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Homocysteine plasma levels in patients treated with antiepileptic drugs depend on folate and vitamin B12 serum levels, but not on genetic variants of homocysteine metabolism

Abstract

Background: Antiepileptic drugs (AEDs) are commonly used in the treatment of epilepsy, psychiatric diseases and pain disorders. Several of these drugs influence blood levels of folate and vitamin B12 and, consequently, homocysteine. This may be relevant for AED effects and side effects. However, not only folate and vitamin B12, but also genetic variants modify homocysteine metabolism. Here, we aimed to determine whether there is a pharmacogenetic interaction between folate, vitamin B12 and genetic variants and homocysteine plasma level in AED-treated patients.

Methods: In this mono-center study, we measured homocysteine, folate and vitamin B12 plasma levels in a population of 498 AED-treated adult patients with epilepsy. In addition, we analyzed the genotypes of seven common genetic variants of homocysteine metabolism: methylenetetrahydrofolate reductase (*MTHFR*) c.677C>T and c.1298A>C, methionine synthase (*MTR*) c.2756A>G, dihydrofolate reductase (*DHFR*) c.594+59del19bp, cystathionine β-synthase (*CBS*) c.844_855ins68, transcobalamin 2 (*TC2*) c.776C>G and methionine synthase reductase (*MTRR*) c.66G>A.

Results: On multivariate logistic regression, folate and vitamin B12 levels, but none of the genetic variants, were predictive for homocysteine levels.

Conclusions: These data suggest that, in AED-treated patients, folate and vitamin B12 play important roles in the development of hyperhomocysteinemia, whereas genetic variants of homocysteine metabolism do not and thus do not contribute to the risk of developing hyperhomocysteinemia during AED treatment.

Keywords: antiepileptic drugs; folate; single nucleotide polymorphism; vitamin B12.

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Introduction

Antiepileptic drugs (AEDs) are widely used to treat epileptic seizures, psychiatric diseases and pain syndromes. Several side effects and risks limit the usage of AEDs. A characteristic of several AEDs is a reduction of folate and vitamin B12 serum levels accompanied by an increase of homocysteine plasma levels [1–4]. A mild to moderate increase in homocysteine plasma levels has been firmly established as an independent risk factor for cardiovascular [5, 6] and neurodegenerative [7–9] diseases in the general population. Epidemiologic studies show that patients with epilepsy have an increased risk for ischemic heart disease, fatal cardiovascular outcome and neurodegenerative diseases such as dementia and Parkinson's disease, which may be attributed to hyperhomocysteinemia [10–13]. In addition, increased homocysteine plasma levels potentially lead to the aggravation of seizures, as indicated by experimental data [14] and in patients with alcohol withdrawal seizures [15]. Therefore, the effect of AEDs on vitamin and homocysteine metabolism and ultimately on cardiovascular and neurological disease is a relevant research area in the patient population receiving chronic AED treatment.

Not only vitamin status, but also genetic variants may modify folate, vitamin B12 and homocysteine metabolism

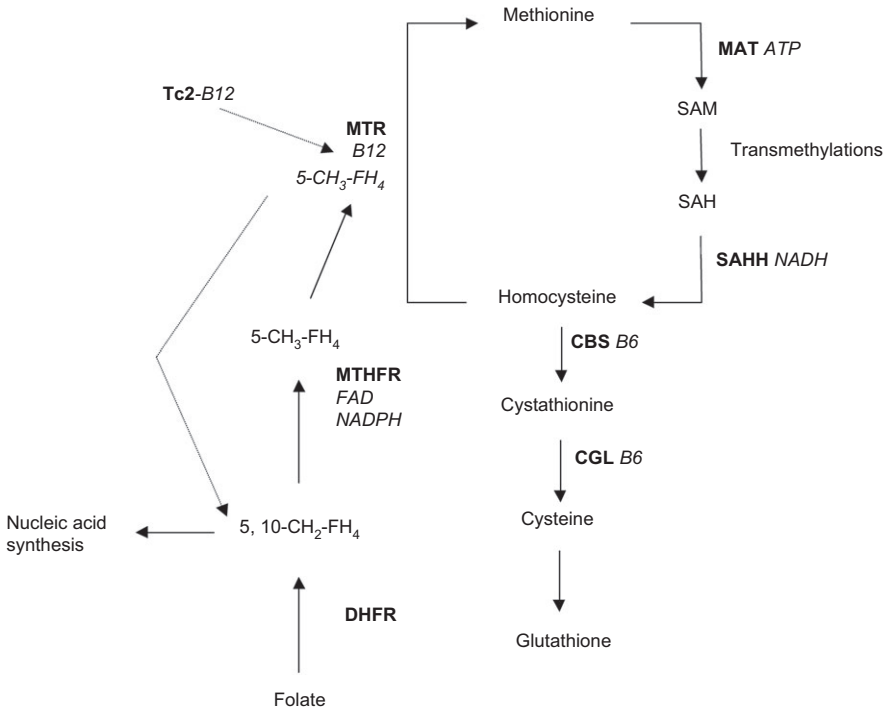

Figure 1 Homocysteine metabolism.
The sulfur-containing amino acid methionine is activated to S-adenosylmethionine (SAM), which is a ubiquitous methyl group donor. The degradation product of SAM is S-adenosylhomocysteine (SAH), which is hydrolyzed to homocysteine. Homocysteine can be remethylated to methionine and SAM via methionine synthase (MTR), which depends on derivatives of folate and vitamin B12 as cofactors. Lack of these vitamins is a common cause of hyperhomocysteinemia [17]. The folate derivative is synthesized by methylenetetrahydrofolate reductase (MTHFR) and dihydrofolate reductase (DHFR), and the derivative of vitamin B12 is transported by transcobalamin 2 (Tc2). Alternatively, homocysteine can be transsulfurated by vitamin B6 dependent cystathionine β-synthase (CBS) and cystathionine gamma-lyase (CGL) to cysteine as a component of glutathione. Due to the existence of several functional variants in the genes involved in homocysteine metabolism, and to differences in dietary vitamin and amino acid uptake, disorders of homocysteine metabolism exhibit marked inter-individual differences.

(Figure 1) [16]. Several studies have reported that elevation of homocysteine plasma levels during AED treatment is enhanced by the presence of genetic risk factors such as the presence of the T allele of the common methylenetetrahydrofolate reductase (*MTHFR*) c.677C>T polymorphism [18–22]. However, these studies are limited due to their small study populations and the small number of genetic variants of homocysteine metabolism tested. In this study, we investigated whether there is a relevant pharmacogenetic relationship between folate, vitamin B12 and seven genetic variants of homocysteine metabolism and homocysteine plasma level in 498 AED-treated patients.

Materials and methods

Patients

Inclusion criteria: This mono-center study included adult serial in- and out-patients with epilepsy seen in the Department for Epileptology of the University Hospital Bonn, Germany. The patients were treated with various commonly used AEDs in mono- or combined therapy [4].

Exclusion criteria: Patients with conditions that could potentially influence folate, vitamin B12 or homocysteine plasma levels, such as renal insufficiency, atrophic gastritis and alcohol or drug abuse, were excluded from the study. Patients who were taking vitamin supplements were also excluded.

This study was approved by the Local Ethics Committee. All patients gave their informed written consent.

Laboratory investigations

Serum concentrations of vitamin B12 and folate were measured by means of a competitive chemiluminescent immunoassay with an Access™ Immunoassay System (Beckman Coulter, Krefeld, Germany). The intra-assay coefficient of variation of the folate assay was 3.1% (mean: 14.1 nmol/L; n=20); the inter-assay coefficient of variation was 3.6% (mean: 14.3 nmol/L; n=20). The intra-assay coefficient of variation of the vitamin B12 assay was 3.8% (mean: 487 pmol/mL; n=20); the inter-assay coefficient was 4.2% (mean: 492 pmol/L; n=20). Homocysteine was determined by fully automated particle-enhanced immunonephelometry with a BN II System (Siemens

Genetic variant	Peptide variant	rs/Genbank no.	Reference
MTHFR c.677C>T	A222V	rs1801133	[23]
MTHFR c.1298A>C	E429A	rs1801131	[24]
MTR c.2756A>G	D919G	rs1805087	[25]
Tc2 c.776C>G	P259R	rs1801198	[26]
DHFR c.594+59del19bp	Change of transcription level?	NM_000791.3	[27]
CBS c.844_855ins68	Change of transcription level?	S78267.1	[28]
MTRR c.66G>A	M22I	rs1801394	[29]

Table 1 The genetic variants of homocysteine metabolism analyzed in this study.

Healthcare Diagnostics, Eschborn, Germany) by enzymatic conversion to S-adenosylhomocysteine. The intra-assay coefficient of variation of the homocysteine assay was 3.4% (mean: 11 µmol/L, n=20); the inter-assay coefficient was 5.6% (mean: µmol/L, n=20) [1].

Genomic DNA prepared from peripheral leukocytes was used for genotyping by PCR amplification and, where applicable, subsequent restriction analysis of the seven genetic variants of homocysteine metabolism (Table 1).

Statistical analysis

Plasma levels of homocysteine plasma and serum levels of folate and vitamin B12 were tested for normal distribution by the Kolmogorov-Smirnov test. The distribution of genotypes was tested with a chi-square goodness-of-fit test (Pearson). Bivariate Pearson's correlation was used to analyze correlations between folate, vitamin B12 and homocysteine levels. To analyze associations between the different genotypes and folate and vitamin B12 levels and folate and vitamin B12 tertiles, univariate analysis of variance (ANOVA) and Pearson's χ^2-tests were used, respectively. To analyze independent associations with homocysteine plasma level as the primary parameter of interest, we applied multivariate linear regression analysis with homocysteine plasma level as the dependent variable and with the genetic variants, folate and vitamin B12 plasma levels and age and sex as covariables. One-way ANOVA was used for exploratory comparison of homocysteine plasma levels between patients with the *MTHFR* c.677C>T genotype treated with either carbamazepine or phenytoin. The threshold was defined as two-sided α=0.05.

Demographic/biochemical data	Mean	SD	
Age, years	40.0	14.0	
Vitamin B12, pmol/L	268	137	
Folate, nmol/L	11.6	7.9	
Homocysteine, µmol/L	15.5	8.6	
Sex, n (%)	Male	Female	
	256 (51.4%)	242 (48.6%)	
Frequency of genotype			
MTHFR c.677C>T	CC	CT	TT
n *(%)*	219 (44%)	224 (45%)	55 (11%)
MTHFR c.1298A>C	AA	AC	CC
n *(%)*	214 (43%)	229 (46%)	55 (11%)
MTR c.2756A>G	AA	AG	GG
n *(%)*	349 (70%)	134 (27%)	15 (3%)
TC2 c.776 C>G	CC	CG	GG
n *(%)*	149 (30%)	229 (46%)	120 (24%)
DHFR c.594+59del19	dd	di	ii
n *(%)*	90 (18%)	244 (49%)	164 (33%)
CBS c.844_855ins68	dd	di	ii
n (%)	418 (84%)	75 (15%)	5 (1%)
MTRR G>A	GG	GA	AA
n (%)	100 (20%)	259 (52%)	139 (28%)

Table 2 Demographic and biochemical data and frequency of genotypes in the study population (n=498).
All genotypes were in Hardy-Weinberg equilibrium. SD, standard deviation.

Results

Demographic, biochemical and genetic data from the 498 patients (51.4% male) enrolled in this study are shown in Table 2. Genotyping succeeded for all genetic variants. Genotype distributions did not deviate from Hardy-Weinberg equilibrium. Homocysteine plasma levels as well as folate and vitamin B12 serum levels were within the normal distribution. Thus, the data were not log-transformed. First, we evaluated the relationships between folate and vitamin B12 levels and homocysteine level by univariate analysis and found negative correlations between homocysteine and folate (Pearson=−0.334; p<0.001) and homocysteine and vitamin B12 (Pearson=−0.236; p=0.001). Therefore, we included folate and vitamin B12 plasma levels along with age, sex and all seven genetic variants as covariables for multivariate analysis of independent associations with homocysteine plasma level as the dependent variable. The associations between folate and vitamin B12 with homocysteine level were confirmed, but none of the genotypes showed an association with homocysteine level (Table 3). In addition, none of the genotypes was associated with folate or vitamin B12 serum level (data not shown). However, *MTHFR* c.677C>T was associated with folate tertiles; i.e., patients with the TT genotype had a higher likelihood of having folate serum

Independent variable	β	p-Value
Sex	0.134	0.61
Age	0.154	0.30
MTHFR c.677C>T	0.08	0.29
MTHFR c.1298A>C	0.03	0.70
MTR c.2756A>G	0.01	0.91
CBS c.844_855ins68	0.05	0.48
DHFR c.594+59del19	0.09	0.20
Tc2 c.776C>G	0.06	0.40
MTRR c.66G>A	0.07	0.33
Folate	−0.27	<0.001
Vitamin B12	−0.17	0.021

Table 3 Multiple logistic regression analysis (R^2=0.249) with homocysteine plasma level as the dependent variable and age, sex, folate, vitamin B12 plasma level and all tested genetic variants as covariables.
Linear data were distributed normally and not log-transformed.

levels in the lowest tertile (χ^2=3.1; p=0.011). Next, we conducted an exploratory analysis of patients who received carbamazepine or phenytoin monotherapy (n=76), looking for an association between *MTHFR* c.677C>T and homocysteine plasma level, which has been reported by previous studies [18–22]. However, we observed no significant differences (ANOVA: F=2.5; p=0.091).

Discussion

In our study cohort of 498 AED-treated epilepsy patients, we observed no associations between any of seven genetic variants of homocysteine metabolism and homocysteine plasma level. Only folate and vitamin B12 serum levels were associated with homocysteine plasma level. This indicates that hyperhomocysteinemia during chronic AED treatment is driven by decreased folate and vitamin B12 levels and not by a pharmacogenetic risk profile.

This is surprising, because genetic variants of homocysteine metabolism are firmly established risk factors for hyperhomocysteinemia in the general population; e.g., *MTHFR* c.677C>T influences homocysteine plasma levels, with differences of approximately 2 μmol/L (15%–20%) between homozygous carriers of the wild-type C versus the mutant T allele [23]. In addition, the T variant also influences folate metabolism, resulting in lower total folate levels [30]. In our study, the association with folate level was weak and was significant only with folate tertiles. We speculate that the effects of the AEDs on folate and homocysteine levels overcame the weaker effects of the genetic variants in our patient population. This is in contrast to previous studies describing genetic risk factors for hyperhomocysteinemia during AED treatment – principally the T allele of *MTHFR* c.677 C>T and the C allele of *MTHFR* c.1298A>C [18–22] – and may be explained by the differing study populations. For example, Yoo et al. described an association between the TT genotype of *MTHFR* c.677 C>T and higher homocysteine plasma levels in AED-treated patients [21]. However, the subjects enrolled in that study were from Korea and were younger (27.5±8.5 years) and had lower mean homocysteine plasma levels (11.2±1.5 μmol/L), higher folate (18.8±10.2 nmol/L) and higher vitamin B12 serum levels (630±252 pmol/L) than the patients in our study (Table 2). Thus, we cannot exclude the possibility that the small subgroup sizes of that study or demographic differences between the populations contributed to the conflicting results.

In conclusion, patients undergoing chronic AED treatment should be screened for folate, vitamin B12 deficiency and hyperhomocysteinemia on a regular basis and any vitamin deficiency should be corrected when necessary [4]. Screening for genetic variants is not feasible for the detection of patients at risk and should not be included in the clinical work-up.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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