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Association between *MTHFR* polymorphism and seizure control in epileptic patients with hyperhomocysteinaemia

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ABSTRACT

Objective. The aim of this study was to investigate possible associations between *MTHFR* polymorphism and seizure control of epileptic patients with hyperhomocysteinaemia.

Methods. A total of 81 epileptic patients with hyperhomocysteinaemia treated with oxcarbazepine monotherapy were enrolled in this study. All patients were offered vitamin B supplementation (2.5 mg/d folate and 1.5 mg/d mecobalamine) for six months. *MTHFR* C677T and A1298C polymorphisms, serum homocysteine, folate and vitamin B12 levels as well as seizure frequency and score based on the Hamilton depression scale (HAMD) were evaluated at baseline and after six months of follow-up.

Results. Spearman correlation analysis showed that the extent of decline of seizure frequency positively correlated with a dynamic change in serum homocysteine concentration between baseline and after six months of follow-up (t=0.241, p=0.015 [Spearman's coefficient]). For the *MTHFR* C677T polymorphism, compared to the CC genotype, the TT genotype was associated with a significant downtrend of homocysteine (19.69 vs 10.28 mmol/L, p=0.006) and uptrend of folate (6.21 vs 2.49 ng/mL; p=0.004). The decrease in homocysteine (17.94 vs 12.52 mmol/L, p=0.001) and increase of folate (5.08 vs 2.86 ng/mL; p=0.003) were significantly greater in patients with the T allele compared to those with the C allele. Also, the TT genotype (2.33 vs 1.4, p=0.056) and T allele (1.95 vs 1.38, p=0.037) were associated with a greater decrease in seizure frequency compared to the CC genotype or C allele. The A1298C polymorphism alone was not associated with elevated homocysteine or decreased folate levels at baseline, and showed little association with response to vitamin B supplementation in epileptic patients with hyperhomocysteinaemia. However, in patients with combined 677TT/1298AA or 677TT/1298AC polymorphisms, the changes in homocysteine and folate levels and seizure frequency were more obvious.

Significance. MTHFR C677T polymorphism was associated with seizure control in epileptic patients with hyperhomocysteinaemia; individuals with the 677TT genotype or T allele demonstrated better seizure control.

Key words: epilepsy, homocysteine, B vitamin supplementation

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Epilepsy is one of the most prevalent serious neurological conditions, affecting approximately 70 million people worldwide [1]. Epidemiological studies show that 10-40% patients with epilepsy experience hyperhomocysteinaemia (HHcy) [2]. HHcy is an elevation of serum homocysteine

(Hcy) concentration due to methionine metabolic disorder [3], which is also a dominant probable risk factor for several disorders including coronary artery disease [4], stroke [5], carotid artery stenosis [6], heart failure [7], mood dysfunction and depression [8, 9]. Moreover, HHcy may cause seizures, increase seizure frequency and severity, and lead to antiepileptic drug resistance [10, 11]. As vitamin B12 and folate are essential cofactors for the remethylation of Hcy, vitamin B supplementation effectively reduces the concentration of Hcy [12]. Early and aggressive treatment may improve the clinical outcome [13, 14]. Both polymorphism of the gene encoding 5,10-methylenetetrahydrofolate reductase (*MTHFR*) and chronic intake of older antiepileptic drugs (AEDs) contribute to HHcy in epileptic patients. Several studies have suggested that treatment with distinct first-generation enzyme-inducing antiepileptic drugs, such as carbamazepine, phenobarbital, primidone and phenytoin, is associated with reduced mean serum levels of folate and vitamin B12 [15], which are believed to disturb the intestinal absorption of folate, influencing CYP450 enzymatic reactions and the subsequent consumption of folate [16]. However, it is believed that the second-generation AEDs are less likely to disrupt Hcy metabolism [11, 17]. Oxcarbazepine (OXC), one of the second antiepileptic drugs used as both monotherapy and adjunctive therapy for the treatment of focal seizures and symptomatic epilepsy [18], is associated with a low incidence of adverse reactions, and causes no increase in serum homocysteine [17].

MTHFR is known to reduce methylene tetrahydrofolate to methyltetrahydrofolate, which is critical for the folate cycle and homocysteine metabolism. *MTHFR* gene polymorphisms are characterized by a base substitution from C to T on residue 677 and A to C on residue 1298. The genotypes of *MTHFR* 677 and 1298 are reported to be associated with higher Hcy levels, effecting the activity of MTHFR (enzyme activity is reduced by 70% and 35% in *MTHFR* C677T homozygotes and heterozygotes, and 35% and 10% in *MTHFR* A1298C homozygotes and heterozygotes, respectively) [19], leading to decreased folate. In addition, *MTHFR* polymorphisms influence the response to vitamin B supplementation. The *MTHFR* 677 genotype is reported to be an independent predictor of response to folic acid supplementation [20]. Individuals with the 677TT genotype have greater responses [21]. The decrease in Hcy associated with the TT genotype was estimated to be 2.4-fold lower than that for the CC genotype [22].

However, whether there is an association between *MTHFR* polymorphism and seizure control of epileptic patients with HHcy remains unknown. The present study was carried out to examine possible associations between *MTHFR* C677T and A1298C polymorphisms and seizure control in epileptic patients with HHcy. Epileptic patients were enrolled with OXC therapy in order to exclude the influence of AEDs on homocysteine levels. Moreover, we set out to further evaluate whether homocysteine levels were associated with particular *MTHFR* genotypes in patients taking vitamin B supplementation.

Material and methods

Participants

From July 2018 to July 2020, a total of 83 epileptic patients with OXC therapy were prospectively recruited in the Second Affiliated Hospital of Soochow University. All of the patients were ≥14 years with serum Hcy concentration >15 μmol/L and the duration of antiepileptic OXC monotherapy lasted for more than six months. Those who had severe cardiac, hepatic, renal and respiratory diseases, stroke, thyroid dysfunction, cancer, severe gastrointestinal diseases, mental disorders and other metabolic diseases were excluded. Additional exclusion criteria were as follows:previous or ongoing vitamin treatment;

- diuretics and other drugs which affect serum Hcy levels;
- non-compliance to treatment;
- and treatments for depression.

Finally, 81 patients were eligible for the analysis. The study was approved by the institutional research ethics committee of the Second Affiliated Hospital of Soochow University, and written informed consent was obtained from all subjects.

Methods

Patients were followed for six months since treatment by trained neurologists unaware of the characteristics of the epileptic patients. Data were collected at baseline and at six months of follow-up. Fasting blood samples were collected from all patients in the morning before recruitment. Determination of C677T and A1298C gene polymorphisms of MTHFR was performed by PCR using melting-fusion curves, before treatment. Immunoassays were used to detect serum Hcy, folate, and vitamin B12 concentrations. All patients were given vitamin B supplementation as 2.5 mg/d folate + 1.5 mg/d mecobalamin for six months. Serum Hcy, folate and vitamin B12 levels were examined before and six months after treatment. The clinical status, including seizure frequency, depressive symptoms and cognitive function were also recorded at recruitment and six months after

treatment. The seizure frequency was calculated based on the total number of seizure attacks within six months before and after treatment. Depressive symptoms were assessed using Hamilton Depression Scale-17 items (HAMD-17).

Statistical analysis

Measurement data were expressed as mean ± standard deviation (SD) or median (interquartile range) for continuous variables, and as number (%) for categorical variables. Comparison was performed using the ANOVA test, Kruskal-Wallis test or the Chi-square test, when appropriate. Correlation between change in seizure frequency and serum Hcy concentration was analysed by Spearman correlation analysis. All statistical analyses were two-tailed. $P<0.05$ was considered statistically significant. SPSS version 19.0 (IBM, Armonk, New York, NY, USA) was used for all analyses.

Results

Clinical characteristics of the participants

During six months, two (2.4%) patients were lost to follow-up. Finally, a total of 81 patients were included with an average age of 49.38 years (62% male). The mean serum Hcy, folate and vitamin B12 levels were 28.69 µmol/L, 6.4 ng/mL, and 308.69 mmol/L, respectively, at baseline.

There were 63 cases with secondary epilepsy, including 25 with central nervous system infection, 29 with traumatic brain injury or following craniocerebral surgery, five presenting with focal cortical dysplasia, two with vascular malformation, and two resulting from cerebral atrophy. Epilepsy in 10 cases was idiopathic, and cryptogenic in eight. Sixty-seven cases presented with partial seizures and 14 cases were diagnosed as generalized seizures. A total of 18 cases had a history of hypertension, while 10 cases had diabetes mellitus.

All patients received EEG examinations at baseline, with 12 cases undergoing long-term EEG. Results were abnormal in 70 cases, of which 41 manifested with sustained or paroxysmal appearance of sharp-wave, spike-wave, sharp-slow-wave or spike-slow-wave activity, and 29 cases exhibited slow-wave activity (increased slow-wave energy). Brain CT or MRI were performed for all patients. Radiographic abnormalities were detected in 63 cases, of which 51 showed cortical lesions, including encephalomalacia, cortical dysplasia, or cortical atrophy and abnormal signals, while 12 cases had subcortical and deep brain lesions.

Frequency of MTHFR polymorphisms

Overall, the 677TT, CT and CC genotype frequencies were 27 (33%), 34 (42%) and 20 (25%), respectively. All patients were divided into a moderate HHcy group (15-30 µmol/L) and intermediate HHcy group (>30 µmol/L). The T allele frequency was 54% in total; 51% in the moderate HHcy group and 62% in the intermediate HHcy group. The frequency of 677TT was higher in the intermediate HHcy group (45%) compared with that of the moderate HHcy group. The 1298AA, AC and CC genotype frequencies were 58 (72%), 22 (27%) and 1 (1%), respectively. In total, the A and C allele frequencies were 85% and 15%, respectively, with no significant differences between moderate HHcy and intermediate HHcy groups (*table 1*).

Association between level of serum Hcy and seizure control

Spearman correlation analysis showed that the decline of seizure frequency over six months positively correlated with the dynamic change in serum Hcy concentration (Spearman's coefficient=0.241, $p=0.015$), suggesting that the decreased Hcy may contribute to the control of seizures.

Association between MTHFR C677T polymorphism and seizure control

At baseline, serum Hcy concentrations among genotypes showed a clear pattern of TT > CT > CC, T>C, and folate concentrations CC > CT > TT, C>T. Hcy level associated with the TT genotype was much higher than that of the CC genotype (33.93±16.47 *vs.* 23.15±8.89), while the folate level associated with the TT genotype was significantly lower than that of the CC genotype (4.04±1.45 vs. 8.27±3.96, all $p<0.05$), suggesting a role of *MTHFR* C677T polymorphism in *in vivo* metabolism of folate and Hcy. Consistently, the dynamic change in Hcy and folate levels associated with the TT genotype was much more obvious than that for the CC genotype before and after vitamin B supplementation over six months (19.69±13.01 *vs.* 10.28±7.28, $p=0.001$; 6.21±4.31 *vs.* 2.49±3.81, $p=0.003$). Similarly, the dynamic change in Hcy and folate levels associated with the T allele was more remarkable than that associated with the C allele (17.94±13.14 *vs.* 12.52±10.63, $p=0.001$; 5.08±4.87 *vs.* 2.86±4.51, $p=0.003$) (*table 2*). Thus, a better response to vitamin B was observed in patients with the TT genotype and T allele.

Furthermore, patients with the TT genotype or T allele demonstrated better control of seizures than those with the CC genotype or C allele (the number of seizures was decreased by 2.33±1.64 *vs* 1.40±1.57 [TT vs CC], $p=0.056$ and 1.95±1.86 *vs.* 1.38±1.79 [T vs C

▼ **Table 1.** General information of the study population.

	Total	Moderate-HHcy	Intermediate -HHcy	*P*
n	81	57	24	
male(%)	50(62%)	33(58%)	17(70%)	
Age	49.38±16.24	48.54±16.88	51.38±14.75	0.477
Smoking	23	13(23%)	10(42%)	0.086
Hypertension	18	12(21%)	6(25%)	0.696
Diabetes	10	6(11%)	4(17%)	0.691
Duration		3.00±2.33	3.54±2.17	0.896
Status epilepticus	12(15%)	7(12%)	5(21%)	0.518
Type of seizure	1			
Generalized seizures Partial seizures	4(17%) 67(83%)	9(16%) 48(84%)	5(21%) 19(79%)	0.821
Seizure frequency	3.16±2.29	3.00±2.34	3.54±2.17	0.334
OXC dose	631.48±238.02	618.42±242.32	662.50±229.48	0.450
OXC concentration	14.27±5.68	14.09±5.21	14.72±6.76	0.65
EEG abnormality	70(86%)	49(86%)	21(87%)	0.854
MRI/CT abnormality	63(78%)	45(79%)	18(75%)	0.696
Hcy	28.69±14.92	21.11±4.38	46.69±15.72	0.001
Folate	6.40±3.72	7.14±3.90	4.66±2.55	0.005
Vitamin B12	308.69±145.87	318.64±152.79	285.07±127.84	0.348
HDMA	7.19±4.99	6.86±4.01	7.96±6.01	0.426
MTHFRC677T				
TT	27(33%)	16(28%)	11(45%)	0.121
CT	34(42%)	26(46%)	8(34%)	0.306
CC	20(25%)	15(26%)	5(21%)	0.601
T	88(54%)	58(51%)	30(62%)	0.175
C	74(46%)	56(49%)	18(38%)	
MTHFRA1298C				
AA	58(72%)	40(70%)	18(75%)	0.66
AC	22(27%)	16(28%)	6(25%)	0.77
CC	1(1%)	1(2%)	0(0)	0.516
A	138(85%)	96(84%)	42(87%)	0.59
C	24(15%)	18(16%)	6(13%)	

allele], p=0.037), however, there was no significant difference between these patients regarding OXC dose and serum OXC concentration (p>0.05) (*table 2*). The results suggest that *MTHFR* C677T polymorphism plays a role in the extent of seizure control of epileptic patients, which may be due to a differential response to vitamin B supplementation, leading to a decrease in serum Hcy concentration.

Moreover, at baseline, there was no significant difference between HAMD scores of patients with the TT and CC genotype (7.52±5.27 vs. 5.85±4.97, p>0.05). However, after six months of treatment, HAMD scores in patients with the TT genotype and T allele were more clearly reduced compared to those with the CC genotype and C allele (1.81±3.06 *vs.* 0.40±1.67, p=0.077; 1.65±2.90 *vs.* 0.85±2.23, p=0.055) (*table 2*).

▼ **Table 2.** Association between *MTHFR* C677T polymorphism and Hcy, folate, B12, seizure frequency, and HAMD at baseline and response to vitamin B supplementation.

	n	Hcy	Folate	B12	Seizures	HAMD	
Baseline	TT	27	33.93±16.47*	4.04±1.45*	266.62±114.28	3.70±2.49	7.52±5.27
	CT	34	27.78±15.45	7.19±3.95	344.65±171.26	2.79±2.24	7.71±4.76
	CC	20	23.15±8.89	8.27±3.96	304.35±126.20	3.05±2.04	5.85±4.97
	T	88	31.55±16.18	5.26±3.09	296.77±142.79	3.35±2.41	7.59±5.02
	C	74	25.28±12.43	7.77±3.93	322.87±148.18	2.93±2.11	6.70±4.89
	P(T*vs*C)		0.001	<0.001	0.139	0.31	0.258
Change	TT	27	19.69±13.01*	6.21±4.31*	273.14±276.96	2.33±1.64	1.81±3.06
	CT	34	15.16±13.26	3.28±5.28	237.25±402.48	1.35±2.06	1.38±2.70
	CC	20	10.28±7.28	2.49±3.81	193.15±253.07	1.40±1.57	0.40±1.67
	T	88	17.94±13.14	5.08±4.87	259.27±328.02	1.95±1.86	1.65±2.90
	C	74	12.52±10.63	2.86±4.51	213.41±327.19	1.38±1.79	0.85±2.23
	P(T*vs*C)		0.001	0.003	0.371	0.037	0.055

*compared with CC genotype, $p<0.05$

Association between MTHFR A1298C +/- C677T polymorphism and seizure control

In total, the 1298AA, AC and CC genotype frequencies were 72%, 27% and 1%, respectively. Among these genotypes, or between A and C alleles, before and after vitamin B supplementation, no significant differences were observed for Hcy, folate and B12 levels, seizure frequency, or HAMD scores (*table 3*). When combined with the C677T polymorphism, a decrease in Hcy, seizure frequency and increase in folate was more obvious in patients with 1298AA/677TT than in those with other genotypes, indicating that the two gene polymorphisms might have a combined effect on the response of epileptic patients to vitamin B treatment and the control of seizures (*table 4*).

Discussion

MTHFR catalyses the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. *MTHFR* polymorphism affects the activity of the MTHFR enzyme and leads to decreased folic acid and increased homocysteine [23]. *MTHFR* C677T and A1298C are the most frequent genetic causes of HHcy. The frequency of *MTHFR* polymorphism is known to vary among different ethnic groups and geographical regions [24]. The 677TT genotype is believed to represent the most important genetic risk factor for HHcy. In different countries, the prevalence of the *MTHFR* 677TT genotype ranges from 10% to 57% [24, 25]. In Chinese Han populations, the frequency of the 677T allele and the 677TT genotype is low (24.0% and 6.4%, respectively) in the south and higher (54.3–60.2% and 30.4-37.0%, respectively) in the north, and in the region reported in this study, the frequencies are documented to be 28.5–43.5% and 8.3–19.7%, respectively [26]. These frequencies are lower than the 54% and 33% shown in this study, which may be related to the fact that the subjects enrolled in this study were a specific population with HHcy. This result indirectly confirms that the 677TT genotype and T allele are associated with increased homocysteine. As to the frequency of the *MTHFR* A1298C polymorphism, few studies have reported this. The prevalence of the 1298C allele is reported to range from 24% to 30% [27, 28]. In the current study, there was only one patient (1%) with the CC genotype, and the frequency of the C allele was 15%, lower than that in previous studies [27, 28], perhaps due to the differences between ethnic groups.

Although *MTHFR* polymorphisms are widely known to affect serum homocysteine level, and an increase in homocysteine demonstrates a convulsive effect, the relationship between *MTHFR* polymorphism and seizure control in epileptic patients has rarely been reported. In a recent study of 71 epileptic patients

▼ **Table 3.** Association between *MTHFR* A1298C polymorphism and Hcy, folate, B12, seizure frequency, and HAMD at baseline and response to vitamin B supplementation.

	n	Hcy	Folate	B12	Seizures	HAMD	
Baseline	AA	58	29.62±15.84	6.32±4.03	323.31±144.22	3.21±2.38	6.83±4.87
	AC	22	26.53±12.57	6.60±2.91	275.81±148.78	2.91±2.04	7.82±5.22
	CC	1	21.9	6.72	184.16	6	14
	A	138	29.13±15.30	6.37±3.85	315.74±144.93	3.16±2.31	6.99±4.90
	C	24	26.14±12.09	6.61±2.78	268.18±144.49	3.17±2.14	8.33±5.29
	P(AvsC)		0.366	0.765	0.140	0.989	0.221
Change	AA	58	16.69±13.12	4.31±5.05	208.08±325.02	1.74±1.93	1.26±2.74
	AC	22	12.81±9.79	3.55±4.37	318.55±339.08	1.50±1.65	1.27±2.49
	CC	1	2.5	1.08	227.42	3	3
	A	138	15.16±11.68	4.18±4.82	242.79±345.79	1.69±1.87	1.30±2.62
	C	24	17.23±15.70	3.37±4.91	206.84±192.35	1.67±1.69	1.17±2.81
	P(AvsC)		0.450	0.445	0.611	0.944	0.814

▼ **Table 4.** Association between *MTHFR* C677T and A1298C polymorphism and response to vitamin B supplementation.

	677	1298	*n*	Hcy	Folate	B12	Seizures	HAMD
Baseline	TT	AA	20	34.49±16.62	3.95±1.42	285.46±125.31	4.20±2.42	8.30±5.49
		AC	7	32.34±17.22	4.29±1.62	212.81±47.99	2.29±2.28	5.29±4.11
	CT	AA	27	28.59±16.49	7.14±4.33	344.85±155.58	2.89±2.47	6.81±.55
		AC	7	24.64±10.88	7.38±2.09	343.86±237.55	2.43±0.98	11.14±4.22
	CC	AA	11	23.29±10.13	8.63±4.61	339.25±146.28	2.18±1.33	4.18±3.46
		AC	8	23.10±8.23	7.95±3.38	271.39±86.25	3.88±2.36	7.13±5.84
		CC	1	21.90	6.72	184.16	6	14
Change	TT	AA	20	20.15±13.38	7.01±4.57	238.59±292.90	2.55±1.57	2.30±3.23
		AC	7	18.40±12.81	3.93±2.46	371.85±213.02	1.71±1.80	0.43±2.15
	CT	AA	27	16.18±14.35	3.14±5.10	207.58±358.31	1.52±2.23	1.07±2.60
		AC	7	11.26±7.20	3.83±6.35	351.60±561.56	0.71±1.11	2.57±2.94
	CC	AA	11	11.71±7.32	2.28±3.92	153.85±316.57	0.82±1.17	0.18±0.87
		AC	8	9.28±7.38	2.96±4.11	242.92±158.89	2.00±1.85	0.87±2.17
		CC	1	0.11	0.16	1.23	0.5	0.21

enrolled with carbamazepine monotherapy, poor seizure control was associated with the 677CT and 677TT genotype [29]. Moreover, *MTHFR* polymorphism (677TT genotype and T allele) is reported to be associated with an increased risk of epilepsy [23, 30] as a result of increased Hcy. In this study, we observed that the decrease in seizure frequency was more significant in individuals with the T allele ($p<0.05$), and slightly greater in individuals with the TT genotype ($p=0.068$). This suggests that epileptic patients with the

T allele and TT genotype may have better seizure control. Although the mechanisms still remain undefined, this may be related to the following factors. Firstly, the convulsive effect of homocysteine was concentration dependent. Although the epileptogenic effect of HHcy (50-200 µmol/L) is very obvious [31], a slight increase in Hcy is not associated with increased seizure frequency [32, 33]. Therefore, reducing serum homocysteine could mitigate the epileptogenic effect, which would be beneficial to seizure control [34]. In this study, the decrease in seizure frequency positively correlated with change in serum Hcy concentration (p=0.015). The greater the decrease in homocysteine, the better the seizure control. Miyaki *et al*. estimated that the effect size of decreased Hcy was 2.4-fold lower in patients with the TT genotype than the CC genotype [22], consistent with this study. On the other hand, many genes are known to influence the response to antiepileptic drug therapy. Whether *MTHFR* polymorphism affected the response to OXC remains to be confirmed in further studies, however, we found no significant difference in dose or plasma concentration of OXC between the different genotypes in this study.

MTHFR A1298C is believed to have marginal or no effect on plasma Hcy concentration [30]. Little information is available on the distribution of *MTHFR* A1298C polymorphism and 677CT /1298AC diplotype in epileptic patients [29]. In this study, neither the homozygous nor the heterozygous state for the A1298C polymorphism was associated with increased plasma homocysteine or better seizure control. Furthermore, the effect on homocysteine and seizure frequency was slightly greater in individuals with 677TT/1298AA, which may be mainly related to the influence of the 677TT genotype. Unfortunately, only one patient had the 1298CC genotype in this study, and therefore statistical analysis could not be performed for this genotype, thus further studies are required.

Epilepsy is a noncommunicable chronic brain disorder and most of the patients also suffer from emotional disturbances, especially depressive symptoms. Homocysteine is also a risk factor for depression [8, 9]. Many studies suggest that individuals with vitamin B supplementation show greater improvements with regards to alleviating depression [35, 36]. Surprisingly, research on the effects of vitamin B supplementation in individuals with depression who have specific *MTHFR* polymorphisms is scarce. In this study, compared with the 677CC genotype and C allele, individuals with the TT genotype and T allele showed a slightly greater improvement in HAMD scores (0.077 and 0.055, respectively). This suggests that *MTHFR* polymorphisms have a mild effect on depression in epileptic patients with vitamin B supplementation. However, further studies are warranted due to the limited nature of the population studied.

Conclusions

Vitamin B supplementation may reduce serum Hcy concentration and attenuate the convulsive effect induced by elevated homocysteine. *MTHFR* C677T polymorphism correlated with response to vitamin B supplementation, and the decrease in serum homocysteine level and seizure frequency was greater in individuals with the TT genotype or T allele. The 677T allele was associated with good seizure control. We believe that vitamin B supplementation is an effective and cost-effective treatment for epileptic patients with HHcy, as it not only decreases serum Hcy level, but also helps to improve seizure outcome, moreover, individuals with the 677T allele, in particular, are likely to benefit from this. ■

Key points

- We investigated the clinical effect of vitamin B supplementation in epileptic patients with HHcy.
- Epileptic patients with intermediate-HHcy (Hcy>30 mmol/L) had a better response to vitamin B supplementation.
- Epileptic patients with *MTHFR* 677TT or the T allele had a better response to vitamin B supplementation.
- Vitamin B supplementation is an effective treatment to decrease Hcy level and improve seizure outcome.

Supplementary material.
Summary slides accompanying the manuscript are available at www.epilepticdisorders.com.

Disclosures.
The authors have no conflicts of interest to disclose.

References

1. Falco WJ. Epilepsy-definition, classification, pathophysiology, and epidemiology. *Semin Neurol* 2020; 40(6): 617-23.

2. Hamed SA, Nabeshima T. The high atherosclerotic risk among epileptics: the atheroprotective role of multivitamins. *J Pharmacol Sic* 2005; 98(4): 340-53.

3. Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, *et al*. Effect modification by population dietary folate on the association between MTHFR genotype,

homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet* 2011; 378(9791): 584-94.

4. Soinio M, Marniemi J, Laakso M, Lehto S, Ronnemaa T. Elevated plasmahomocysteine level is an independent predictor of coronary heart diseaseevents in patients with type 2 diabetes mellitus. *Ann Intern Med* 2004; 140(2): 94-100.

5. Iso H, Moriyama Y, Sato S, Kitamura A, Tanigawa T, Yamagishi K, *et al*. Serumtotal homocysteine concentrations and risk of stroke and its subtypes in Japanese. *Circulation* 2004; 109(22): 2766-72.

6. McQuillan BM, Beilby JP, Nidorf M, Thompson PL, Hung J. Hyperhomocysteinemia but not the C677T mutation of methylenetetrahydrofolate reductase isan independent risk determinant of carotid wall thickening. The Perth CarotidUltrasound Disease Assessment Study (CUDAS). *Circulation* 1999; 99(18): 2383-8.

7. Vasan RS, Beiser A, D'Agostino RB, Levy D, Selhub J, Jacques PF, *et al*. Plasmahomocysteine and risk for congestive heart failure in adults without priormyocardial infarction. *JAMA* 2003; 289(10): 1251-7.

8. Koning EJ, Zwaluw NL, Wijingaarden JP, Sohl E, Brouwer-Brolsma EM, Marwijk HWJ, *et al*. Effects of two-year vitamin B12 and folic acid supplementation on depressive symptoms and quality of life in older adults with elevated homocysteine concentrations: additional results from the B-PROOF study, an RCT. *Nutrients* 2016; 8(11): 748-64.

9. Kwok T, Wu Y, Lee J, Lee R, Yung CY, Choi G, *et al*. A randomized placebo-controlled trial of using B vitamins to prevent cognitive decline in older moderate cognitive impairment patients. *Clin Nutr* 2019; 39(8): 2399-405.

10. Jakubas T, Michalska-Jakubas M, Lukawski K, Janowska A, Czuczwar SJ. Athero-sclerosis risk among children taking antiepileptic drugs. *Pharmacol Rep* 2009; 61: 411-23.

11. Rezaei S, Shab-Bidar S, Abdulahi AA, Djafarian K. Oxcarbazepine administration and the serum levels of homocysteine, vitamin B12 and folate in epileptic patients: a systematic review and meta-analysis. *Seizure* 2017; 45: 87-94.

12. Huang T, Chen Y, Yang B, Yang J, Wahlqvist MK, Li D. Meta-analysis of B vitamin supplementation on plasma homocysteine, cardiovascular and all-cause mortality. *Clin Nutr* 2012; 31(4): 448-54.

13. Deopa B, Parakh M, Dara P, Payal V, Chordiya K, Panday A, *et al*. Effect of folic acid supplementation on seizure control in epileptic children receiving long term antiepileptic therapy. *Indian J Pediatr* 2018; 85(7): 493-7.

14. Bochynska A, Lipczynska-Lojkowska W, Gugała-Iwaniuk M, Lechowicz W, Restel M, Graban A, *et al*. The effect of vitamin B supplementation on homocysteine metabolism and clinical state of patients with chronic epilepsy treated with carbamazepine and valproic acid. *Seizure* 2012; 21(4): 276-81.

15. Emeksiz HC, Serdaroglu A, Biberoglu G, Gulbahar O, Arhan E, Cansu A, *et al*. Assessment of atherosclerosis risk due to the homocysteine-asymmetric dimethylarginine-nitric oxide cascade in children taking antiepileptic drugs. *Seizure* 2013; 22: 124-7.

16. Belcastro V, Striano P, Gorgone G, Costa C, Ciampa C, Caccamo D, *et al*. Hyperhomocysteinemia in epileptic patients on new antiepileptic drugs. *Epilepsia* 2010; 51: 274-9.

17. Sun M, Zhou R, Wang X, Cheng Y. Influence of different antiepileptic drugs on blood ammonia and homocysteine levels in children with epilepsy. *Evid Based Complement Alternat Med* 2021; 2021: 5698765.

18. Rabasseda X. Oxcarbapezine: anticonvulsant profile and safety. *Drugs Today (Barc)* 2001; 37(5): 333-55.

19. Kumar J, Das SK, Sharma P, Karthikeyan G, Ramakrishnan L, Sengupta S, *et al*. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian population. *J Hum Genet* 2005; 50: 655-63.

20. Crider KS, Zhu JH, Hao L, Yang QH, Yang TP, Gindler J, *et al*. MTHFR 677C-T genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation. *Am J Clin Nutr* 2011; 93: 1365-72.

21. Anderson CA, Beresford SA, McLerran D, Lampe JW, Deeb S, Feng Z, *et al*. Response of serum and red blood cell folate concentrations to folic acid supplementation depends on methylenetetrahydrofolate reductase C677T genotype: results from a crossover trial. *Mol Nutr Food Res* 2013; 57: 637-44.

22. Miyaki K, Murata M, Kikuchi H, Takei I, Nakayama T, Watanabe K, *et al*. Assessment of tailor-made prevention of atherosclerosis with folic acid supplementation: randomized, double-blind, placebo-controlled trials in each MTHFR C677T genotype. *J Hum Genet* 2005; 50: 241-8.

23. Rai V, Kumar P. Methylenetetrahydrofolate reductase C677T polymorphism and susceptibility to epilepsy. *Neurol Sci* 2018; 39(12): 2033-41.

24. Binia A, Contreras AV, Canizales-Quinteros S, Alonzo VA, Tejero ME, Silva-Zolezzi I. Geographical and ethnic distribution of single nucleotide polymorphisms within genes of the folate /homocysteine pathway metabolism. *Genes Nutr* 2014; 9(5): 421.

25. Iida K, Tomita K, Okada R, Kawai S, Morita E, Hishida A, *et al*. Applicability of allele/genotype frequency from documented controls for case-control studies on genotypes among Japanese: MTHFR C677T as an example. *Asian Pac J Cancer Prev* 2009; 10(2): 231-6.

26. Yang B, Liu Y, Li Y, Fan S, Zhi X, Lu X, *et al*. Geographical distribution of MTHFR C677T, A1298C and MTRR A66G gene polymorphisms in China: findings from 15357 adults of Han nationality. *PLoS One* 2013; 8(3): e57917.

27. Reyes-Engel A, Munoz E, Gaitan MJ, Fabra E, Gallo M, Dieguez JL, *et al*. Implications on human fertility of the 677CT and 1298AC polymorphisms of the MTHFR gene: consequences of a possible genetic selection. *Mol Hum Reprod* 2002; 8(10): 952-7.

28. Markan S, Sachdeva M, Sehrawat BS, Kumari S, Jain S, Khullar M. MTHFR 677 CT/MTHFR 1298 CC genotypes are

associated with increased risk of hypertension in Indians. *Mol Cell Biochem* 2007; 302(1-2): 125-31.

29. Ullah S, Ali N, Khan A, Ali S, Nazish HR, Uddin Z. Epilepsy control with carbamazepine monotherapy from a genetic perspective. *BMC Pharmacol Toxicol* 2018; 19(1): 73.

30. Caccamo D, Condello S, Gorgone G, Crisafulli G, Belcastro V, Gennaro S, *et al*. Screening for C677T and A1298C MTHFR polymorphisms in patients with epilepsy and risk of hyperhomocysteinemia. *Neuromolecular Med* 2004; 6: 117-26.

31. Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, *et al*. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet* 1985; 37: 1-31.

32. Hamed SA, Hamed EA, Hamdy R, Nabeshima T. Vascular risk factors and oxidative stress as independent predictors of asymptomatic atherosclerosis in adult patients with epilepsy. *Epilepsy Res* 2007; 74: 183-92.

33. Reynolds EH. Vitamin B12, folic acid, and the nervous system. *Lancet* 2006; 2: 473-81.

34. Belcastro V, Striano P. Antiepileptic drugs, hyperhomocysteinemia and B-vitamins supplementation in patients with epilepsy. *Epilepsy Res* 2012; 102: 1-7.

35. Coppen A, Bailey J. Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial. *J Affect Disord* 2000; 60(2): 121-30.

36. Resler G, Lavie R, Campos J, Mata S, Urbina M, Garcia A, *et al*. Effect of folic acid combined with fluoxetine in patients with major depression on plasma homocysteine and vitamin B12, and serotonin levels in lymphocytes. *Neuroimmunomodulation* 2008; 15(3): 145-52.

TEST YOURSELF

(1) Is *MTHFR* polymorphism associated with seizure control in epileptic patients?

(2) How might *MTHFR* C677T polymorphism affect seizure control?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com.