.12	0.08 ± 0.01	q_A	q_A	q_A	[0]	[0]	[0]	7	180.21	188.21	23.79 (0.0012)
<i>Three distribution</i>															
K. Codominant	80.11 ± 4.72	2.39 ± 0.04	2.59 ± 0.10	3.57 ± 0.13	0.07 ± 0.01	[1.0]	[0.5]	[0.0]	0.35 ± 0.24	-0.03 ± 0.11	0.17 ± 0.10	3	171.08	187.08	14.66 (0.0021)
L. Equal transmission	6.47 ± 2.06	4.08 ± 0.15	3.03 ± 0.10	2.41 ± 0.03	0.07 ± 0.01	q_A	q_A	q_A	0.34 ± 0.27	0.11 ± 0.11	0.36 ± 0.09	3	161.51	177.51	5.09 (0.1653)
M. τ_{AA} , τ_{BB} fixed and τ_{AB} free	78.72 ± 5.66	2.38 ± 0.04	2.59 ± 0.10	3.57 ± 0.13	0.07 ± 0.01	[1.0]	0.55 ± 0.09	[0.0]	0.37 ± 0.25	-0.03 ± 0.12	0.18 ± 0.11	2	170.8	188.8	14.38 (0.0008)
<i>General model</i>	98.46 ± 3.28	2.41 ± 0.03	3.04 ± 0.09	4.11 ± 0.15	0.07 ± 0.01	0.94 ± 0.02	0.62 ± 0.22	0.00 ± ^b	0.18 ± 0.25	0.02 ± 0.10	0.37 ± 0.10		156.42	176.42	

Bold values indicate the models with lowest AIC, that is, the most parsimonious model.

^aAdjusted by age, age², sex, sex*age, B-vitamin supplements.

^bMeans the SE is fixed by maxfun at bound.

Two distribution models including both dominant (Model E–G) and recessive model (Model H–J) were also strongly rejected, yielding no evidence of Mendelian control of in preloading homocysteine level. The codominant model (K) with residual familial correlations was also strongly rejected, but the model of equal transmission could not be rejected (LRT = 5.09 with 3 df, $P = 0.1653$). Of the models examined here, the best fitting and most parsimonious model for preloading homocysteine levels was a nongenetic model with equal transmission.

Postloading (Table 5)

There was again significant evidence for multiple distributions underlying the observed distribution of log-homocysteine among these 429 individuals in that all single distribution models were rejected. Single distribution models confirmed the strong familial correlations seen in Table 2.

Among the two distribution models, there were still substantial residual familial correlations, suggesting any major gene cannot account for all of the observed familial correlations in postloading homocysteine. The recessive model gave exactly the same log-likelihood value as did the dominant model, and these two models were effectively mirror image of on another ($\mu_{AA} = 4.16$ versus 3.51 for genotypes AB and BB in the recessive model, $\mu_{BB} = 4.16$ versus 3.51 for genotypes AB and AA in the dominant model). These two distribution models gave no evidence of non-Mendelian transmission and fit these 51 families much better than did the equal transmission model with similar 'high' and 'low' distributions.

When three distribution models were fit, a general codominant Mendelian model did not give ordered effects ($\mu_{AA} = 3.53$, $\mu_{AB} = 4.17$ and $\mu_{BB} = 3.23$) for the hypothesized genotypes at the major locus, and the substantial residual correlations changed very little when three distributions were considered compared to the two distribution models. These three genotypic means were 'medium', 'high' and 'low' for the genotypes under a single locus model, and while this is not impossible from a biological perspective, it is difficult to interpret and further calls into question the validity of the underlying model. However, even though recessive, dominant, codominant Mendelian model and more general transmission models (eg the free τ_{AB} model) had very similar $-2\ln L$ values, the AIC value of the two distribution models (either dominant or recessive) were smaller than any of three distribution models. Therefore, a two-distribution model with residual familial correlation can be considered the most parsimonious model for postloading log-homocysteine levels in these 51 families.

Discussion

Results of this study suggests fasting homocysteine level is not under direct genetic control even though MTHFR

genotypes at C667T variant did influence the levels of fasting homocysteine. On the other hand, postload homocysteine levels appear to have a high heritability and showed evidence for a Mendelian major locus controlling this complex quantitative phenotypes.

Heritability

Several studies have demonstrated a substantial heritability for hyperhomocysteinemia. In twin studies, the correlation coefficient between plasma homocysteine levels has been reported as between 0.53 and 0.56 for monozygotic twins compared with 0.19 for dizygotic twins.^{9,10} Three studies on families with a hyperhomocysteinemic proband suggested that plasma homocysteine levels are under some degree of genetic control.^{11–13} However, these studies did not quantify the magnitude of this control.

Recently, two studies were published to assess the heritability of fasting homocysteine in family studies. Souto *et al*¹⁴ measured levels of several thrombophilic risk factors, including plasma homocysteine in 398 individuals from 21 pedigrees (12 pedigrees were selected through a proband with idiopathic thrombosis). They found, using a variance component model, a strong genetic correlation for homocysteine levels ($r = 0.652$, $P = 0.0015$) and a much lower environmental correlation ($r = 0.28$, $P = \text{NS}$). These families were selected for the presence of thrombophilia, which might have increased the power to detect genetic component that controls homocysteine levels, but does create a potential ascertainment bias. It remains a challenge to incorporate appropriate corrections for such ascertainment bias in family studies. A second study was published by Jee *et al*¹⁵ using 112 families of probands who underwent coronary arteriography. They found a heritability of 47%, similar to the heritability estimate for adjusted homocysteine (considering age, sex and vitamin B intake) in the present study ($h^2 = 33.5 \pm 13.5$).

This is the first study to estimate the heritability for postload homocysteine levels. It is very interesting that postload homocysteine levels showed a much greater heritability (67.5% (SE 14.6)) than did fasting homocysteine (33.5% (SE 15.5)) in this 51 families. The methionine loading test was originally developed to detect heterozygosity for cystathionine-synthase deficiency. With increasing sensitivity of the homocysteine measurements and with the use of prospective studies presenting only data on fasting homocysteine, methionine loading has become less popular. Although some polymorphisms are reported to influence only postload homocysteine levels,²¹ little attention has been given to the genetics of postload homocysteine levels in individuals or families.

It is important to realize that heritability is a ratio and is determined by a numerator (genetically explained variance) and a denominator (total variance). Although Ubbink *et al*²² showed that the variability coefficients for the 6 h postload homocysteine are somewhat lower than

Table 5 Segregation analysis of adjusted homocysteine in 306 persons of 51 families (postloading)^a

Model	q_A	μ_{AA}	μ_{AB}	μ_{BB}	σ^2	τ_{AA}	τ_{AB}	τ_{BB}	ρ_{MF}	ρ_{PO}	ρ_{SS}	DF	$-2 \ln(L)$	AIC	χ^2 (P)
<i>Single distribution</i>															
A. Single dis., no familial correlation.	[100%]	3.60 ± 0.02	= μ_{AA}	= μ_{AA}	0.12 ± 0.01				[0]	[0]	[0]	9	206.77	210.77	69.16 (<0.0001)
B. Single dis., ρ	[100%]	3.59 ± 0.03	= μ_{AA}	= μ_{AA}	0.12 ± 0.01				[0]	0.29 ± 0.05	= ρ_{PO}	8	172.40	178.40	34.79 (<0.0001)
^{PO = ρ_{SS}} C. Single dis., father-child and mother-child correlation	[100%]	3.60 ± 0.03	= μ_{AA}	= μ_{AA}	0.12 ± 0.01				0.36 ± 0.19	ρ_{FO} = 0.26 ± 0.13	0.29 ± 0.07	5	169.01	181.01	31.4 (<0.0001)
D. Single dis., arbitrary familial correlation	[100%]	3.60 ± 0.03	= μ_{AA}	= μ_{AA}	0.12 ± 0.01				0.38 ± 0.18	ρ_{MO} = 0.34 ± 0.08 0.32 ± 0.08	0.29 ± 0.07	6	169.40	179.40	31.79 (<0.0001)
<i>Two distribution</i>															
E. Dominant, no familial correlation.	6.38 ± 1.99	4.16 ± 0.06	= μ_{AA}	3.51 ± 0.03	0.07 ± 0.01	[1.0]	[0.5]	[0.0]	[0]	[0]	[0]	7	152.88	160.88	15.27 (<0.0327)
F. Dominant, familial correlation	63.62 ± 5.12	3.51 ± 0.03	= μ_{AA}	4.16 ± 0.06	0.06 ± 0.01	[1.0]	[0.5]	[0.0]	0.21 ± 0.21	0.35 ± 0.10	0.19 ± 0.09	4	142.57	156.57	4.96 (0.2914)
G. Dominant, no familial correlation, no transmission	6.98 ± 1.72	4.19 ± 0.06	= μ_{AA}	3.51 ± 0.02	0.06 ± 0.01	q_A	q_A	q_A	[0]	[0]	[0]	7	176.5	184.5	38.89 (<0.0001)
H. Recessive, no familial correlation.	93.62 ± 1.99	3.51 ± 0.02	= μ_{BB}	4.16 ± 0.06	0.07 ± 0.01	[1.0]	[0.5]	[0.0]	[0]	[0]	[0]	7	152.88	160.88	15.27 (<0.0327)
I. Recessive, familial correlation	36.38 ± 5.12	4.16 ± 0.06	= μ_{BB}	3.51 ± 0.03	0.06 ± 0.01	[1.0]	[0.5]	[0.0]	0.21 ± 0.21	0.35 ± 0.10	0.19 ± 0.09	4	142.57	156.57	4.96 (0.2914)
J. Recessive, no familial correlation, no transmissions	36.71 ± 4.40	4.19 ± 0.06	= μ_{BB}	3.51 ± 0.02	0.06 ± 0.01	q_A	q_A	q_A	[0]	[0]	[0]	7	176.5	184.5	38.89 (<0.0001)
<i>Three distribution</i>															
K. Codominant	93.72 ± 2.13	3.53 ± 0.03	4.17 ± 0.07	3.23 ± 0.20	0.07 ± 0.01	[1.0]	[0.5]	[0.0]	0.23 ± 0.22	0.33 ± 0.13	0.16 ± 0.08	3	142.48	158.48	4.87 (0.1816)
L. Equal transmission	68.89 ± 4.36	3.41 ± 0.04	3.71 ± 0.05	4.18 ± 0.06	0.07 ± inf ^b	q_A	q_A	q_A	0.55 ± 0.03	0.63 ± inf	0.58 ± 0.01	3	148.78	164.78	11.17 (0.0108)
M. τ_{AA} τ_{BB} fixed and τ_{AB} free	68.47 ± 6.00	3.44 ± 0.05	3.57 ± 0.05	4.19 ± 0.06	0.06 ± 0.01	[1.0]	0.40 ± 0.09	[0.0]	0.20 ± 0.26	0.27 ± 0.13	0.14 ± 0.10	2	141.36	159.36	3.75 (0.1534)
<i>General model</i>	78.61 ± 7.03	3.43 ± 0.05	3.55 ± 0.04	4.13 ± 0.15	0.06 ± 0.01	0.33 ± 0.10	0.96 ± 0.08	0.04 ± 0.03	0.14 ± 0.22	0.39 ± 0.08	0.22 ± 0.10		137.61		

Bold values indicate the models with lowest AIC, that is, the most parsimonious model.

^aAdjusted by age, age², sex, sex*age, B-vitamin supplements.

^bmeans infinite values.

for fasting homocysteine, Table 1 shows that the standard deviations of log-transformed fasting and postload homocysteine are very similar. So, a possible difference in measurement accuracy does not explain the differences in heritability found in this study. The most likely explanation for the difference in heritability is that the capacity methionine handling is more dependent of (genetically determined) enzyme activities, while fasting (basal) homocysteine levels are stronger influenced by environmental factors.

MTHFR

In 1995, the MTHFR 677C>T polymorphism was reported as a common genetic cause of hyperhomocysteinemia.¹⁹ Further research on genes for key enzymes in the homocysteine metabolic pathway did reveal a lot of polymorphisms, but none of them has shown any substantial evidence for genetic control in the general population.²³

The drop in estimated heritability after adjustment for observed MTHFR 677TT genotype confirmed previous studies that candidate genes such as MTHFR can modify fasting homocysteine levels. This study showed 14.7% drop in estimated heritability (from 34 to 19%) after adjustment for MTHFR 677TT genotype, which suggests MTHFR genotype can account for a substantial proportion of variance.

Segregation

Segregation analysis of these 51 families for fasting homocysteine showed that the distribution of fasting homocysteine level fit to a nongenetic model with equal transmission better than any Mendelian model. Jee *et al*¹⁵ also suggested an equal transmission model for fasting homocysteine levels. To explore the possibility of a separate major gene (independent of MTHFR) could exist, we also conducted the segregation analysis with adjustment for each individual's MTHFR genotype (data not shown). However, there was no evidence to support the presence of major gene as is suggested by Jee *et al*.¹⁵ In addition, we considered a simpler polygenic model for fasting homocysteine, but this model was also rejected.

For the postload homocysteine, our data did suggest a major gene may be controlling response to methionine load, although we could not discriminate between a recessive and dominant major locus model. These two distribution models fit these 51 families much better than did the equal transmission model with similar 'high' and 'low' distributions, further strengthening the argument for a genetic mechanism controlling postloading homocysteine levels. When three distribution models were fit, however, three genotypic means were 'medium', 'high' and 'low' for the genotypes under a single locus model, and while this is not impossible from a biological perspective, it is difficult to interpret and further calls into question the validity of the underlying model. Such problems with

interpretation are not at all uncommon in segregation analysis, and may result from unrecognized confounders that influence homocysteine level. These findings suggest that there are differences of genetical determinants for fasting and postloading homocysteine levels.

Limitation

Our study has several limitations. The sample size was modest (306 individuals in 51 families). More than half of those families spanned three generations and can be considered informative for testing hypothesis regarding transmission of genes. We also could not exclude the potential for ascertainment bias beyond that corrected for here, because probands were identified through their homocysteine levels. However, we feel such bias is minimal because these probands were recruited at the earliest stages of risk for cardiovascular disease.

Conclusion

In conclusion, this study of 51 Dutch families found evidence for a major gene controlling levels of postload homocysteine, but no evidence for Mendelian single gene control fasting homocysteine levels. Our findings suggest different genetic control mechanisms for fasting and methionine load homocysteine levels. Further investigation into the genetic control of postload homocysteine level is warranted.

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