Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Effect of enalapril on plasma homocysteine levels in patients with essential hypertension

Fang-fang FAN[1], Yong HUO[1], Xu WANG[2], Xin XU[2], Bin-yan WANG[2], Xi-ping XU[2], Jian-ping LI[†‡1]

([1]*Department of Cardiology, Peking University First Hospital, Beijing 100034, China*)

([2]*Institute of Biomedicine, Anhui Medical University, Hefei 230032, China*)

[†]E-mail: lijianping@medmail.com.cn

Received June 11, 2010; Revision accepted June 27, 2010; Crosschecked July 2, 2010

Abstract: Objective: To investigate the effect of enalapril on plasma homocysteine (Hcy) levels and the association of *methylenetetrahydrofolate reductase* (*MTHFR*) *C677T* polymorphism with the changes of Hcy levels in response to enalapril among patients with essential hypertension. Methods: A total of 130 patients with mild-to-moderate essential hypertension were enrolled and enalapril was orally administered at a dose of 10 mg/d for eight weeks. Plasma Hcy levels were measured by denaturing high-performance liquid chromatography (DHPLC) at baseline and after eight weeks of treatment. Genotyping of *MTHFR C677T* polymorphism was performed by TaqMan probe technique. Results: Compared with baseline, plasma Hcy levels did not change significantly after eight weeks (P=0.81). Stratified by baseline Hcy levels, a significant increase in plasma Hcy levels (P=0.02) among those with Hcy <10 μmol/L was observed, in contrast to no significant changes in plasma Hcy levels (P=0.54) among those with Hcy ≥10 μmol/L. No significant association was observed between *MTHFR C677T* polymorphism and changes in Hcy levels in response to enalapril. Conclusions: Enalapril may cause an increase in plasma Hcy levels among the hypertensives with low baseline Hcy levels. There was no significant association between *MTHFR C677T* genotypes and changes in Hcy levels in response to enalapril among subjects with essential hypertension.

Key words: Essential hypertension, *Methylenetetrahydrofolate reductase* (*MTHFR*) *C677T* polymorphism, Enalapril, Homocysteine

doi:10.1631/jzus.B1001003 **Document code:** A **CLC number:** R54

1 Introduction

Over the past 20 years, cardiovascular disease (CVD) has become the leading cause of death in both urban and rural China (Gu *et al*., 2006). Data from the monitoring trends and determinants in a cardiovascular ongoing trial (MONICA) have revealed that ischemic stroke in China is increasing by 8.7% per year (Zhao *et al*., 2008). The classical risk factors for CVD are widely known, among which hypertension is very important, being closely associated with stroke (Kjeldsen *et al*., 2001). In recent years, hyperhomocysteinemia has received increasing attention as a new risk factor for CVD. Many studies have shown that homocysteine (Hcy) is positively correlated with CVD: subjects with higher Hcy levels are more susceptible to CVD (Boushey *et al*., 1995; Homomcysteine Studies Collaboration, 2002; Wald *et al*., 2002), and lowering Hcy could significantly lower the risk of CVD, especially that of stroke (Yang *et al*., 2006; Wang *et al*., 2007; Saposnik *et al*., 2009). In China, the percentage of hypertension patients with hyperhomocysteinemia is as high as 75% (Hu and Xu, 2008), and several studies have revealed that hyperhomocysteinemia interacts with hypertension in significantly increasing the risk of vascular events (Graham *et al*., 1997). Therefore, hypertension with hyperhomocysteinemia has been defined as "H-type hypertension" in order to promote a multi-risk

‡ Corresponding author

factor-intervention strategy and to emphasize the benefit from lowering both blood pressure (BP) and Hcy level, especially for a large portion of the hypertensive patients in China (Li *et al*., 2007; Hu and Xu, 2008; Wang *et al*., 2008).

Many factors may affect the metabolism of Hcy, including several medications widely used in the treatment of CVD (Ueland *et al*., 2001; Desouza *et al*., 2002; Dierkes and Westphal, 2005). The Framingham offspring study (Jacques *et al*., 2001) examined the relationship between fasting plasma Hcy concentrations and several health factors in 5135 persons. The results showed that the Hcy concentrations were 9% higher in those who were using antihypertensive medications than in those who were not using these medications (P<0.001). The increase in Hcy concentrations after antihypertensive medications may be clinically relevant and counteract the desired cardiovascular protection conferred by lowering the BP, given that higher Hcy concentrations increase the risk of CVD. This effect may depend on the type of antihypertensive agents. Several studies have already shown that thiazide-type diuretics can significantly increase plasma Hcy levels (Westphal *et al*., 2003; Atar *et al*., 2005), and the β-receptor blocker, metoprolol, decreases plasma Hcy levels. However, findings from several studies on the association between angiotensin-converting enzyme inhibitor (ACEI) and Hcy (Šebeková *et al*., 2003; Westphal *et al*., 2003; Poduri *et al*., 2008) remain controversial. Additional trials to understand the effect of ACEI on plasma Hcy levels are needed.

The metabolism of Hcy is also under genetic control. Methylenetetrahydrofolate reductase (MTHFR) represents a key enzyme in the folate cycle, which provides the methyl group in the remethylation of Hcy. Frosst *et al*. (1995) observed that the *MTHFR 677C→T* genotype influences the activity of MTHFR: *TT* genotype with decreased activity of MTHFR was associated with higher plasma Hcy levels as compared to the heterozygote or homozygote of wild genotype. For individuals with the *TT* genotype, the effect of folate on the metabolism of Hcy is more significant than for those without the homozygote of the mutation, leading to a remarkable decrease in Hcy level in response to folate supplement or Hcy-lowering therapy with folic acid combinants (Jacques *et al*., 1996; Xu *et al*., 2006; McNulty *et al*., 2008). Jiang *et al*. (2004) found an association between *MTHFR C677T* genotypes and changes in BP levels in response to benazepril among subjects with essential hypertension. Thus, there may be gene-nutrient and gene-gene interactions, which indicate that there may be an association of *MTHFR C677T* genotypes and plasma Hcy levels in response to ACEI.

In the present study, we examined the effect of ACEI on plasma Hcy levels in a cohort of mild and moderate essential hypertensive patients. We also studied the genetic modification effect on the changes of Hcy levels after enalapril treatment.

2 Patients and methods

2.1 Study population

We used the data of subjects treated with enalapril 10 mg/d in the phase II clinical trial on enalapril maleate and folic acid (Li *et al*., 2007). Hypertensive patients were recruited from seven hospitals and centers in several cities in China. Participants who met the following criteria were recruited as mild and moderate hypertensives: (1) systolic blood pressure (SBP) between 140 and 180 mmHg, and/or diastolic blood pressure (DBP) between 90 and 110 mmHg; and, (2) aged from 18 to 75 years. To avoid potentially severe adverse effects, patients with secondary hypertension, chronic CVD, chronic cerebrovascular disease, chronic lung, liver or renal disease, chronic liver or renal insufficiency, and folate intake, as well as pregnant and lactating women, were excluded. Written informed consent was obtained from all subjects.

2.2 Enalapril treatment

After a washout period of 7–10 d, all subjects were treated orally with enalapril (Shanghai modern pharmaceutical Co., Ltd., China) at a daily fixed dosage of 10 mg for eight consecutive weeks. Subjects were required to take their enalapril between 8:00 a.m. and 10:00 a.m. During treatment, subjects were required to visit our clinical center every two weeks to have BP and heart rate measured, and to report any adverse effect. This study was approved by the Ethics Committee of the Peking University First Hospital.

2.3 Blood pressure measurement

The subjects were invited to our clinic center at 8:00 a.m. to 10:00 a.m. after fasting overnight. After a 5-min rest, sitting BPs were measured via mercury sphygmomanometer according to a standard operation protocol (SOP). All subjects had an empty bladder before the measurements, and no coffee or cigarettes were allowed. Three consecutive measurements were taken with a 30-s interval between replicates. If the difference between the measurements was more than 4 mmHg, the patient was asked to rest for another 5 min, and then the measurements were repeated. The average of three consecutive BP readings was used.

2.4 Plasma Hcy examination

Venous blood samples (8 ml) were drawn and collected in ethylenediaminetetraacetic acid (EDTA) tubes after a 10-h fast at 8.00 a.m. to 10.00 a.m. The samples were then centrifuged at 3000 r/min for 10 min to obtain the serum, this being carried out within 15 min of collection. A portion of the serum (3 ml) was sent to the laboratories of local hospitals within 30 min, where the blood biochemical criterion was measured according to the unified SOP on the automatic biochemistry analyzer by technician in charge. The residual serum (5 ml) was immediately snap frozen at −20 °C, and kept at −80 °C for one week before the collective measurement of Hcy levels by the central laboratory.

Total plasma Hcy levels were measured by high performance liquid chromatography (HPLC, Agilent HP 1100 type) coupled with a fluorescence detector (HP 1046 type). The chromatographic column was Hypersil C18 (4.6 mm×250 mm, 5 μm). We used a 0.07 mol/L HAc buffer as the mobile phase with a flow-rate of 0.8 ml/min. The inject volume was 15 μl. The column temperature was 25 °C. The analysis time was 15 min. The excitation and emission wavelengths were set at 390 and 470 nm, respectively. The intra- and inter-assay coefficient variations (CVs) for total Hcy were 3.5% and 4.2%, respectively.

2.5 Genotyping of *MTHFR*

The central laboratory was responsible for the genotyping of *MTHFR* by TaqMan technique. The probe was E-rs1801133. The assay was performed under universal conditions, with each reaction containing 4 ng dried DNA, 0.08 μl 40× assay mix, and 2.0 μl TaqMan universal polymerase chain reaction (PCR) master mix made to a final volume of 4 μl with 1.92 μl sterile water. The PCR cycle conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 50 cycles of 92 °C for 15 s and 60 °C for 1 min. Concordance of 100% was observed for all samples.

2.6 Statistical analysis

EpiData 3.1 was used to input data. SAS 8.2 software was used to perform all statistical analysis. All results were analyzed by using per protocol set (PPS). Quantitative variables were assessed via the geometric mean and categorical variables by the constituent ratio. The *t*-test was used to compare quantitative variables among two groups, and analysis of variance (ANOVA) was used to compare quantitative variables among more than two groups. The Chi-square test was used for categorical variables. Paired *t*-test was used to compare Hcy levels before and after treatment. Using the generalized estimating equation (GEE) correction, a multivariate linear regression model was used to evaluate the modification effect of the baseline Hcy levels and *MTHFR C677T* polymorphism on the changes of Hcy levels after enalapril treatment before and after adjusting for possible confounders, including age, gender, body mass index (BMI), and center. Probability values <0.05 were considered statistically significant.

3 Results

3.1 Baseline characteristics of participants

A total of 160 patients with mild and moderate hypertension were enrolled. After excluding patients with the prescribed regimen as described in Section 2.1, 130 subjects with complete information were used in the final data analysis. The age, BMI, and baseline BP were not significantly different between the male and female subjects. Males had higher baseline Hcy levels [(16.3±1.7) μmol/L] than female [(11.2±1.4) μmol/L] (P=0.00). The percentages of hyperhomocysteinemia (Hcy ≥10 μmol/L) were 91.8% and 66.7% in male and female patients, respectively (P=0.00). The distribution of *MTHFR C677T* genotypes was not significantly different

between males and females (P=0.84), where the percentages of *TT* genotype were 30.6% and 25.9%, respectively (Table 1). The baseline Hcy levels for *CC*, *CT*, and *TT* genotypes were (11.5±1.4), (11.2±1.4), and (18.0±1.8) μmol/L respectively. Subjects with the *TT* genotype had higher Hcy levels than others (P=0.00) (Table 2).

3.2 Effect of enalapril treatment on Hcy levels

Among all subjects, no significant changes were observed in the Hcy levels after enalapril treatment (P=0.81). Stratified by baseline Hcy levels, the patients with baseline Hcy <10 μmol/L had a significant increase in plasma Hcy levels (P=0.02). By contrast, those with baseline Hcy ≥10 μmol/L had no remarkable changes in plasma Hcy levels (P=0.54) (Table 3). The results of multivariate linear regression demonstrate the effect of the baseline Hcy levels on the changes of Hcy levels after enalapril treatment, and this is shown in Table 4. However, no significant association of the baseline Hcy levels and the changes of Hcy levels was found in either unadjusted or adjusted GEE model.

3.3 Modification effect of *MTHFR C677T* polymorphism on the changes of Hcy levels after enalapril treatment

There were 34, 60, and 36 patients with *CC*, *CT* and *TT* genotypes, respectively. The changes in the percentages of Hcy after eight weeks of treatment were (1±16)%, (3±15)%, and (0±30)%, respectively. However, there were no significant changes after enalapril treatment for all genotypes, and no correlation between *MTHFR C677T* polymorphism and the changes of Hcy levels was found in either unadjusted or adjusted GEE model (Table 5).

Table 1 Baseline characteristics of the subjects

Group	n	Age (year)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	Baseline Hcy			*MTHFR C677T* genotypes		
						Mean level (μmol/L)	<10 μmol/L	≥10 μmol/L	CC	CT	TT
Male	49	57.9±9.7	25.1±2.9	152.7±10.1	94.7±8.3	16.3±1.7	4 (8.2%)	45 (91.8%)	12 (24.5%)	22 (44.9%)	15 (30.6%)
Female	81	56.5±9.5	26.0±3.4	154.9±11.7	92.8±7.5	11.2±1.4	27 (33.3%)	54 (66.7%)	22 (27.2%)	38 (46.9%)	21 (25.9%)
P		0.41	0.14	0.29	0.17	0.00	0.00		0.84		

Values are expressed as $\bar{X}$ ±s or n (%). BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure

Table 2 Baseline Hcy levels of subjects with the three different *MTHFR C677T* genotypes

Group	Baseline Hcy level (μmol/L)			P
	CC	CT	TT	
Total	11.5±1.4	11.2±1.4	18.0±1.8	0.00
Male	13.5±1.3	13.3±1.3	23.6±2.1	0.00
Female	10.0±1.3	10.3±1.4	14.7±1.5	0.00

Values are expressed as $\bar{X}$ ±s

Table 3 Effect of enalapril treatment on Hcy levels

Group	n	Baseline Hcy level (μmol/L)	Hcy changes (μmol/L)	Hcy changes (%)	P
Total	130	12.9±1.5	−0.1±3.9	1±20	0.81
Baseline Hcy level					
<10 μmol/L	31	7.9±1.2	0.5±1.2	6±15	0.02
≥10 μmol/L	99	15.0±1.5	−0.3±4.4	0±21	0.54

Values are expressed as $\bar{X}$ ±s

Table 4 Multivariate linear regression of the baseline Hcy level modification effect on the changes of Hcy levels after enalapril treatment

Group	n	Value of Hcy changes (μmol/L)	Unadjusted GEE			Adjusted GEE		
			β	SE	P	β	SE	P
Baseline Hcy level <10 μmol/L	31	0.5±1.2	0	0		0	0	
Baseline Hcy level ≥10 μmol/L	99	−0.3±4.4	−0.79	0.80	0.32	−0.76	0.85	0.38

Adjusted for age, gender, BMI, and center. GEE: generalized estimating equation; β: partial regression coefficient; SE: standard error

Table 5 Modification effect of *MTHFR C677T* polymorphism on the changes of Hcy levels after enalapril treatment

Genotype	*n*	Baseline Hcy level (μmol/L)	Value of Hcy changes (μmol/L)	Hcy changes (%)	Unadjusted GEE			Adjusted GEE		
					β	SE	*P*	*β*	SE	*P*
CC	34	11.5±1.4	0.21±2.17	1±16	0	0		0	0	
CT	60	11.2±1.4	0.40±2.01	3±15	0.18	0.82	0.82	0.23	0.82	0.78
TT	36	18.0±1.8	−1.16±6.56	0±30	−1.37	0.91	0.14	−1.35	0.90	0.14

Adjusted for age, gender, BMI, and center. GEE: generalized estimating equation; *β*: partial regression coefficient; SE: standard error

4 Discussion

4.1 Baseline characteristics

Hcy is a sulpher-containing amino acid produced by conversion of methionine, an essential amino acid present in foods regularly consumed within the diet. Generally speaking, when by Hcy, we mean the total Hcy, including all the forms of Hcy in plasma. Low levels of Hcy (5–15 μmol/L) are normally found in the plasma. There is no uniform critical value for hyperhomocysteinemia as of yet (Guilliams, 2004). In 2006, the American Heart Association/American Stroke Association (AHA/ASA) issued guidelines for the prevention of stroke in patients with ischemic stroke (IS) or transient ischemic attack (TIA), which recommended that for patients with IS or TIA and hyperhomocysteinemia (Hcy levels >10 μmol/L), daily standard multivitamin preparations were reasonable to reduce the levels of homocysteine, given their safety and low cost (Class I, level A) (Sacco *et al*., 2006). Many epidemiological and clinical studies also revealed that the risk of developing CVD increased significantly when plasma Hcy level was more than 10 μmol/L (Boushey *et al*., 1995; Guilliams, 2004; Sun *et al*., 2009), and that the reduction to lower Hcy levels by multivitamin preparations could benefit these patients (Spence *et al*., 2001). Therefore, our study also defined hyperhomocysteinemia as Hcy ≥10 μmol/L.

We observed that Hcy levels were related to gender. The males had higher Hcy levels, and the percentage of hyperhomocysteinemia of males was up to 91.8%, which is consistent with studies from home to abroad (Andersson *et al*., 1992; Brattstrom *et al*., 1994; Nygård *et al*., 1995; Huang *et al*., 2006), probably because estrogen is involved in the metabolism of Hcy.

Hcy is metabolized via two major pathways (Zhu *et al*., 2001): the first is a trans-sulphuration pathway resulting in the production of cystathionine, which requires vitamin B_6 as a cofactor, and the other is a methionine-conserving pathway involving remethylation of Hcy to methionine, which requires methyltetrahydrofolate as the carbon donor for the remethylation of Hcy to methionine and vitamin B_{12} as a cofactor. In the second process, methyltetrahydrofolate comes from the folic acid cycle (Ueland *et al*., 1993), requiring MTHFR as a rate-limiting enzyme, which catalyzes the reduction of methylenetetrahydrofolate to methyltetrahydrofolate. *C677T* is a common missense mutation of *MTHFR* gene. The mutation of 677-nucleotide (C→T) of this gene causes a 222-amino acid substitution (A→V), which results in reduced activity and increased thermolability of MTHFR. Finally, this mutation influences the remethylation of Hcy, resulting in the elevation of Hcy (Frosst *et al*., 1995). As reported in earlier studies (Jacques *et al*., 1996; Verhoef *et al*., 1997; Sunder-Plassmann and Födinger, 2003), we also found that those with the *TT* genotype had significantly higher plasma Hcy levels than subjects with other genotypes.

4.2 Effect of enalapril treatment on Hcy levels and the mechanism

Many factors may affect Hcy metabolism. As mentioned above, folate, and vitamins B_6 and B_{12} are all involved in the conversion of Hcy. Factors which affect B-vitamins will influence the metabolism of Hcy (Trabetti, 2008). Plasma Hcy is mainly cleared by the kidney, so patients with elevated creatinine will have higher Hcy levels (Dierkes and Westphal, 2005; Rong *et al*., 2009).

ACEIs are recommended as the first-line medicine for hypertension treatment. The main pharmacology action of ACEIs is to inhibit the activity of ACE, resulting in the reduction of angiotensin II. ACEIs can also reduce the degradation of bradykinin,

leading to the elevation of bradykinin in plasma, and enhancing the vasodilatation affected by bradykinin, which finally causes vascular dilatation and pressure reduction. ACEIs can be used to treat mild and moderate hypertension, and also severe hypertension in combination with other anti-hypertension medications. Additionally, ACEIs can protect some patients with cardiovascular heart disease (CHD), diabetes mellitus, or chronic kidney disease (CKD) from heart failure and end-stage renal disease. Thus, ACEIs are widely used in clinical treatment (Zhan *et al*., 2001). However, studies related to the effect of ACEI treatment on Hcy levels have produced conflicting results, with varying ACEIs.

Šebeková *et al*. (2003) observed the anti-oxidative effect of ACEIs in patients with non-diabetic nephropathy during short-term administration of ramipril, in which Hcy was used as a parameter of oxidative stress. Ramipril (2.5–5.0 mg/d) was administered to 12 newly diagnosed patients for two months and data were compared with a patient group (n=7) under conventional therapy (diuretic/β-blockers). The results revealed that Hcy levels remained unaffected, and ramipril treatment did not affect renal function (as evaluated by plasma creatinine concentration, creatinine clearance, and cystatin C concentration (Madero *et al*., 2006)) either.

Westphal *et al*. (2003) investigated not only Hcy levels, but also the major determinants (vitamins B_6 and B_{12}, folic acid, creatinine, and cystatin C) of Hcy concentrations after treatment with hydrochorothiazide (HCT) (n=21, 25 mg/d) and captopril (n=19, twice daily doses of 25 mg) for 29 and 31 d, respectively. They found that there were no significant changes after captopril treatment. The ACEI did not increase or decrease any of these parameters, except cystatin C, which showed an increasing trend (P=0.053).

Yet, in the study by Poduri *et al*. (2008), there was a significant decrease of Hcy levels in mild essential hypertensive patients (n=94) after ramipril treatment (5 mg/d) for six weeks. The Hcy levels before and after ramipril treatments were (19.12±6.94) and (14.39±5.75) μmol/L, respectively. There were no significant changes of folate and vitamin B_{12}. Thus, the authors concluded that the reduction in Hcy levels with the ACEI did not appear to be influenced by these factors.

We investigated the changes of Hcy levels of 130 mild and moderate hypertensive subjects after enalapril treatment for eight weeks. As with the first two studies, we did not find an increase or decrease in Hcy levels. But stratified by baseline Hcy levels, we found that those with baseline Hcy <10 μmol/L had a significant increase in plasma Hcy levels (P=0.02). There were no stratified baseline Hcy levels in any of the three studies previously mentioned, and the baseline Hcy levels were not the same as each other. Two of the former studies had a baseline level of 13–14 μmol/L, but in the study of Poduri *et al*. (2008), the baseline Hcy level was (19.12±6.94) μmol/L, suggesting that this may explain the difference in results among the studies. Therefore, we did a multivariate linear regression of the baseline Hcy level modification effect on the changes of Hcy levels after enalapril treatment, and there were no additional effects, probably because there were few patients with Hcy <10 μmol/L (n=31). We did not examine folate, vitamins B_6 and B_{12}, and cystatin C, so we could not investigate these parameters' effect on Hcy levels.

The Rotterdam study (Bots *et al*., 1999), involving 7983 subjects, from 1990 to 1994 found that the risk of stroke and myocardial infarction increased directly with total homocysteine. The linear coefficient suggested a risk increase of 6% to 7% for every 1 μmol/L. Saposnik *et al*. (2009) additionally analyzed the heart outcomes prevention evaluation 2 (HOPE2) study. After five-year follow-up, there were 258 strokes in 5522 patients. The incidence of stroke in patients with or without B-vitamin supplements was 0.88% or 1.15% (hazard ratio (HR) 0.75, 95% confidence interval (CI) 0.59–0.97), and the Hcy level decreased to 2.2 μmol/L in the former team. This result suggests that we should pay attention to mild elevations in Hcy levels and consider starting B-vitamin supplement.

4.3 Modification effect of *MTHFR C677T* polymorphism on the changes of Hcy levels after enalapril treatment

There are few studies on the modification effect of *MTHFR C677T* polymorphism on the changes of Hcy levels after anti-hypertension treatment. Poduri *et al*. (2008) did not find any correlation between *MTHFR C677T* genotypes with changes in plasma Hcy levels following any anti-hypertension medications,

which suggested that the observed changes in Hcy concentrations with anti-hypertension medications were independent of *MTHFR* polymorphisms. However, the *MTHFR C677T* genotypes were limited to *CC* and *CT*, without *TT* genotype. We observed the modification effect of *MTHFR C677T* polymorphism involving all the three genotypes, but did not find any correlation either.

However, these findings need to be replicated and expanded to a large population of essential hypertensive individuals to understand the effect of anti-hypertension treatment on Hcy levels. One can also examine folate, vitamins B_6 and B_{12}, and cystatin C to investigate the relationship between these parameters and Hcy levels.

5 Conclusions

To summarize, our data suggest that enalapril can cause an increase in plasma Hcy levels among hypertensives with low baseline Hcy levels, and there is no significant association of *MTHFR C677T* genotypes with changes in Hcy levels in response to enalapril among the subjects with essential hypertension. The changes of Hcy levels after enalapril treatment were not modulated by *MTHFR C677T* polymorphism. In consideration of the correlation between Hcy and CVD, one should pay attention to the mild elevation of Hcy levels after anti-hypertensive treatment, and consider starting B-vitamin supplement.

References

Andersson, A., Brattström, L., Israelsson, B., Isaksson, A., Hamfelt, A., Hultberg, B., 1992. Plasma homoeysteine before and after methionine loading with regard to age, gender and menopausal status. *European Journal of Clinical Investigation*, **22**(2):79-87. [doi:10.1111/j.1365-2362.1992.tb01940.x]

Atar, I., Korkmaz, M.E., Demircan, S., Atar, I.A., Bozbaş, H., Aydinalp, A., Ozin, B., Yildirir, A., Müderrisoğlu, H., 2005. Beta blocker effects on plasma homocysteine levels in patients with hypertension. *Atherosclerosis*, **181**(2):399-402. [doi:10.1016/j.atherosclerosis.2005.01.035]

Bots, M.L., Launer, L.J., Lindemans, J., Hoes, A.W., Hofman, A., Witteman, J.C., Koudstaal, P.J., Grobbee, D.E., 1999. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam study. *Archives of Internal Medicine*, **159**(1):38-44. [doi:10.1001/archinte.159.1.38]

Boushey, C.J., Beresford, S.A., Omenn, G.S., Motulsky, A.G., 1995. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *The Journal of the American Medical Association*, **274**(13):1049-1057. [doi:10.1001/jama.274.13.1049]

Brattstrom, L., Lindgren, A., Israelsson, B., Andersson, A., Hultberg, B., 1994. Homocysteine and cysteine: determinants of plasma levels in middle-aged and elderly subjects. *Journal of Internal Medicine*, **236**(6):633-641. [doi:10.1111/j.1365-2796.1994.tb00856.x]

Desouza, C., Keebler, M., McNamara, D.B., Fonseca, V., 2002. Drugs affecting homocysteine metabolism: impact on cardiovascular risk. *Drugs*, **62**(4):605-616. [doi:10.2165/00003495-200262040-00005]

Dierkes, J., Westphal, S., 2005. Effect of drugs on homocysteine concentrations. *Seminars in Vascular Medicine*, **5**(2):124-139. [doi:10.1055/s-2005-872398]

Frosst, P., Blom, H.J., Milos, R., Goyette, P., Sheppard, C.A., Matthews, R.G., Boers, G.J., den Heijer, M., Kluijtmans, L.A., van den Heuvel, L.P., *et al*., 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Genetics*, **10**(1):111-113. [doi:10.1038/ng0595-111]

Graham, I.M., Daly, L.E., Refsum, H.M., Robinson, K., Brattström, L.E., Ueland, P.M., Palma-Reis, R.J., Boers, G.H., Sheahan, R.G., Israelsson, B., *et al*., 1997. Plasma homocysteine as a risk factor for vascular disease. The Eumpean Concerted Action Project. *The Journal of the American Medical Association*, **277**(22):1775-1781. [doi:10.1001/jama.277.22.1775]

Gu, D.F., He, J., Wu, X.G., Duan, X.F., Yao, C.H., Wang, J.L., Reynolds, K., Chen, C.S., Klag, M.J., Whelton, P.K., 2006. Main causes and its modifiable risks of death among men and women in China. *Chinese Journal of Prevention and Control of Chronic Non-communicable Diseases*, **14**(3):149-154 (in Chinese).

Guilliams, T.G., 2004. Homocysteine—a risk factor for vascular diseases: guidelines for the clinical practice. *The Journal of the American Nutraceutical Association*, **7**(1):11-24.

Homomcysteine Studies Collaboration, 2002. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *The Journal of the American Medical Association*, **288**(16):2015-2022.

Hu, D.Y., Xu, X.P., 2008. Prevention of stroke relies on valid control "H" type hypertension. *Chinese Journal of Internal Medicine*, **47**(12):976-977 (in Chinese).

Huang, H.W., Guo, M.H., Huang, J.X., Lin, R.J., Zhang, Y., Chen, Y.L., 2006. The analysis of plasma homocysteine among 1020 residents in community. *Chinese Journal of Epidemiology*, **27**(8):721-724 (in Chinese).

Jacques, P.F., Bostom, A.G., Williams, R.R., Ellison, R.C., Eckfeldt, J.H., Rosenberg, I.H., Selhub, J., Rozen, R., 1996. Relation between folate status, a common mutation

in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation*, **93**(1):7-9.

Jacques, P.F., Bostom, A.G., Wilson, P.W., Rich, S., Rosenberg, I.H., Selhub, J., 2001. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. *The American Journal of Clinical Nutrition*, **73**(3):613-621.

Jiang, S., Hsu, Y.H., Xu, X., Xing, H., Chen, C., Niu, T., Zhang, Y., Peng, S., Xu, X., 2004. The C677T polymorphism of the methylenetetrahydrofolate reductase gene is associated with the level of decrease on diastolic blood pressure in essential hypertension patients treated by angiotensin-converting enzyme inhibitor. *Thrombosis Research*, **113**(6):361-369. [doi:10.1016/j.thromres.2004.04.005]

Kjeldsen, S.E., Julius, S., Hedner, T., Hansson, L., 2001. Stroke is more common than myocardial infarction in hypertension: analysis based on 11 major randomized intervention trials. *Blood Pressure*, **10**(4):190-192. [doi:10.1080/08037050152669684]

Li, J.P., Huo, Y., Liu, P., Qin, X.H., Guan, D.M., Ge, J.B., Hu, J., Wang, Y.N., Zhang, F.M., Mao, G.Y., *et al*., 2007. Efficacy and safety of enalapril-folate acid tablets in lowering blood pressure and plasma homocysteine. *Journal of Peking University: Health Sciences*, **39**(6):614-618 (in Chinese).

Madero, M., Sarnak, M.J., Stevens, L.A., 2006. Serum cystatin C as a marker of glomerular filtration rate. *Current Opinion in Nephrology and Hypertension*, **15**(6):610-616. [doi:10.1097/01.mnh.0000247505.71915.05]

McNulty, H., Pentieva, K., Hoey, L., Ward, M., 2008. Homocysteine, B-vitamins and CVD. *The Proceedings of the Nutrition Society*, **67**(2):232-237. [doi:10.1017/S0029665108007076]

Nygård, O., Vollset, S.E., Refsum, H., Stensvold, I., Tverdal, A., Nordrehaug, J.E., Ueland, M., Kvåle, G., 1995. Total plasmahomocystein and cardiovaseular risk Profile. The Hordaland Homoeystein study. *The Journal of the American Medical Association*, **274**(19):1526-1533. [doi:10.1001/jama.274.19.1526]

Poduri, A., Kaur, J., Thakur, J.S., Kumari, S., Jain, S., Khullar, M., 2008. Effect of ACE inhibitors and beta-blockers on homocysteine levels in essential hypertension. *Journal of Human Hypertension*, **22**(4):289-294. [doi:10.1038/sj.jhh.1002325]

Rong, R., Zhang, A.M., Fan, C.H., Zhang, Z., 2009. The analysis of the relationship between serum homocysteine (HCY) and chemistries in patients of coronary heart disease. *Chinese Journal of Laboratory*, **13**(1):77-80 (in Chinese).

Sacco, R.L., Adams, R., Albers, G., Alberts, M.J., Benavente, O., Furie, K., Goldstein, L.B., Gorelick, P., Halperin, J., Harbaugh, R., *et al*., 2006. Guidelines for prevention of stroke in patients with ischemic stroke or transient ischemic attack: a statement for healthcare professionals from the American Heart Association/American Stroke Association Council on Stroke: co-sponsored by the Council on Cardiovascular Radiology and Intervention. *Stroke*, **37**(2):577-617. [doi:10.1161/01.STR.0000199147.30016.74]

Saposnik, G., Ray, J.G., Sheridan, P., McQueen, M., Lonn, E., Heart Outcomes Prevention Evaluation 2 Investigators, 2009. Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE 2 trial. *Stroke*, **40**(4):1365-1372. [doi:10.1161/STROKEAHA.108.529503]

Šebeková, K., Gazdíková, K., Syrová, D., Blazícek, P., Schinzel, R., Heidland, A., Spustová, V., Dzúrik, R., 2003. Effects of ramipril in nondiabetic nephropathy: improved parameters of oxidatives stress and potential modulation of advanced glycation end products. *Journal of Human Hypertension*, **17**(4):265-270. [doi:10.1038/sj.jhh.1001541]

Spence, J.D., Howard, V.J., Chambless, L.E., Malinow, M.R., Pettigrew, L.C., Stampfer, M., Toole, J.F., 2001. Vitamin intervention for stroke prevention (VISP) trial: rationale and design. *Neuroepidemiology*, **20**(1):16-25. [doi:10.1159/000054753]

Sun, Y., Chien, K.L., Hsu, H.C., Su, T.C., Chen, M.F., Lee, Y.T., 2009. Use of serum homocysteine to predict stroke, coronary heart disease and death in Ethnic Chinese: a 12-year prospective cohort study. *Circulation Journal*, **73**(8):1423-1430. [doi:10.1253/circj.CJ-08-1077]

Sunder-Plassmann, G., Födinger, M., 2003. Genetic determinants of the homocysteine level. *Kidney International*, **63**(s84):141-144. [doi:10.1046/j.1523-1755.63.s84.52.x]

Trabetti, E., 2008. Homocysteine, MTHFR gene polymorphisms, and cardio-cerebrovascular risk. *Journal of Applied Genetics*, **49**(3):267-282.

Ueland, P.M., Refsum, H., Stabler, S.P., Malinow, M.R., Andersson, A., Allen, R.H., 1993. Total homocysteine in plasma or serum: methods and clinical applications. *Clinical Chemistry*, **39**(9):1764-1779.

Ueland, P.M., Hustad, S., Schneede, J., Refsum, H., Vollset, S.E., 2001. Biological and clinical implications of the MTHFR C677T polymorphism. *Trends in Pharmacological Sciences*, **22**(4):195-201. [doi:10.1016/S0165-6147(00)01675-8]

Verhoef, P., Kok, F.J., Kluijtmans, L.A., Blom, H.J., Refsum, H., Ueland, P.M., Kruyssen, D.A., 1997. The 677C→T mutation in the methylenetetrahydrofolate reductase gene: associations with plasma total homocysteine levels and risk of coronary atherosclerotic disease. *Atherosclerosis*, **132**(1):105-113. [doi:10.1016/S0021-9150(97)00084-1]

Wald, D.S., Law, M., Morris, J.K., 2002. Homocysteine and CVD: evidence on causality from a meta-analysis. *BMJ (Clinical Research Ed.)*, **325**(7374):1202. [doi:10.1136/bmj.325.7374.1202]

Wang, X., Qin, X., Demirtas, H., Li, J., Mao, G., Huo, Y., Sun, N., Liu, L., Xu, X., 2007. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet*, **369**(9576):1876-1882. [doi:10.1016/S0140-6736(07)60854-X]

Wang, Y.J., Liu, L.S., Rao, K.Q., Xu, X.P., 2008. Thoughts for prevention of stroke in China: control both hypertension and hyperhomocysteinemia. *National Medical Journal of China*, **88**(47):3316-3318 (in Chinese).

Westphal, S., Rading, A., Luley, C., Dierkes, J., 2003. Antihypertensive treatment and homocysteine concentrations. *Metabolism*, **52**(3):261-263. [doi:10.1053/meta.2003.50060]

Xu, Y.L., Zhang, S.C., Li, J.P., Wang, M.D., Xing, H.X., Zang, T.H., Huo, Y., Xu, X.P., 2006. The modification of MTHFR gene polymorphism effect of the correlation between serum folic acid and plasma homocysteine. *Acta Universitatis Medicinalis Anhui*, **41**(6):639-642 (in Chinese).

Yang, Q., Botto, L.D., Erickson, J.D., Berry, R.J., Sambell, C., Johansen, H., Friedman, J.M., 2006. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. *Circulation*, **113**(10):1335-1343. [doi:10.1161/CIRCULATIONAHA.105.570846]

Zhan, Y.Q., Jin, Y., Li, Y.Z., 2001. A general introduction of clinical application of angiotension converting enzyme inhibitors. *China Licensed Pharmacist*, **7**(1):3-6 (in Chinese).

Zhao, D., Liu, J., Wang, W., Zeng, Z., Cheng, J., Liu, J., Sun, J., Wu, Z., 2008. Epidemiological transition of stroke in China: twenty-one-year observational study from the Sino-MONICA-Beijing Project. *Stroke*, **39**(6):1668-1674. [doi:10.1161/STROKEAHA.107.502807]

Zhu, L.H., Xu, G.B., Yang, H.Y., 2001. Homocysteine and atherosclerosis. *Chinese Journal of Laboratory Medicine*, **24**(2):121-123 (in Chinese).