ORIGINAL RESEARCH

The effect of MTHFR c.677C>T on plasma homocysteine levels depends on health, age and smoking

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Abstract

Purpose: The role of homocysteine in the pathogenesis of arteriosclerosis and stroke is under debate. It is important to determine the interplay of factors that influence homocysteine plasma levels, such as age, gender, smoking and the genetic background. The T-allele of the common variant methylenetetrahydrofolate reductase (MTHFR) c.677C>T is the most prevalent known genetic cause of elevated plasma homocysteine levels, but the association of this allele with vascular disease has been controversial. The aim of the present study was to examine whether the influence of MTHFR c.677C>T on homocysteine levels depends on individual factors.

Methods: From an ongoing study on atherosclerosis, we analyzed 523 Caucasian individuals, including patients with cerebrovascular disease (n=141), their healthy spouses (n=106) and the offspring (n=276). ANOVA and regression analyses were employed to separately analyze the effect of MTHFR c.677C>T on homocysteine levels in patients, spouses and offspring as well as in subgroups defined by age, gender and smoking.

Results: MTHFR c.677C>T was associated with homocysteine plasma levels in the study sample ($P<0.001$), but not in the patients with cerebrovascular disease, if analyzed separately. Analyses of subgroups divided by the age of 55 years revealed that the MTHFR c.677 C>T genotype was

associated with homocysteine plasma levels in the younger ($P<0.001$), but not in the older individuals. In addition, if individuals with at least less than ten cigarette package years were analyzed separately, MTHFR c.677C>T was associated with plasma homocysteine levels only in the group with the lower cigarette consumption ($P=0.002$).

Conclusion: In our study, the association of the MTHFR c.677C>T genotype with plasma homocysteine levels was weakened by other factors that impact homocysteine levels. The effect of MTHFR c.677C>T on plasma homocysteine levels may, thus, be of major importance for healthy, young, non-smoking persons. Such specifications may explain the controversial results of epidemiological studies on the relevance of MTHFR c.677C>T.

Increased plasma homocysteine levels are considered to be an independent risk factor for vascular disease. However, the precise role of homocysteine in the etiology of arteriosclerosis and vascular disease is under debate. Thus, it is necessary to explore factors that influence homocysteine plasma levels. It is known that plasma homocysteine levels are higher in men than in women, and this has been attributed to differences in muscle mass and sex hormones.¹ In addition,

the availability of folate, vitamin B6 and vitamin B12, smoking and other lifestyle factors as well as renal function impact homocysteine levels.²⁻⁴ Further, the T-allele of a polymorphic genetic variant, the missense mutation methylenetetrahydrofolate reductase (MTHFRy) c.677C>T (A222V), is associated with elevated homocysteine plasma levels. On average, carriers of the TT genotype have fasting total plasma homocysteine levels that are approximately 2 µmol/L higher than carriers of the CC-genotype, whereas the normal range is up to 12-17 µmol/L, depending on the laboratory.^{5,6} However, despite a clear association between MTHFR 677TT and elevated homocysteine levels, the impact of this genotype for vascular disease was conflicting in different study samples.⁷⁻⁹ The aim of the present study was to examine whether the influence of MTHFR c.677C>T on homocysteine levels varies by age, sex or cigarette smoking.

Methods

Patients and study participants

We recruited 141 consecutive Caucasian patients (mean age $\pm$ SD; 64.3 $\pm$ 8.9 yr; 29.1% female) from the ultrasound division of the Department of Neurology, University of Bonn. Patients had been referred due to cardiovascular disease and had been diagnosed with at least unilateral 30% carotid stenosis. In addition, we recruited the patients' spouses (n = 106; 61.8 $\pm$ 8.6 yr, 79.2% female) if they had no history of vascular events and no carotid stenosis and also the offspring (n = 276, 36.0 $\pm$ 8.3 yr, 52.5% female). Overall, 523 individuals were recruited. Subjects with concomitant diseases or medications that alter homocysteine, such as renal insufficiency or multivitamin-therapy, were not eligible.

The study was approved by the local ethics committee. All participants gave written informed consent. Study details such as personal data, ultrasonic findings, medical history and laboratory parameters were determined as previously described.¹⁰

Genotyping

Genomic DNA prepared from peripheral leukocytes was used for genotyping by PCR amplification and restriction analysis of MTHFR c.677C>T (p.A222V; rs1801133) as previously published.¹¹

Homocysteine Assay

Homocysteine was determined by means of fully automated particle-enhanced immunonephelometry with a BN II System (Dade Behring). Bound homocysteine in the heparinized plasma sample is reduced to free homocysteine by the action of dithiothreitol, and then converted enzymatically to S-adenosyl-homocysteine (SAH) in the next step. Conjugated S-adenosyl-cysteine (SAC), added at the onset of the reaction, competes with SAH in the sample for bonding by anti-SAH antibodies bound to polystyrene particles. The result is evaluated by comparison with a standard of known concentration. The intra-assay coefficient of variation of the homocysteine assay was 3.4% (mean: 11 µmol/l, n=20), while the inter-assay coefficient was 5.6% (mean: 11 mg/dl, n=20).

Statistics

Multiple linear regression with homocysteine fasting plasma levels as the dependent variable and age, sex, smoking package years and MTHFR c.677C>T as co-variables was utilized to confirm the independent correlation of these factors with homocysteine plasma levels in the entire study population. ANOVA was employed to analyze separately the effect of MTHFR c.677C>T in subgroups defined by sex, package years or age (< 55 yr vs. > 54 yr) as previously published for the Framingham Offspring Study Cohort.¹² Due to multiple testing (11 different ANOVA stratifications or calculations), significance threshold was restrictively defined as $\alpha < 0.004$.

TABLE 1. MTHFR c.677C>T genotypes and homocysteine plasma. Plasma homocysteine levels ($\mu\text{mol/L}$) are displayed as mean $\pm$ standard deviation [95% confidence interval].

	CC	CT	TT	ANOVA
All participants (n=523)	12.90 $\pm$ 4.00 [12.39 – 13.40]	13.65 $\pm$ 4.02 [13.13 – 14.16]	15.20 $\pm$ 5.37 [13.57 – 16.87]	F=6.37; <i>P</i> =0.002
All $\geq$ 55 yr (n=207)	14.38 $\pm$ 5.01 [13.39 – 15.38]	15.09 $\pm$ 4.43 [14.16 – 16.02]	14.95 $\pm$ 4.64 [12.71 – 17.18]	F=0.542; <i>P</i> =0.582
men (n=111)	15.74 $\pm$ 5.49 [14.24 – 17.24]	16.48 $\pm$ 4.76 [14.99 – 17.96]	15.13 $\pm$ 4.3 [12.41 – 17.86]	F=0.638; <i>P</i> =0.451
women (n=96)	12.76 $\pm$ 3.81 [11.61 – 13.90]	13.85 $\pm$ 3.73 [12.76 – 14.95]	14.25 $\pm$ 3.86 [8.10 – 20.40]	F=1.09; <i>P</i> =0.342
All < 55 yr (n=315)	11.88 $\pm$ 2.74 [11.43 – 12.33]	12.77 $\pm$ 3.49 [12.20 – 13.34]	15.58 $\pm$ 6.01 [13.04 – 18.12]	F=12.38; <i>P</i> <0.001
men (n=141)	12.79 $\pm$ 2.63 [12.14 – 13.14]	13.52 $\pm$ 3.43 [12.67 – 14.37]	17.00 $\pm$ 5.68 [12.94 – 21.06]	F=12.38; <i>P</i> <0.001
women (n=174)	11.13 $\pm$ 2.61 [10.54 – 11.71]	12.16 $\pm$ 3.44 [11.40 – 12.92]	14.57 $\pm$ 6.25 [10.96 – 18.18]	F=6.57; <i>P</i> =0.002
All, <10 package years (n=256)	12.19 $\pm$ 3.42 [11.60 – 12.78]	12.79 $\pm$ 3.84 [12.03 – 13.55]	15.41 $\pm$ 6.08 [12.71 – 18.11]	F=6.56; <i>P</i> =0.002
All, $\geq$ 10 package years (n=215)	13.81 $\pm$ 4.62 [12.84 – 14.78]	14.14 $\pm$ 3.98 [13.38 – 14.90]	15.63 $\pm$ 4.71 [13.36 – 17.90]	F=1.40; <i>P</i> =0.250

Results

Multiple linear regression confirmed a correlation of homocysteine levels with age (Beta=0.258; *P*<0.001) and cigarette consume measured in package years (Beta=0.098; *P*<0.001) as well as an association with sex (*P*<0.001) and the MTHFR c.677C>T genotype (*P*<0.001). Homocysteine levels were higher in men (14.52 $\pm$ 4.43), than in women (12.41 $\pm$ 3.67; *P*<0.001), whilst the men were elder than the women contributing to this difference. Analyses of subgroups revealed that, in the patients with a history of cardiovascular disease, MTHFR c.677C>T was not associated with plasma homocysteine levels. In (all) individuals < 55 yr, the association of the MTHFR c.677C>T genotype with homocysteine plasma levels was significant for both women (*P*=0.002) and men (*P*<0.001), but not for women or men >55 yr (table 1).

Homocysteine levels increased with cigarette consumption measured in package years (Beta=0.098; *P*<0.001). Stratification of participants for cigarette consumption showed an influence of the MTHFR c.677C>T variant on homocysteine levels for subjects with < ten package years (*P*=0.002), but not for persons with more than ten package years.

Discussion

In our study sample, individual factors such as vascular disease, higher age and smoking eliminated the significance of the influence of the MTHFR c.677C>T genotype on plasma homocysteine levels. Previous studies on the effect of MTHFR c.677C>T on homocysteine plasma levels yielded conflicting results regarding the influence of age and sex.¹²⁻¹⁷ The effect of MTHFR c.677C>T genotype becomes more relevant when low folate plasma levels are present.¹⁸⁻²⁰ As low folate plasma levels are more common in the elderly,^{2,21} our finding that the effect of MTHFR c.677C>T on homocysteine levels is limited to younger subjects, is surprising. However, in the elderly, other factors such as renal function, vascular disease and hormone status that also influence homocysteine levels may override the effect of the MTHFR c.677C>T genotype in the elderly.^{3,22,23} In addition to age and sex, smoking may overcome the effect of MTHFR c.677C>T. The missing association of MTHFR c.677C>T with homocysteine plasma levels in patients with vascular disease argues against a major relevance of MTHFR c.677C>T for the further progression of vascular

disease, at least in regard of homocysteine plasma levels as a risk factor. Although some of our results are limited by the family-based approach of the study, the results suggest that confounding factors such as age, smoking status and vascular disease must be regarded in association studies investigating the relevance for MTHFR c.677C>T for homocysteine levels and associated diseases.

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