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Increased homocysteine levels in valproate treated epileptic patients: a meta-analysis

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Title

Increased homocysteine levels in valproate treated epileptic patients: a meta-analysis

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Keywords: Homocysteine, Valproate, Meta-analysis, Epilepsy

Word count: 2355 words

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Abstract

Objective: To determine whether valproate monotherapy influences homocysteine metabolism in epileptic patients.

Design: Systematic review and meta-analysis.

Data sources: We search all articles in English through PubMed, Web of Science, EMBASE published up to August 2013 concerning the homocysteine levels in VPA monotherapeutic patients with epilepsy.

Participants: Valproate treated epileptic patients (n=229) and matched healthy control (n=258).

Outcome measures: Heterogeneity between studies was assessed using I^2 statistics. Pooled standardized mean difference (SMD) and 95% confidence intervals (95% CI) were calculated by using random effects or fixed effect models.

Results:A total of 7 eligible studies were enrolled in our meta-analysis. We compared the plasma levels of homocysteine in valproate treated epileptic patients and healthy controls. There was significant heterogeneity in the estimates according to I^2 test ($I^2 =$ 66.9%, P =0.006). Plasma homocysteine levels in VPA treated epileptic patients was significantly higher than healthy controls under a random effect model. [SMD, 0.65; 95% confidence interval (CI), 0.30–0.99, $P < 0.001$]. Moreover, in the subgroup analysis based on ethnic, we found that the plasma homocysteine levels is significantly higher in epileptic patients than healthy controls in all subgroups [European group:SMD, 0.87; 95% confidence interval (CI), 0.43–1.32, $P < 0.001$; West- Asian group:SMD, 0.45; 95% confidence interval (CI), 0.08–0.81, P=0.016; East- Asian group:SMD, 1.26; 95% confidence interval (CI), 0.85–1.66, $P < 0.001$].

Conclusions: Our meta-analysis indicates that VPA monotherapy is associated with the increase in plasma homocysteine in patients with epilepsy and this association was influenced by race.

Strengths and limitations of this study

This study found valproate may play an important role in development of

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hyperhomocysteinemia in epileptic patients.

The number of published studies in our meta-analysis were relatively limited.

After subgroup analysis, most of studies were included in West-Asian group, so the conclusions may not be apply to other ethnic groups.

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Introduction

Homocysteine, a thiol-containing amino acid formed by demethylation of methionine, is an intermediate product in one-carbon metabolism. It is metabolized via two pathways: the remethylation pathway and the transsulfuration pathway. Folic acid, vitamin B-12 and vitamin B-6 are cofactors of these metabolic pathways. Both folic acid and vitamin B-12 are essential for remethylation of homocysteine to methionine. Homocysteine may form cystathionine in transsulfuration pathway which requires vitamin B-6, as a cofactor.[1] Thus, a deficiency of folic acid and vitamin B-12 may lead to elevated plasma homocysteine concentrations. Karabiber et al.[2] reported that long-term treatment with some antiepileptic drugs (AEDs) for epileptic patients, including sodium valproate (VPA) and carbamazepine (CBZ), may lead to hyperhomocysteinemia by affecting the blood concentrations of folate, Vitamin B-12 which have a role in the metabolism of homocysteine. Sener et al.[3] compared epileptic patients not receiving AEDs with healthy controls, and found that there were no significant differences in homocysteine levels. For this reason, they thought that suffering from epilepsy is unlikely to directly interfere with homocysteine metabolism. Therefore, rather than being epileptic in origin, the AEDs may play an important role in development of hyperhomocysteinemia in epileptic patients.

The older generation AEDs can be classified into inducer AEDs(CBZ, phenytoin, phenobarbital) and inhibitor AEDs(VPA) according to whether they can affect cytochrome P450 isozymes[4]. Several studies showed that taking inducer AEDs were associated with a significant elevation in homocysteine concentration, as well as a significant reduction in folic acid in serum, but the effects on the serum concentration of vitamin B-12 remained unclear[1, 5]. Although the mechanisms by which AEDs induce folic acid deficiency are still unclear, the proposed mechanisms can be summarized as interference with the intestinal absorption of folic acid, induction of enzymes in the liver and finally deplete folic acid, and interference with the metabolism of folic acid co-enzymes[6]. The deficiency of folic acid mainly causes an increase in homocysteine level in epileptic patients.

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An elevated plasma homocysteine concentration is a marker of low folate status and an independent risk factor for arteriosclerosis and fetal malformations. The relationship between hyperhomocysteinemia and vaso-occlusive diseases is known for a long time. Recent researches have found that long-term use of older generation AEDs with prominent effects on the enzyme system,including CBZ, PHT, and VPA, may contribute to the progression of atherosclerosis in patients with epilepsy,which is due to the increase in plasma homocysteine levels[7]. High concentrations of total homocysteine is also related to potential teratogenic effects as there is a tenfold increased risk for major congenital malformations including neural tube defects in children whose mothers receive AEDs, during the first trimester[8, 9].

VPA is one of the commonly prescribed AEDs in children, as well as in adults. The literatures hold controversial views on the homocysteine status in patients under treatment with VPA[5, 10]. Many of these researches used relatively small sample sizes, therefore, we performed a meta-analysis of all studies published until August 2013 to elucidate whether VPA treatment could lead to the elevation of plasma homocysteine levels in epileptic patients.

Materials and methods

Identification of studies

We carried out a systematic search for studies reporting the association between plasma homocysteine and valproate monotherapy in PubMed, Web of Science and EMBASE until August 2013. Using the following terms: 'Epilepsy', 'Valproate', 'Homocysteine'. The obtained articles were examined by a quick view at the titles and abstracts and inappropriate articles were rejected in the initial screening. Any study lacking information regarding specific effect of valproate on homocysteine in epileptic patients was also rejected. Definitely, non-controlled design studies, reviews, and animal or in vitro studies were excluded. Two investigators independently reviewed full text eligibility and reached a consensus on which studies to include for review. We also checked the reference of selected articles for any further relevant

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studies.

The enrolled studies had to be in accordance with the following major criteria: a) effect of VPA on homocysteine in patients with epilepsy; b) controlled design studies, VPA compared with healthy controls; c) data of interest (Homocysteine concentration) presented as continuous (mean value and SD or SE).

The studies were excluded if one of the following existed: a) metabolic diseases: diabetes mellitus;b) vitamin supplementation; c) renal and hepatic impairment;d) malignancy; e) vascular diseases: cerebrovascular disease; f) endocrine diseases: hypothyroidism, thyroid dysfunction; g) psychiatric disorders: major depression and schizophrenia; h) smoking and chronic alcohol consumption;i) drugs: thiazide diuretics, azathioprine[11].

Data extraction

The data elements of interest in the studies were extracted by two investigators. Any discrepancies in extracted data were resolved by a consensus conference between the two investigators. The extracted data elements was listed as: first author, publication year, country in which the study was conducted, ethnicity, characteristics of cases and controls (number of cases and controls, mean value and SD of homocysteine in cases and controls).

Statistical analysis

The data of interest presented as continuous (mean value and SD) was analyzed by used either the weighted mean difference (WMD) or the standardized mean difference (SMD) as effect measures. The presence of heterogeneity was assessed using the I-square (I^2) test. When significant heterogeneity was present, source of heterogeneity was further investigated with subgroup analysis and sensitivity analysis was performed as well. Additionally, publication bias was assessed with Begg's and Egger's test. The results of meta-analysis were shown in forest plots. All analyses were conducted using Stata software, version 12.0 (Stata Corp, College Station,TX, USA).

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Results

Literature search and characteristics of eligible studies

In line with our research strategy, a total of 30 potentially relevant researches were identified in our initial literature search. Finally, our research enrolled 7 eligible studies met the inclusion criteria[1-3, 7, 10, 12, 13]. A flow chart showing the study selection is presented in **Fig. 1**. Characteristics of eligible studies including VPA therapeutic dosage, duration of VPA monotherapy, plasma homocysteine concentrations (mean ± SD), were listed in **Table 1**, involving 229 epileptic patients receiving VPA monotherapy and 258 healthy controls. Age and gender were matched between epileptic patients and healthy controls.

Meta analysis of plasma homocysteine levels

There was significant difference between study when all comparisons were considered ($I^2 = 66.9\%$, $P = 0.006$).Based on the comparison of plasma homocysteine levels between epileptic patients receiving VPA monotherapy and healthy controls we found that the plasma homocysteine levels in VPA treated epileptic patients were significantly higher than those in controls. [**Fig. 2**: SMD, 0.65; 95% confidence interval (CI), 0.30–0.99, $P < 0.001$].

Albert et al[14] found that genetic determination of differential concentrations of homocysteine differed among various ethnic groups. Furthermore, we performed the subgroup analysis based on race, the 7 eligible studies were divided into three subgroups (European, West-Asian, East-Asian) according to the race of the participants in studies.

After subgroup analysis, we found that the plasma homocysteine levels is significantly higher in epileptic patients than healthy controls in all subgroups[**Fig. 3**: European group:SMD, 0.87; 95% confidence interval (CI), 0.43–1.32, $P < 0.001$; West- Asian group:SMD, 0.45; 95% confidence interval (CI), 0.08–0.81, $P=0.016$; East- Asian group:SMD, 1.26; 95% confidence interval (CI), 0.85–1.66, $P < 0.001$].

Sensitivity analysis and publication bias

We conducted sensitivity analysis to evaluate the stability of the meta-analysis. When

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any single study was deleted, the corresponding pooled SMD was not substantially altered. Publication bias was assayed by the Begg's funnel plot and Egger's test. The shape of the funnel plots was seemed symmetrical in recessive model (**Fig. 4**). Then, *P* values were 0.368 in Begg's test and 0.211 in Egger's test separately, also suggesting no obvious publication bias.

Discussion

VPA is one of first-line AEDs for controlling most subtypes of epileptic seizures since its antiepileptic properties was recognized in early 1960s. As we know, long-term or lifelong VPA therapy is usually required for most patients with epilepsy. However, prolonged VPA therapy is often associated with a wide range of chronic adverse effects including metabolic and endocrine disturbances, atherosclerotic vascular diseases and major congenital malformations[7, 15]. As an important metabolic product in one-carbon metabolism, homocysteine is a risk factor for atherosclerotic vascular diseases (e.g stroke, myocardiac infarction) and major congenital malformations (e.g neural tube defects)[16, 17]. We noted that conflict existed in reports on the association between VPA and disruption of homocysteine metabolism. Therefore, we conducted a meta-analysis of plasma homocysteine, focusing on VPA treatment in epileptic patients. The results indicated that there was significant higher levels of homocysteine in VPA monotherapeutic patients with epilepsy than those in controls.

In the circle of one-carbon metabolism, homocysteine participates in two metabolic pathways: the remethylation pathway and the transsulfuration pathway. Some cofactors such as folic acid, Vitamin B-12 and Vitamin B-6 play important roles in these metabolic pathways. A significant negative correlation was found between the levels of homocysteine and folic acid in patients using AEDs.[18] Data on VPA effects on folic acid, an enzyme inhibitor AED, is conflicting. Most researches indicated that the VPA decreased the levels of folic acid [1, 2], but other studies found that the levels of folic acid were not significantly lower in the VPA treated patients

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compared with healthy controls[3, 5]. In contrast to inducer AEDs that have various effects on enzyme induction in the liver with folic acid, VPA has no effect on hepatic enzyme induction. VPA may impair intestinal absorption of folic acid, and directly interfere with the metabolism of folic acid co-enzymes[19].

Hyperhomocysteinemia is frequently caused not only by folic acid deficiency but also associated with genetic polymorphisms coding for enzymes involved in the one-carbon metabolism. Compared the plasma homocysteine, folate level and methylentetrahydrofolate reductase (MTHFR) C677T mutation in epileptic patients with those in normal controls , Yoo and Hong found that a common MTHFR C677T mutation was a determinant of hyperhomocysteinemia in epileptic patients receiving AEDs, which suggests that a gene-drug interaction induced hyperhomocysteinemia[20]. MTHFR is a key enzyme in homocysteine remethylation pathway, which plays an important role in transmethylation of homocysteine to methionine. Ono et al. indicated that there was a relationship between the hyperhomocysteinemia and homozygote MTHFR gene variant in epileptic patients receiving multidrug therapy, but not in those receiving monotherapy[21]. However, Vurucu et al which found that the variations in the MTHFR gene had no significant contribution on hyperhomocysteinemia in epileptic patients receiving AEDs therapy[10]. Therefore, further studies are warranted to clarify the mechanism as to how homozygous genotype of thermolabile MTHFR affects plasma homocysteine concentrations in patients with epilepsy when they receive VPA monotherapy or other AEDs treatment.

Although our meta-analysis found that the levels of plasma homocysteine were significantly higher in VPA treated epileptic patients than those in controls, the heterogeneity was also existed. After using the subgroup analysis according to the race, we found the main outcome remained unchanged but the heterogeneity did not exist in West-Asian group. MTHFR as a key enzyme in homocysteine metabolism has been proved correlated with the plasma levels of homocysteine. The presence of the T allele (MTHFR C677T) renders the enzyme thermolabile reducing its enzymatic activity which may cause elevated plasma levels of homocysteine. However, the ethnic composition and the location of sampling can influence the frequency of

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MTHFR genotypes[14, 22]. Cappuccio et al. found that the difference in the practice of folic acid fortification for commonly consumed foods and dietary habits between the countries may explain the plasma levels of homocysteine heterogeneity in different countries[23].In our research, the heterogeneity may be caused by the different races or countries.

Elevated circulating homocysteine, irrespective of the underlying metabolic abnormality, can be detrimental to vascular structure and function through a number of mechanism. Hyperhomocysteinemia is a well-established risk factor for vascular disease such as stroke, myocardiac infarction and peripheral arterial disease[24]. As we know, ultrasonographic determination of mean common carotid artery intima media thickness (CCA IMT) is a marker to stratify the risk of atherosclerosis. Chuang et al. indicated that the levels of plasma homocysteine and the mean CCA IMT were significantly increased in monotherapy with VPA. Moreover, their research demonstrated that the mean CCA IMT was correlated with the duration monotherapy with VPA. Therefore, long-term monotherapy with VPA has been associated with hyperhomocysteinemia that lead to an increase in risk of atherosclerosis in patients with epilepsy[7, 25].

Increased levels of homocysteine are associated with NTD-affected pregnancies, which was regarded as the major risk factor for NTDs[26]. As an important intermediate product in one-carbon metabolism, homocysteine may be a better indicator of methyl group supply and therefore more accurately reflect functional transmethylation in genome-wide methylation. Hyperhomocysteinemia was usually associated with the genome-wide hypo-methylation as assessed using mean long interspersed nucleotide element-1 (LINE-1) methylation in human. Wang et al. found that the reduction in LINE-1 methylation was accompanied by an increased risk of NTDs, indicating that LINE-1 hypomethylation is likely to contribute to the development of NTDs. Because of aberrant genomic methylation underlies the complex pathogenesis of NTDs, high level of homocysteine may interfere genome wide methylation which further induces NTDs[27, 28]. Although recent study confirmed a specific increase in the risk of NTDs associated with maternal use of VPA, the mechanism VPA initiating the molecular

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and biochemical events is still unclear[29]. It is probable that therapy with VPA during pregnancy has been associated with hyperhomocysteinemia that lead to an increase in risk of NTD-affected pregnancies.

Some limitations of this meta-analysis should be considered. First, number of published studies in our meta-analysis were relatively limited. Second, a significant heterogeneity was found in our study. After subgroup analysis, most of studies were included in West-Asian group, so the conclusions may not be apply to other ethnic groups. Third, characteristics of eligible studies was incomplete information about the dosage of valproate monotherapy. Finally, only the studies in English language were considered.

Conclusions

In conclusion, this meta-analysis suggested that VPA monotherapy is associated with the increase in plasma homocysteine in patients with epilepsy.Hyperhomocysteinemia is an independent risk factor for thrombosis, atherosclerosis, and neural tube defects in the offspring of affected women.Therefore, we advise to measure plasma homocysteine in all epileptic patients received valproate treatment. In addition, folate supplementation to reduce homocysteine levels may be useful in valproate treated epileptic patients.

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Acknowledgments

Competing Interests: None

Data Sharing Statement: No additional data are available.

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Table1   Summary of studies included in the meta-analysis

Study	Year	Country	Ethnicity	VPA treated group				Healthy controls	
				Dose	Duration	Cases	Hcy(umol/l)	Cases	Hcy(umol/l)
Verrotti et al.[1]	2000	Italy	European	21.7±6.8 mg/kg	12m	32	12.7±7.1	63	7.9±4.5
Karabiber et al.[2]	2002	Turkey	West-Asian	no	>12m	30	14±6.8	29	9.2±2.7
Sener et al.[3]	2006	Turkey	West-Asian	no	6.5±6.2y	22	17±8.0	11	11.5±11.4
Vurucu et al.[10]	2007	Turkey	West-Asian	no	27.36±21.12m	64	6.88±2.24	62	5.52±2.53
Kurul et al.[13]	2007	Turkey	West-Asian	no	4.78±2.07y	8	7.18±2.54	10	7.66±2.34
Yildiz et al.[12]	2010	Turkey	West-Asian	54.49mg/ml	>6m	19	6.73±2.81	23	6.79±1.95
Chuang et al.[7]	2012	China(Taiwan)	East-Asian	750-1000mg/d	8.7±5.2 y	54	13.84±4.29	60	9.41±2.65

30 relevant studies were enrolled from initial search

22 studies were excluded:
tHcy not presented as continuous data (n=8)
self control of pre and post VPA (n=5)
not clinical research (n=4)
reviews (n=2)
case reports (n=2)
commentary article (n=1)

8 studies were related to VPA and Hcy

1 study was exclude:
the controls were not healthy controls

7 studies were included in our meta-analysis finally
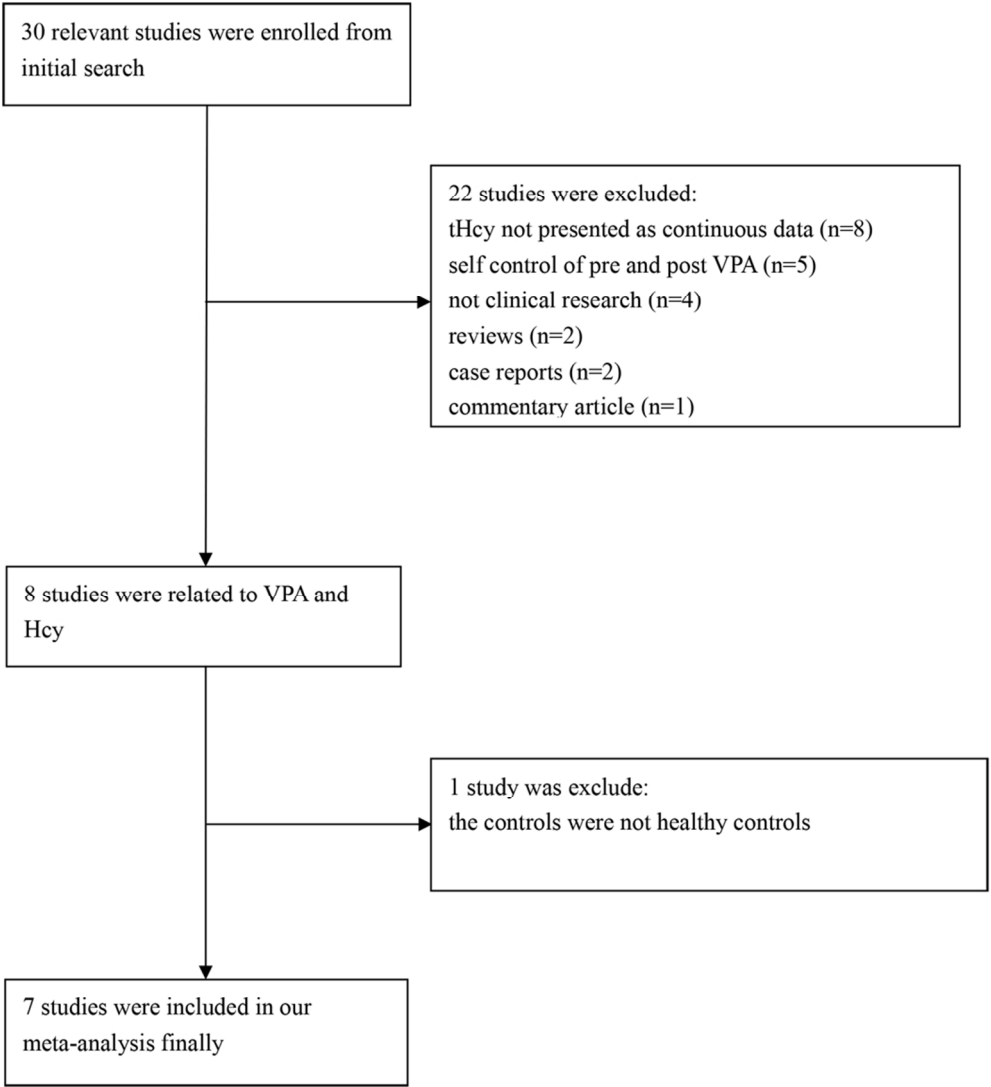
Fig.1. Search strategy for meta-analysis
90x98mm (300 x 300 DPI)
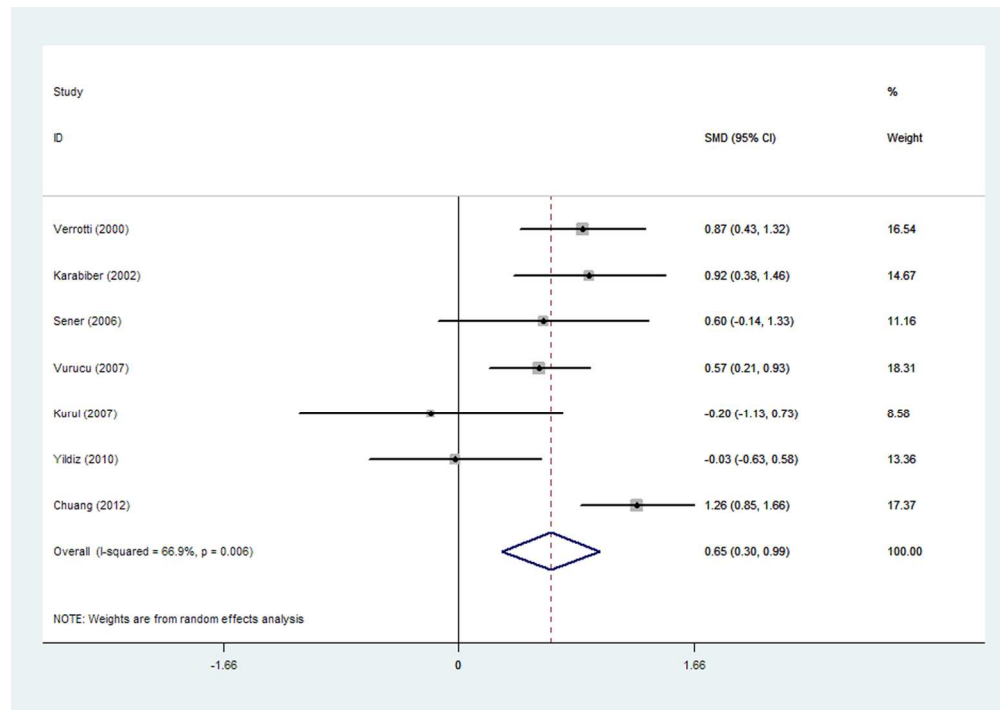
Fig.2. Pooled estimate of SMD and 95% CI of plasma homocysteine levels in epileptic patients received VPA monotherapy
140x98mm (300 x 300 DPI)
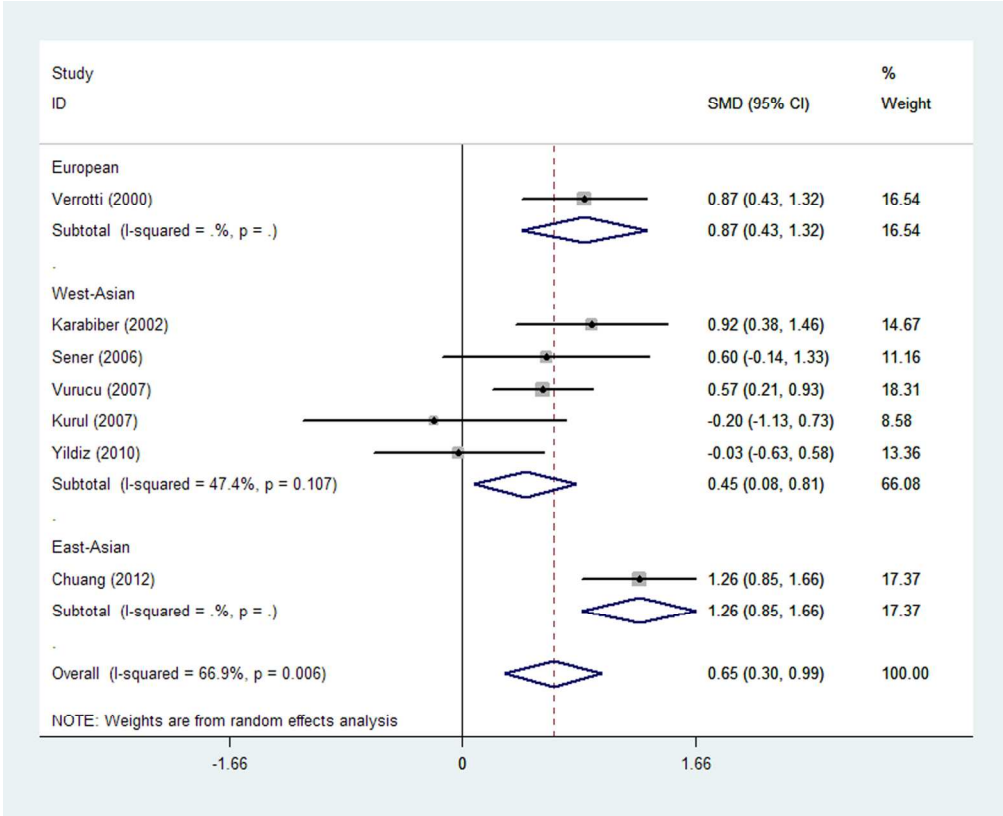
Fig.3. Pooled estimate of SMD and 95% CI of plasma homocysteine levels in epileptic patients received VPA monotherapy among different ethnicity populations.
140x113mm (300 x 300 DPI)
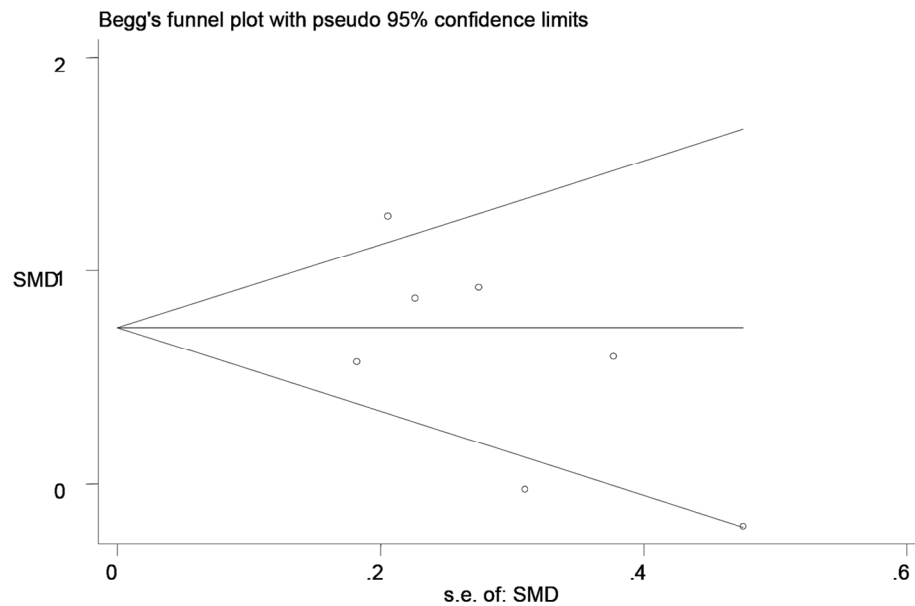
Fig.4. Funnel plot for publication bias in selection of studies on the plasma homocysteine in epileptic patients receive VPA monotherapy
140x90mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5,6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5,6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

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Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7,8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7,8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8,9,10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	11

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Increased homocysteine levels in valproate treated patients with epilepsy: a meta-analysis

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Title

Increased homocysteine levels in valproate treated patients with epilepsy: a

meta-analysis

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Keywords: Homocysteine, Valproate, Meta-analysis, Epilepsy

Word count: 2228 words

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Abstract

Objective: To determine whether valproate (VPA) monotherapy influences homocysteine metabolism in patients with epilepsy.

Design: Systematic review and meta-analysis.

Data sources: We search all articles in English through PubMed, Web of Science, EMBASE published up to August 2013 concerning the homocysteine levels in VPA monotherapeutic patients with epilepsy.

Participants:VPA treated patients with epilepsy (n=266) and matched healthy controls (n=489).

Outcome measures: Heterogeneity between studies was assessed using I^2 statistics. Pooled standardized mean difference (SMD) and 95% confidence intervals (95% CI) were calculated by using random effect model.

Results:A total of 8 eligible studies were enrolled in our meta-analysis. We compared the plasma levels of homocysteine in VPA treated patients with epilepsy and healthy controls. There was significant heterogeneity in the estimates according to I^2 test ($I^2 = 65.6\%$, $P =0.005$). Plasma homocysteine levels in VPA treated patients with epilepsy was significantly higher than healthy controls under a random effect model. [SMD, 0.62; 95% CI, 0.32–0.92]. Further subgroup analyses suggested that no significant differences were present when grouped by ethnicity and age, but the risk of heterogeneity in West-Asian group ($I^2 = 47.4\%$, $P =0.107$) was diminished when compare with over-all groups ($I^2 = 65.6\%$, $P =0.005$).

Conclusions: Our meta-analysis indicates that VPA monotherapy is associated with the increase in plasma homocysteine levels in patients with epilepsy.Whether this association is influenced by ethnicity needs further research.

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Strengths and limitations of this study

This study found VPA may play an important role in development of hyperhomocysteinemia in patients with epilepsy.

The number of included studies in our meta-analysis were relatively limited.

In subgroup analysis, the subgroup may be underpowered as the East-Asian group had only one study.

Introduction

Homocysteine, a thiol-containing amino acid formed by demethylation of methionine, is an intermediate product in one-carbon metabolism(OCM). Folic acid and vitamin B-12 are cofactors of OCM.[1]Deficiency of folic acid and vitamin B-12 may lead to elevated plasma homocysteine concentrations. Karabiber et al.[2] reported that long-term treatment with some antiepileptic drugs (AEDs) for patients with epilepsy might lead to hyperhomocysteinemia by affecting the blood concentrations of folate, Vitamin B-12. Sener et al.[3]compared patients with epilepsy not receiving AEDs with healthy controls, and found that there were no significant differences in homocysteine levels. For this reason, they thought that suffering from epilepsy was unlikely to directly interfere with homocysteine metabolism. Therefore, rather than being epileptic in origin, the AEDs may play an important role in development of hyperhomocysteinemia in patients with epilepsy.

An elevated plasma homocysteine concentration is a marker of low folate status and an independent risk factor for arteriosclerosis and fetal malformations. The relationship between hyperhomocysteinemia and vaso-occlusive diseases is known for a long time. Recent researches have found that long-term use of older generation AEDs with prominent effects on the enzyme system,including carbamazepine (CBZ), phenytoin (PHT), and valproate (VPA), may contribute to the progression of atherosclerosis in patients with epilepsy,which is due to the increase in plasma homocysteine levels.[4] High concentrations of total homocysteine is also related to potential teratogenic effects as there is a tenfold increased risk for major congenital malformations including neural tube defects in children whose mothers receive AEDs, especially during the first trimester.[5, 6]

VPA is one of the commonly prescribed AEDs in children, as well as in adults. The literatures hold controversial views on the homocysteine status in patients under treatment with VPA.[7, 8] Many of these researches used relatively small sample sizes, therefore, we performed a meta-analysis of all studies published until August 2013 to elucidate whether VPA treatment could lead to the elevation of plasma homocysteine

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levels in patients with epilepsy.

Materials and methods

Identification of studies

We developed a protocol prior to conducting this research. Eligible studies met the following criteria: a) effect of VPA on homocysteine in patients with epilepsy; b) controlled design studies, VPA compared with healthy controls; c) data of interest (Homocysteine concentration) presented as continuous (mean value and SD or SE). The studies were excluded if one of the following existed: a) metabolic diseases: diabetes mellitus;b) vitamin supplementation; c) renal and hepatic impairment;d) malignancy; e) vascular diseases: cerebrovascular disease; f) endocrine diseases: hypothyroidism, thyroid dysfunction; g) psychiatric disorders: major depression and schizophrenia; h) smoking and chronic alcohol consumption;i) drugs: thiazide diuretics, azathioprine.[9]

We carried out a systematic search for studies reporting the association between plasma homocysteine and VPA monotherapy in PubMed, Web of Science and EMBASE until August 2013, using the following terms: 'Epilepsy', 'Valproate', 'Homocysteine'and 'Epilep*' . The obtained articles were examined by a quick view at the titles and abstracts and inappropriate articles were rejected in the initial screening. We selected observational case-control studies that evaluated homocysteine levels in subjects with epilepsy compared to controls. Any study lacking information regarding specific effect of VPA on homocysteine in patients with epilepsy was also rejected. Definitely, non-controlled design studies, reviews, and animal or in vitro studies were excluded. Two investigators independently reviewed full text eligibility and reached a consensus on which studies to include for review. We also checked the reference of selected articles for any further relevant studies.

Data extraction

The data elements of interest in the studies were extracted by two investigators. Any

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discrepancies in extracted data were resolved by a consensus conference between the two investigators. The extracted data elements was listed as: first author, publication year, ethnicity, characteristics of cases and controls (number of cases and controls, mean value and SD of homocysteine in cases and controls).

Quality appraisal of the included research

Two investigators independently assessed the methodological quality of included studies using the Newcastle–Ottawa quality assessment scale (NOS) for case–control studies, which contained nine items that categorized into three major categories. The maximum for selection was 4, for comparability was 2 and for exposure was 3. The ultimate score of 6 or more was regarded as high-quality.

Statistical analysis

The data of interest presented as continuous (mean value and SD) was analyzed by using either the weighted mean difference (WMD) or the standardized mean difference (SMD) as effect measures. The presence of heterogeneity was assessed using the I-square (I^2) test. If there was no statistical difference about heterogeneity (p >0.05), a fixed-effect model was applied to analyze the data. Otherwise, a random-effect model would be put into use.To explore the sources of heterogeneity, further subgroup analysis was performed according to ethnicity and age. The stability of the study was also detected by sensitivity analysis.The results of meta-analysis were shown in forest plots. All analyses were conducted using Stata software, version 12.0 (Stata Corp, College Station,TX, USA).

Results

Literature search and characteristics of eligible studies

In line with our research strategy, a total of 30 potentially relevant researches were identified in our initial literature search. Finally, our research enrolled 8 eligible studies met the inclusion criteria.[1-4, 7, 10-12] A flow chart showing the study selection was presented in **Fig. 1**. Characteristics of eligible studies including VPA therapeutic dosage, duration of VPA monotherapy, plasma homocysteine concentrations (mean ±

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SD) were listed in **Table 1**, which involved 266 patients with epilepsy receiving VPA monotherapy and 489 healthy controls.

Quality assessment of included literature

The results of quality assessment of included studies using the Newcastle–Ottawa Scale (NOS) were shown in **Table 2**. All studies reported that diagnoses of cases and controls were based on criteria and clinical records, and thus all studies were assigned points for "adequate definition of cases" and "definition of controls." Three studies reported consecutive participants. All studies reached a total quality score of 6 or greater.

Meta analysis of plasma homocysteine levels

There was marked heterogeneity when all comparisons were considered (I^2 = 65.6%, P =0.005).Based on the comparison of plasma homocysteine levels between patients with epilepsy receiving VPA monotherapy and healthy controls, we found that the plasma homocysteine levels in VPA treated patients with epilepsy were significantly higher than those in controls. [**Fig. 2**: SMD, 0.62; 95%CI, 0.32-0.92].

To explore the sources of heterogeneity among the studies, we performed subgroup analyses by ethnicity (European,West-Asian and East-Asian) and participants' age (<18 years and≧18 years) **Table 3**. Although subgroup analyses suggested that no significant differences were present when grouped by ethnicity and age, the risk of heterogeneity in West-Asian group (I^2 = 47.4%, P =0.107) was diminished as compared with over-all groups (I^2 = 65.6%, P =0.005). We also conducted sensitivity analysis to evaluate the stability of the meta-analysis. When any single study was deleted, the corresponding pooled SMD was not substantially altered.

Discussion

The results of random-effects meta-analysis indicate that VPA treated patients with epilepsy have a higher levels of homocysteine than healthy controls. It suggests that the high plasma level of homocysteine seems to be an important risk factor for VPA

monotherapy in patients with epilepsy. This result is in keeping with some data showing that VPA monotherapy may have harmful effects on arteriosclerosis and fetal malformations.

VPA is one of the first-line AEDs for controlling most subtypes of epileptic seizures since its antiepileptic properties was recognized in early 1960s. As we know, long-term or lifelong VPA therapy is usually required for most patients with epilepsy. However, prolonged VPA therapy is often associated with a wide range of chronic adverse effects including metabolic and endocrine disturbances, atherosclerotic vascular diseases and major congenital malformations.[4, 13] As an important metabolic product in OCM, homocysteine is a risk factor for atherosclerotic vascular diseases (e.g stroke, myocardiac infarction) and major congenital malformations (e.g neural tube defects-NTDs).[14, 15]

In the circle of OCM, homocysteine participates in two metabolic pathways: the remethylation pathway and the transsulfuration pathway. Some cofactors such as folic acid, Vitamin B-12 play important roles in these metabolic pathways. A significant negative correlation was found between the levels of homocysteine and folic acid in patients using AEDs.[16] Data on VPA effects on folic acid is conflicting. Most researches indicated that the VPA decreased the levels of folic acid, but other studies found that the levels of folic acid were not significantly lower in the VPA treated patients compared with healthy controls.[1-3, 8] In contrast to inducer AEDs that have various effects on enzyme induction in the liver with folic acid, VPA has no effect on hepatic enzyme induction. VPA may impair intestinal absorption of folic acid, and directly interfere with the metabolism of folic acid co-enzymes.[17]

Hyperhomocysteinemia is frequently caused not only by folic acid deficiency but also by genetic polymorphisms coding for enzymes involved in the OCM. Comparing the plasma homocysteine, folate level and methylentetrahydrofolate reductase (MTHFR) C677T mutation in patients with epilepsy with those in normal controls , Yoo and Hong found that a common MTHFR C677T mutation was a determinant of hyperhomocysteinemia in patients with epilepsy receiving AEDs, which suggests that a gene-drug interaction induced hyperhomocysteinemia.[18] MTHFR is a key enzyme in

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homocysteine remethylation pathway, which plays an important role in transmethylation of homocysteine to methionine. Ono et al. indicated that there was a relationship between the hyperhomocysteinemia and homozygote MTHFR gene variant in patients with epilepsy receiving multidrug therapy, but not in those receiving monotherapy.[19] However, Vurucu et al found that the variations in the MTHFR gene had no significant contribution on hyperhomocysteinemia in patients with epilepsy receiving AEDs therapy.[7] Therefore, further studies are required to clarify the mechanism as to how homozygous genotype of thermolabile MTHFR affects plasma homocysteine concentrations in patients with epilepsy when they receive VPA monotherapy or other AEDs treatment.

Although our meta-analysis found that the levels of plasma homocysteine were significantly higher in VPA treated patients with epilepsy than those in controls, the heterogeneity was also existed. After using the subgroup analysis according to the ethnicity,the risk of heterogeneity in West-Asian group was diminished as compared with over-all groups. MTHFR as a key enzyme in homocysteine metabolism has been proved correlated with the plasma levels of homocysteine. The presence of the T allele (MTHFR C677T) renders the enzyme thermolabile reducing its enzymatic activity which may cause elevated plasma levels of homocysteine. However, the ethnic composition and the location of sampling can influence the frequency of MTHFR genotypes.[20, 21] One research indicated that the prevalence of the 677T allele varied from 9.34-40.53% in different ethnic groups, the lowest being demonstrated for Southern Asians and the highest for East-Asian.[22] Whether the risk of heterogeneity between studies is influenced by MTHFR polymorphism needs further research.

Elevated circulating homocysteine, irrespective of the underlying metabolic abnormality, can be detrimental to vascular structure and function through a number of mechanism. Hyperhomocysteinemia is a well-established risk factor for vascular disease such as stroke, myocardiac infarction and peripheral arterial disease.[23] As we know, ultrasonographic determination of mean common carotid artery intima media thickness (CCA IMT) is a marker to stratify the risk of atherosclerosis. Chuang et al. indicated that the levels of plasma homocysteine and the mean CCA IMT were

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significantly increased in monotherapy with VPA. Moreover, their research demonstrated that the mean CCA IMT was correlated with the duration monotherapy with VPA. Therefore, long-term monotherapy with VPA has been associated with hyperhomocysteinemia that lead to an increase in risk of atherosclerosis in patients with epilepsy.[4, 24]

Increased levels of homocysteine are associated with NTD-affected pregnancies, which is regarded as the major risk factor for NTDs.[25] As an important intermediate product in OCM, homocysteine may be a better indicator of methyl group supply and therefore more accurately reflects functional transmethylation in genome-wide methylation. Hyperhomocysteinemia is usually associated with the genome-wide hypo-methylation as assessed using mean long interspersed nucleotide element-1 (LINE-1) methylation in human. Wang et al. found that the reduction in LINE-1 methylation was accompanied by an increased risk of NTDs, indicating that LINE-1 hypomethylation was likely to contribute to the development of NTDs. Because of aberrant genomic methylation underlies the complex pathogenesis of NTDs, high level of homocysteine may interfere genome wide methylation which further induces NTDs.[26, 27] Although recent study confirmed a specific increase in the risk of NTDs associated with maternal use of VPA, the mechanism VPA initiating the molecular and biochemical events was still unclear.[28] It is probable that therapy with VPA during pregnancy is associated with hyperhomocysteinemia that leads to an increase in risk of NTD-affected pregnancies.

Some limitations of this meta-analysis should be considered. First, the number of published studies in our meta-analysis is relatively limited. Second, a significant heterogeneity is found in our study. In subgroup analyses, the subgroup may be underpowered as the East-Asian group has only one study.Third, characteristics of eligible studies have incomplete information about the dosage of VPA monotherapy. Finally, only the studies in English are considered.

Conclusions

In conclusion, this meta-analysis suggests that VPA monotherapy is associated with

the increase in plasma homocysteine levels in patients with epilepsy. Hyperhomocysteinemia is an independent risk factor for thrombosis, atherosclerosis, and neural tube defects in the offspring of affected women.Therefore, we advise to measure plasma homocysteine in all patients with epilepsy received VPA treatment. In addition, folate supplementation to reduce homocysteine levels may be useful in VPA treated patients with epilepsy.

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Table1 Summary of studies included in the meta-analysis

Study	Year	Ethnicity	VPA treated group				Healthy controls	
			Dose	Duration	Cases	Hcy(umol/l)	Cases	Hcy(umol/l)
Verrotti et al.	2000	European	21.7±6.8 mg/kg	12m	32	12.7±7.1	63	7.9±4.5
Karabiber et al.	2002	West-Asian	no	>12m	30	14±6.8	29	9.2±2.7
Sener et al.	2006	West-Asian	no	6.5±6.2y	22	17±8.0	11	11.5±11.4
Vurucu et al.	2007	West-Asian	no	27.36±21.12m	64	6.88±2.24	62	5.52±2.53
Kurul et al.	2007	West-Asian	no	4.78±2.07y	8	7.18±2.54	10	7.66±2.34
Yildiz et al.	2010	West-Asian	54.49mg/ml	>6m	19	6.73±2.81	23	6.79±1.95
Belcastro et al	2010	European	946.4±172mg/d	>6m	37	10.4±3.04	231	9.1±3.04
Chuang et al.	2012	East-Asian	750-1000mg/d	8.7±5.2 y	54	13.84±4.29	60	9.41±2.65

Table2 Results of quality assessment by Newcastle–Ottawa Scale

Study	Selection				Comparability		Exposure			Total
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Control for additional factor	Ascertainment of exposure	Same method to ascertain	Non-response rate	
Verrotti et al	1	0	1	1	1	1	1	1	0	7
Karabiber et al	1	0	1	1	1	0	1	1	0	6
Sener et al	1	0	1	1	1	0	1	1	0	6
Vurucu et al	1	0	1	1	1	0	1	1	0	6
Kurul et al	1	0	1	1	1	1	1	1	0	7
Yildiz et al	1	1	1	1	1	0	1	1	0	7
Belcastro et al	1	1	1	1	1	0	1	1	0	7
Chuang et al	1	1	1	1	1	0	1	1	0	7

Table 3 Differences between studies by subgroup analysis

Subgroups	No. of studies	SMD (95% CI)	I^2(%)	p
Ethnicity				
European	2	0.63 (0.19 to 1.06)	58.0	0.005
West-Asian	4	0.45 (0.08 to 0.81)	47.4	0.016
East-Asian	1	1.26 (0.85 to 1.66)	0	<0.001
Age (years)				
<18	5	0.52 (0.15 to 0.89)	58.8	0.006
≧18	2	0.77 (0.18 to 1.36)	79.0	0.01

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Figure legends

Fig.1 Search strategy for meta-analysis

Fig.2 Pooled estimate of SMD and 95%CI of plasma homocysteine levels in patients

with epilepsy received VPA monotherapy

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Title

Increased homocysteine levels in valproate treated patients with epilepsy epileptic patients: a meta-analysis

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Keywords: Homocysteine, Valproate, Meta-analysis, Epilepsy

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1

Abstract

Objective: To determine whether valproate (VPA) monotherapy influences homocysteine metabolism in patients with epilepsy epileptic patients.

Design: Systematic review and meta-analysis.

Data sources: We search all articles in English through PubMed, Web of Science, EMBASE published up to August 2013 concerning the homocysteine levels in VPA monotherapeutic patients with epilepsy.

Participants: Valproate VPA treated patients with epilepsy epileptic patients (n=266229) and matched healthy controls (n=489258).

Outcome measures: Heterogeneity between studies was assessed using I^2 statistics. Pooled standardized mean difference (SMD) and 95% confidence intervals (95% CI) were calculated by using random effects or fixed effect models effect model.

Results:A total of 87 eligible studies were enrolled in our meta-analysis. We compared the plasma levels of homocysteine in VPA valproate treated patients with epilepsy epileptic patients and healthy controls. There was significant heterogeneity in the estimates according to I^2 test (I^2 = 65.666.9%, P =0.0056). Plasma homocysteine levels in VPA treated patients with epilepsy epileptic patients was significantly higher than healthy controls under a random effect model. [SMD, 0.620.65; 95% confidence interval (CI), 0.32-0.920.30 0.99, P < 0.001]. Further subgroup analyses suggested that no significant differences were present when grouped by ethnicity and age, the risk of heterogeneity in West-Asian group (I^2 = 47.4%, P =0.107) was diminished when compare with over-all groups (I^2 = 65.6%, P =0.005) Moreover, in the subgroup analysis based on ethnic, we found that the plasma homocysteine levels is significantly higher in epileptic patients than healthy controls in all subgroups [European group:SMD, 0.87; 95% confidence interval (CI), 0.43 1.32, P < 0.001; West Asian group:SMD, 0.45; 95% confidence interval (CI), 0.08 0.81, P=0.016; East Asian group:SMD, 1.26; 95% confidence interval (CI), 0.85 1.66, P < 0.001].

Conclusions: Our meta-analysis indicates that VPA monotherapy is associated with the increase in plasma homocysteine levels in patients with epilepsy and this

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association was influenced by race.Whether this association is influenced by ethnicity needs further research.

Strengths and limitations of this study

This study found VPA valproate may play an important role in development of hyperhomocysteinemia in patients with epilepsy epileptic patients.

The number of included published studies in our meta-analysis were relatively limited.

In subgroup analysis, the subgroup may be underpowered as the East-Asian group had only one studyAfter subgroup analysis, most of studies were included in West Asian group, so the conclusions may not be apply to other ethnic groups.

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Introduction

Homocysteine, a thiol-containing amino acid formed by demethylation of methionine, is an intermediate product in one-carbon metabolism(OCM). ~~It is metabolized via two pathways: the remethylation pathway and the transsulfuration pathway.~~ Folic acid, ~~and~~ vitamin B-12 ~~and vitamin B-6~~ are cofactors of ~~these metabolic pathways~~ OCM. ~~Both folic acid and vitamin B-12 are essential for remethylation of homocysteine to methionine. Homocysteine may form cystathionine in transsulfuration pathway which requires vitamin B-6, as a cofactor.[1]~~ ~~Thus, a d~~Deficiency of folic acid and vitamin B-12 may lead to elevated plasma homocysteine concentrations. Karabiber et al. [2]reported that long-term treatment with some antiepileptic drugs (AEDs) for patients with epilepsy ~~epileptic patients, including sodium valproate (VPA) and carbamazepine (CBZ), may~~ might lead to hyperhomocysteinemia by affecting the blood concentrations of folate, Vitamin B-12 ~~which have a role in the metabolism of homocysteine~~. Sener et al.[3] compared ~~epileptic~~ patients with epilepsy not receiving AEDs with healthy controls, and found that there were no significant differences in homocysteine levels. For this reason, they thought that suffering from epilepsy was~~is~~ unlikely to directly interfere with homocysteine metabolism. Therefore, rather than being epileptic in origin, the AEDs may play an important role in development of hyperhomocysteinemia in ~~epileptic~~ patients with epilepsy.

~~The older generation AEDs can be classified into inducer AEDs(CBZ, phenytoin, phenobarbital) and inhibitor AEDs(VPA) according to whether they can affect cytochrome P450 isozymes[4]. Several studies showed that taking inducer AEDs were associated with a significant elevation in homocysteine concentration, as well as a significant reduction in folic acid in serum, but the effects on the serum concentration of vitamin B-12 remained unclear[1, 5]. Although the mechanisms by which AEDs induce folic acid deficiency are still unclear, the proposed mechanisms can be summarized as interference with the intestinal absorption of folic acid, induction of enzymes in the liver and finally deplete folic acid, and interference with the metabolism of folic acid co-enzymes[6]. The deficiency of folic acid mainly causes an~~

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increase in homocysteine level in epileptic patients.

An elevated plasma homocysteine concentration is a marker of low folate status and an independent risk factor for arteriosclerosis and fetal malformations. The relationship between hyperhomocysteinemia and vaso-occlusive diseases is known for a long time. Recent researches have found that long-term use of older generation AEDs with prominent effects on the enzyme system,including carbamazepine (CBZ), phenytoin (PHT), and valproate (VPA),CBZ, PHT, and VPA, may contribute to the progression of atherosclerosis in patients with epilepsy,which is due to the increase in plasma homocysteine levels[7]. High concentrations of total homocysteine is also related to potential teratogenic effects as there is a tenfold increased risk for major congenital malformations including neural tube defects in children whose mothers receive AEDs, especially during the first trimester[8, 9].

VPA is one of the commonly prescribed AEDs in children, as well as in adults. The literatures hold controversial views on the homocysteine status in patients under treatment with VPA[5, 10]. Many of these researches used relatively small sample sizes, therefore, we performed a meta-analysis of all studies published until August 2013 to elucidate whether VPA treatment could lead to the elevation of plasma homocysteine levels in patients with epilepsy epileptic patients.

Materials and methods

Identification of studies

We developed a protocol prior to conducting this research. Eligible studies met the following criteria: a) effect of VPA on homocysteine in patients with epilepsy; b) controlled design studies, VPA compared with healthy controls; c) data of interest (Homocysteine concentration) presented as continuous (mean value and SD or SE). The studies were excluded if one of the following existed: a) metabolic diseases: diabetes mellitus;b) vitamin supplementation; c) renal and hepatic impairment;d) malignancy; e) vascular diseases: cerebrovascular disease; f) endocrine diseases: hypothyroidism, thyroid dysfunction; g) psychiatric disorders: major depression and

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schizophrenia; h) smoking and chronic alcohol consumption;i) drugs: thiazide diuretics, azathioprine[11].

We carried out a systematic search for studies reporting the association between plasma homocysteine and VPA ~~valproate~~ monotherapy in PubMed, Web of Science and EMBASE until August 2013~~. Using~~, using the following terms: 'Epilepsy', 'Valproate', 'Homocysteine'and 'Epilep*'. The obtained articles were examined by a quick view at the titles and abstracts and inappropriate articles were rejected in the initial screening. We selected observational case-control studies that evaluated homocysteine levels in subjects with epilepsy compared to controls. Any study lacking information regarding specific effect of ~~valproate~~ VPA on homocysteine in patients with epilepsy ~~epileptic patients~~ was also rejected. Definitely, non-controlled design studies, reviews, and animal or in vitro studies were excluded. Two investigators independently reviewed full text eligibility and reached a consensus on which studies to include for review. We also checked the reference of selected articles for any further relevant studies.

~~The enrolled studies had to be in accordance with the following major criteria: a) effect of VPA on homocysteine in patients with epilepsy; b) controlled design studies, VPA compared with healthy controls; c) data of interest (Homocysteine concentration) presented as continuous (mean value and SD or SE).~~

~~The studies were excluded if one of the following existed: a) metabolic diseases: diabetes mellitus;b) vitamin supplementation; c) renal and hepatic impairment;d) malignancy; e) vascular diseases: cerebrovascular disease; f) endocrine diseases: hypothyroidism, thyroid dysfunction; g) psychiatric disorders: major depression and schizophrenia; h) smoking and chronic alcohol consumption;i) drugs: thiazide diuretics, azathioprine[11].~~

Data extraction

The data elements of interest in the studies were extracted by two investigators. Any discrepancies in extracted data were resolved by a consensus conference between the two investigators. The extracted data elements was listed as: first author, publication year, ~~country in which the study was conducted,~~ ethnicity, characteristics of cases and

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controls (number of cases and controls, mean value and SD of homocysteine in cases and controls).

Quality appraisal of the included research

Two investigators independently assessed the methodological quality of included studies using the Newcastle–Ottawa quality assessment scale (NOS) for case–control studies, which contained nine items that categorized into three major categories. The maximum for selection was 4, for comparability was 2 and for exposure was 3. The ultimate score of 6 or more was regarded as high-quality.

Statistical analysis

The data of interest presented as continuous (mean value and SD) was analyzed by using~~used~~ either the weighted mean difference (WMD) or the standardized mean difference (SMD) as effect measures. The presence of heterogeneity was assessed using the I-square (I^2) test. If there was no statistical difference about heterogeneity (p >0.05), a fixed-effect model was applied to analyze the data. Otherwise, a random-effect model would be put into use.To explore the sources of heterogeneity, further subgroup analysis was performed according to ethnicity and age. The stability of the study was also detected by sensitivity analysis. ~~When significant heterogeneity was present, source of heterogeneity was further investigated with subgroup analysis and sensitivity analysis was performed as well. Additionally, publication bias was assessed with Begg's and Egger's test.~~ The results of meta-analysis were shown in forest plots. All analyses were conducted using Stata software, version 12.0 (Stata Corp, College Station,TX, USA).

Results

Literature search and characteristics of eligible studies

In line with our research strategy, a total of 30 potentially relevant researches were identified in our initial literature search. Finally, our research enrolled 8~~7~~ eligible studies met the inclusion criteria[1-3, 7, 10, 12, 13]. A flow chart showing the study selection was~~is~~ presented in **Fig. 1**. Characteristics of eligible studies including VPA

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therapeutic dosage, duration of VPA monotherapy, plasma homocysteine concentrations (mean ± SD), were listed in **Table 1**, which involved involving 266229 patients with epilepsy epileptic patients receiving VPA monotherapy and 489258 healthy controls. Age and gender were matched between epileptic patients and healthy controls.

Quality assessment of included literature

The results of quality assessment of included studies using the Newcastle–Ottawa Scale (NOS) were shown in **Table 2**. All studies reported that diagnoses of cases and controls were based on criteria and clinical records, and thus all studies were assigned points for "adequate definition of cases" and "definition of controls." Three studies reported consecutive participants. All studies reached a total quality score of 6 or greater.

Meta analysis of plasma homocysteine levels

There was significant difference between study marked heterogeneity when all comparisons were considered (I^2 = 65.666.9%, P =0.0056).Based on the comparison of plasma homocysteine levels between patients with epilepsy epileptic patients receiving VPA monotherapy and healthy controls, we found that the plasma homocysteine levels in VPA treated patients with epilepsy epileptic patients were significantly higher than those in controls. [**Fig. 2**: SMD, 0.620.65; 95% confidence interval (CI), 0.32-0.920.30–0.99, P < 0.001].

To explore the sources of heterogeneity among the studies, we performed subgroup analyses by ethnicity (European,West-Asian and East-Asian) and participants' age (<18 years and ≧18 years) **Table 3**. Although subgroup analyses suggested that no significant differences were present when grouped by ethnicity and age, the risk of heterogeneity in West-Asian group (I^2 = 47.4%, P =0.107) was diminished as compared with over-all groups (I^2 = 65.6%, P =0.005). We also conducted sensitivity analysis to evaluate the stability of the meta-analysis. When any single study was deleted, the corresponding pooled SMD was not substantially altered.

Albert et al[14] found that genetic determination of differential concentrations of

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homocysteine differed among various ethnic groups. Furthermore, we performed the subgroup analysis based on race, the 7 eligible studies were divided into three subgroups (European, West Asian, East Asian) according to the race of the participants in studies.

After subgroup analysis, we found that the plasma homocysteine levels is significantly higher in epileptic patients than healthy controls in all subgroups[**Fig. 3**: European group:SMD, 0.87; 95% confidence interval (CI), 0.43–1.32, $P < 0.001$; West Asian group:SMD, 0.45; 95% confidence interval (CI), 0.08–0.81, $P=0.016$; East Asian group:SMD, 1.26; 95% confidence interval (CI), 0.85–1.66, $P < 0.001$].
Sensitivity analysis and publication bias
We conducted sensitivity analysis to evaluate the stability of the meta-analysis. When any single study was deleted, the corresponding pooled SMD was not substantially altered. Publication bias was assayed by the Begg's funnel plot and Egger's test. The shape of the funnel plots was seemed symmetrical in recessive model (**Fig. 4**). Then, P values were 0.368 in Begg's test and 0.211 in Egger's test separately, also suggesting no obvious publication bias.

Discussion

The results of random-effects meta-analysis indicate that VPA treated patients with epilepsy have a higher levels of homocysteine than healthy controls. It suggests that the high plasma level of homocysteine seems to be an important risk factor for VPA monotherapy in patients with epilepsy. This result is in keeping with some data showing that VPA monotherapy may have harmful effects on arteriosclerosis and fetal malformations.

VPA is one of the first-line AEDs for controlling most subtypes of epileptic seizures since its antiepileptic properties was recognized in early 1960s. As we know, long-term or lifelong VPA therapy is usually required for most patients with epilepsy. However, prolonged VPA therapy is often associated with a wide range of chronic adverse effects including metabolic and endocrine disturbances, atherosclerotic

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vascular diseases and major congenital malformations[7, 15]. As an important metabolic product in OCM ~~one-carbon metabolism~~, homocysteine is a risk factor for atherosclerotic vascular diseases (e.g stroke, myocardiac infarction) and major congenital malformations (e.g neural tube defects)[16, 17]. ~~We noted that conflict existed in reports on the association between VPA and disruption of homocysteine metabolism. Therefore, we conducted a meta-analysis of plasma homocysteine, focusing on VPA treatment in epileptic patients. The results indicated that there was significant higher levels of homocysteine in VPA monotherapeutic patients with epilepsy than those in controls.~~

In the circle of OCM ~~one-carbon metabolism~~, homocysteine participates in two metabolic pathways: the remethylation pathway and the transsulfuration pathway. Some cofactors such as folic acid, Vitamin B-12 ~~and Vitamin B-6~~ play important roles in these metabolic pathways. A significant negative correlation was found between the levels of homocysteine and folic acid in patients using AEDs.[18]. Data on VPA effects on folic acid~~, an enzyme inhibitor AED,~~ is conflicting. Most researches indicated that the VPA decreased the levels of folic acid [1, 2], but other studies found that the levels of folic acid were not significantly lower in the VPA treated patients compared with healthy controls[3, 5]. In contrast to inducer AEDs that have various effects on enzyme induction in the liver with folic acid, VPA has no effect on hepatic enzyme induction. VPA may impair intestinal absorption of folic acid, and directly interfere with the metabolism of folic acid co-enzymes[19].

Hyperhomocysteinemia is frequently caused not only by folic acid deficiency but also ~~associated with~~by genetic polymorphisms coding for enzymes involved in the OCM ~~one-carbon metabolism~~. Comparing ~~Compared~~ the plasma homocysteine, folate level and methylentetrahydrofolate reductase (MTHFR) C677T mutation in patients with epilepsy ~~epileptic patients~~ with those in normal controls , Yoo and Hong found that a common MTHFR C677T mutation was a determinant of hyperhomocysteinemia in patients with epilepsy ~~epileptic patients~~ receiving AEDs, which suggests that a gene-drug interaction induced hyperhomocysteinemia[20]. MTHFR is a key enzyme in homocysteine remethylation pathway, which plays an

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important role in transmethylation of homocysteine to methionine. Ono et al. indicated that there was a relationship between the hyperhomocysteinemia and homozygote MTHFR gene variant in ~~patients with epilepsy~~ ~~epileptic patients~~ receiving multidrug therapy, but not in those receiving monotherapy[21]. However, Vurucu et al ~~which~~ found that the variations in the MTHFR gene had no significant contribution on hyperhomocysteinemia in ~~patients with epilepsy~~ ~~epileptic patients~~ receiving AEDs therapy[10]. Therefore, further studies are ~~required~~ ~~warranted~~ to clarify the mechanism as to how homozygous genotype of thermolabile MTHFR affects plasma homocysteine concentrations in patients with epilepsy when they receive VPA monotherapy or other AEDs treatment.

Although our meta-analysis found that the levels of plasma homocysteine were significantly higher in VPA treated ~~patients with epilepsy~~ ~~epileptic patients~~ than those in controls, the heterogeneity was also existed. After using the subgroup analysis according to the ~~ethnicity~~ ~~race~~, ~~the risk of heterogeneity in West-Asian group was diminished as compared with over-all groups~~ ~~we found the main outcome remained unchanged but the heterogeneity did not exist in West-Asian group~~. MTHFR as a key enzyme in homocysteine metabolism has been proved correlated with the plasma levels of homocysteine. The presence of the T allele (MTHFR C677T) renders the enzyme thermolabile reducing its enzymatic activity which may cause elevated plasma levels of homocysteine. However, the ethnic composition and the location of sampling can influence the frequency of MTHFR genotypes[14, 22]. ~~One research indicated that the prevalence of the 677T allele varied from 9.34-40.53% in different ethnic groups, the lowest being demonstrated for Southern Asians and the highest for East-Asian?. Whether the risk of heterogeneity between studies is influenced by MTHFR polymorphism needs further research~~ ~~Cappuccio et al. found that the difference in the practice of folic acid fortification for commonly consumed foods and dietary habits between the countries may explain the plasma levels of homocysteine heterogeneity in different countries[23].In our research, the heterogeneity may be caused by the different races or countries.~~

Elevated circulating homocysteine, irrespective of the underlying metabolic

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abnormality, can be detrimental to vascular structure and function through a number of mechanism. Hyperhomocysteinemia is a well-established risk factor for vascular disease such as stroke, myocardiac infarction and peripheral arterial disease[24]. As we know, ultrasonographic determination of mean common carotid artery intima media thickness (CCA IMT) is a marker to stratify the risk of atherosclerosis. Chuang et al. indicated that the levels of plasma homocysteine and the mean CCA IMT were significantly increased in monotherapy with VPA. Moreover, their research demonstrated that the mean CCA IMT was correlated with the duration monotherapy with VPA. Therefore, long-term monotherapy with VPA has been associated with hyperhomocysteinemia that lead to an increase in risk of atherosclerosis in patients with epilepsy[7, 25].

Increased levels of homocysteine are associated with NTD-affected pregnancies, which is~~was~~ regarded as the major risk factor for NTDs[26]. As an important intermediate product in OCM ~~one-carbon metabolism~~, homocysteine may be a better indicator of methyl group supply and therefore more accurately reflects functional transmethylation in genome-wide methylation. Hyperhomocysteinemia ~~was~~ is usually associated with the genome-wide hypo-methylation as assessed using mean long interspersed nucleotide element-1 (LINE-1) methylation in human. Wang et al. found that the reduction in LINE-1 methylation was accompanied by an increased risk of NTDs, indicating that LINE-1 hypomethylation ~~is~~ was likely to contribute to the development of NTDs. Because of aberrant genomic methylation underlies the complex pathogenesis of NTDs, high level of homocysteine may interfere genome wide methylation which further induces NTDs[27, 28]. Although recent study confirmed a specific increase in the risk of NTDs associated with maternal use of VPA, the mechanism VPA initiating the molecular and biochemical events ~~is~~ was still unclear[29]. It is probable that therapy with VPA during pregnancy ~~has been~~is associated with hyperhomocysteinemia that leads to an increase in risk of NTD-affected pregnancies.

Some limitations of this meta-analysis should be considered. First, the number of published studies in our meta-analysis ~~were~~ is relatively limited. Second, a significant heterogeneity ~~was~~ is found in our study. In subgroup analyses, the subgroup may be

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underpowered as the East-Asian group has only one study. ~~After subgroup analysis, most of studies were included in West-Asian group, so the conclusions may not be apply to other ethnic groups.~~ Third, characteristics of eligible studies ~~was~~ have incomplete information about the dosage of VPA ~~valproate~~ monotherapy. Finally, only the studies in English ~~language were~~ are considered.

Conclusions

In conclusion, this meta-analysis ~~suggested~~ suggests that VPA monotherapy is associated with the increase in plasma homocysteine levels in patients with epilepsy. Hyperhomocysteinemia is an independent risk factor for thrombosis, atherosclerosis, and neural tube defects in the offspring of affected women. Therefore, we advise to measure plasma homocysteine in all patients with epilepsy ~~epileptic patients~~ received ~~valproate~~ VPA treatment. In addition, folate supplementation to reduce homocysteine levels may be useful in VPA ~~valproate~~ treated patients with epilepsy ~~epileptic patients~~.

Contributors   GN and LZ contributed to conception and design the experiments. GN, JQ, ZF and YC contributed to the data acquisition and analysis of the data. GN, ZC and JZ contributed reagents / materials/ analysis tools. GN, JQ and LZ wrote the manuscript.

Competing interests   None

Data sharing statement   All original data extraction are available from the corresponding author at lmzhou56@163.com.

Figure legends

Fig.1 Search strategy for meta-analysis

Fig.2 Pooled estimate of SMD and 95%CI of plasma homocysteine levels in patients with epilepsy received VPA monotherapy

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Table1   Summary of studies included in the meta-analysis

Study	Year	Ethnicity	VPA treated group				Healthy controls	
			Dose	Duration	Cases	Hcy(umol/l)	Cases	Hcy(umol/l)
Verrotti et al.[1]	2000	European	21.7±6.8 mg/kg	12m	32	12.7±7.1	63	7.9±4.5
Karabiber et al.[2]	2002	West-Asian	no	＞12m	30	14±6.8	29	9.2±2.7
Sener et al.[3]	2006	West-Asian	no	6.5±6.2y	22	17±8.0	11	11.5±11.4
Vurucu et al.[11]	2007	West-Asian	no	27.36±21.12m	64	6.88±2.24	62	5.52±2.53
Kurul et al.[13]	2007	West-Asian	no	4.78±2.07y	8	7.18±2.54	10	7.66±2.34
Yildiz et al.[12]	2010	West-Asian	54.49mg/ml	＞6m	19	6.73±2.81	23	6.79±1.95
Belcastro et al	2010	European	946.4±172mg/d	≥6m	37	10.4±3.04	231	9.1±3.04
Chuang et al.[7]	2012	East-Asian	750-1000mg/d	8.7±5.2 y	54	13.84±4.29	60	9.41±2.65

Table2   Results of quality assessment by Newcastle–Ottawa Scale

Study	Selection				Comparability		Exposure			Total
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Control for additional factor	Ascertainment of exposure	Same method to ascertain	Non-response rate	
Verrotti et al	1	0	1	1	1	1	1	1	0	7
Karabiber et al	1	0	1	1	1	0	1	1	0	6
Sener et al	1	0	1	1	1	0	1	1	0	6
Vurucu et al	1	0	1	1	1	0	1	1	0	6
Kurul et al	1	0	1	1	1	1	1	1	0	7
Yildiz et al	1	1	1	1	1	0	1	1	0	7
Belcastro et al	1	1	1	1	1	0	1	1	0	7
Chuang et al	1	1	1	1	1	0	1	1	0	7

Table 3   Differences between studies by subgroup analysis

Subgroups	No. of studies	SMD (95% CI)	I^2(%)	p
Ethnicity				
European	2	0.63 (0.19 to 1.06)	58.0	0.005
West-Asian	4	0.45 (0.08 to 0.81)	47.4	0.016
East-Asian	1	1.26 (0.85 to 1.66)	0	<0.001
Age (years)				
<18	5	0.52 (0.15 to 0.89)	58.8	0.006
≧18	2	0.77 (0.18 to 1.36)	79.0	0.01

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Identification

Records identified
through Pubmed
(n=32)

Records identified
through Web of Science
(n=33)

Records identified
through Embase
(n=28)

Records after duplicates removed
(n=30)

Screening

Records screened
(n=30)

9 records excluded, based on
title and abstract,due to
review,case report,not clinical
research and commentary article

Eligibility

Full-text articles assessed
for eligibility
(n=21)

13 studies excluded in full-text
review,based on non-continuous
data (n=7), self control of pre
and post VPA (n=5) and controls
were patients with newly
diagnosed epilepsy (n=1)

Studies included in
qualitative synthesis
(n=8)

Included

Studies included in
quantitative synthesis
(meta-analysis)
(n=8)

Fig.1. Search strategy for meta-analysis
173x165mm (300 x 300 DPI)

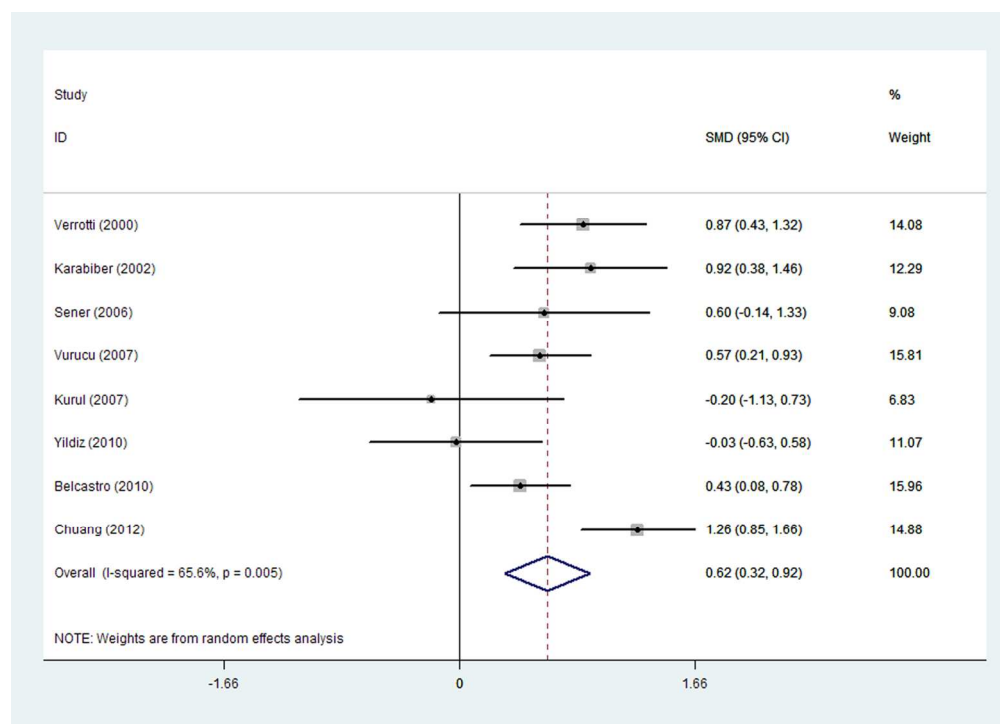| Study				
ID			SMD (95% CI)	% Weight
Verrotti (2000)			0.87 (0.43, 1.32)	14.08
Karabiber (2002)			0.92 (0.38, 1.46)	12.29
Sener (2006)			0.60 (-0.14, 1.33)	9.08
Vurucu (2007)			0.57 (0.21, 0.93)	15.81
Kurul (2007)			-0.20 (-1.13, 0.73)	6.83
Yildiz (2010)			-0.03 (-0.63, 0.58)	11.07
Belcastro (2010)			0.43 (0.08, 0.78)	15.96
Chuang (2012)			1.26 (0.85, 1.66)	14.88
Overall (I-squared = 65.6%, p = 0.005)			0.62 (0.32, 0.92)	100.00

NOTE: Weights are from random effects analysis

Fig.2 Pooled estimate of SMD and 95%CI of plasma homocysteine levels in patients with epilepsy received VPA monotherapy
173x124mm (300 x 300 DPI)

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Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Identification of studies
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Identification of studies
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Identification of studies
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Identification of studies
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Identification of studies
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Data extraction
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Data extraction

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Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Quality appraisal of the included research
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Statistical analysis
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Statistical analysis

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Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Statistical analysis
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Statistical analysis
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Literature search and characteristics of eligible studies
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Literature search and characteristics of eligible studies
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Quality assessment of included literature
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Meta analysis of plasma homocysteine levels

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Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Meta analysis of plasma homocysteine levels
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Meta analysis of plasma homocysteine levels
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Meta analysis of plasma homocysteine levels
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Discussion section Par.1
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Discussion section Par.8
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Conclusions
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Acknowledgments

For more information, visit: **www.prisma-statement.org**.

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