Low Plasma Pyridoxal 5'-phosphate Concentration and MTHFR 677C→T Genotypes are Associated with Increased Risk of Hypertension

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Abstract: Few studies have linked homocysteine, B vitamins and/or genetic defects to the risk of hypertension. The purpose of this study was to investigate homocysteine, B-vitamins, and genetic mutation in relation to the risk of hypertension. Subjects were assigned to the hypertension (HTN) group ($n = 50$) or non-hypertension (non-HTN) group ($n = 123$). All subjects' blood pressure (systolic blood pressure, SBP; diastolic blood pressure, DBP), biochemical values, plasma homocysteine, pyridoxal 5'-phosphate (PLP), serum folate, vitamin B_{12} concentrations, and methylenetetrafolate reductase (MTHFR) 677C→T gene polymorphism were measured. Results showed that subjects with T-allele were positively associated with DBP ($\beta = 4.22$, $p = 0.04$) but the significance became weaker ($p = 0.06$) after homocysteine and B vitamins were additionally adjusted. A significant association of plasma PLP with SBP remained ($\beta = -0.06$, $p = 0.01$) even after homocysteine and T-allele genotypes were additionally adjusted ($\beta = -0.07$, $p = 0.02$). The combined presence of low PLP (< 30 nmol/L) and carried T-allele enhanced the risk of hypertension and the risk magnitude was substantially greater (OR, 16.44, $p < 0.001$). Taken together, the results show that low plasma PLP levels and MTHFR 677C→T genotypes might be significant risk factors for hypertension.

Key words: Hypertension, pyridoxal 5'-phosphate, methylenetetrahydrofolate reductase, homocysteine, folate

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Introduction

Hypertension is a condition that greatly increases the risk for cardiovascular disease and thus is a major public health problem, with approximately 20% of the adult population affected in Western countries [1]. Pyridoxal 5'-phosphate (PLP), the physiologically active form of vitamin B_6, has been proposed to have an antihypertensive effect by influencing calcium influx [2–4], facilitating production of an aldehyde-binding thiol scavenger (i.e., cysteine) [5], acting directly on catecholamine to inhibit sympathetic activity [6], or possibly by reducing plasma homocysteine, which may cause endothelial cell injury [7–8]. Much of the research on the relationship between vitamin B_6 and blood pressure has been conducted with animal models; however, data on the association between vitamin B_6 status and blood pressure in humans is scant.

Hyperhomocysteinemia has been demonstrated to be an independent risk factor for cardiovascular disease [9]. In addition, the association between plasma homocysteine and blood pressure has recently been the focus of attention [10–13]. Sutton-Tyrrell *et al* [11] indicated that elevated levels of homocysteine were related to isolated systolic hypertension in some individuals after adjustment for age, gender, body mass index, high-density lipoprotein-3, smoking, cholesterol, and alcohol use ($p = .019$). However, this relationship was not significant after adjustment for age, gender, body mass index, and smoking in Iranian adults. [14]. In the homocysteine metabolism, methylenetetrahydrofolate reductase (MTHFR) catalyzes folate-dependent remethylation of homocysteine to methionine. The MTHFR 677 C→T mutation (Ala 222 Val) has been demonstrated to be thermolabile and mildly dysfunctional *in vivo*, and it may contribute to hyperhomocysteinemia [15, 16]. Studies [1, 17, 18] have shown that the MTHFR 677 C→T mutation (677TT genotype) is associated with an increased risk of hypertension. However, Williams *et al* [19] reported that blood pressure and arterial stiffness responses were independent of the MTHFR genotypes. Nakata *et al* [20] indicated that the 677TT genotype was even associated with lower blood pressure. It seemed that discrepancies exist in the data on the relationship of homocysteine and gene mutation with blood pressure.

The association between B vitamins (folate, vitamins B_6, and B_{12}), homocysteine, or genetic defects and blood pressure is still poorly understood and highly controversial. Therefore, the purpose of this study was to investigate the relationship of B vitamins, genetic mutation, and homocysteine with the risk of hypertension.

Materials and Methods

Subjects

Healthy subjects who exhibited normal blood biochemical values, including fasting blood glucose < 110 mg/dL, blood urea nitrogen (BUN) < 7.9 mmol/L, creatinine < 1.4 mg/dL, alkaline phosphates < 190 U/L, glutamic oxoloacetic transaminase (GOT) < 35 U/L, and glutamic pyruvate transaminase (GPT) < 45 U/L were recruited from the physical examination unit of Taichung Veterans Hospital. Exclusion criteria were illness, history of gastrointestinal disorder, cardiovascular disease, hyperlipidemia, liver and renal disease, diabetes, cancer, alcoholism, or other metabolic disease. All subjects' age, gender, smoking and drinking habits, and family history, were recorded. Body weight and height were measured; the body mass index (BMI; kg/m^2) was then calculated. Blood pressure [systolic and diastolic blood pressure (SBP and DBP)] was measured twice with a 30-minute interval between measurements and after a resting period of at least 5 minutes. The hypertension criteria were defined as systolic blood pressure (SBP) > 140 mm Hg, diastolic blood pressure (DBP) > 90 mm Hg, and/or receiving antihypertensive therapy ($n = 31$; 20 men and 11 women); or SBP > 160 mm Hg and/or DBP > 100 mm Hg without taking antihypertensive medication ($n = 19$; 11 men and 8 women). The normal blood pressure criteria were defined as no history of, and no current, hypertension with SBP < 130 mm Hg and DBP < 85 mm Hg. Fifty subjects who were matched to the hypertension criteria were assigned to the hypertension group (HTN group), while 123 subjects who had normal blood pressure were assigned to the non-hypertension group (non-HTN group). Both groups were recruited from the same population and were matched by age.

Biochemical analyses

Blood specimens (15 mL) were collected in Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing EDTA as an anticoagulant or no anticoagulant as required to estimate hematological and vitamin status. Plasma homocysteine was measured by using high-performance liquid chromatography (HPLC) according to the method of Araki and Sako [21]. Plasma PLP was determined by HPLC based on the method of Bates *et al* [22]. Serum folate and vitamin B_{12} were analyzed by standard competitive immunochemiluminometric methods. Hematological entities [i.e., BUN, GOT, GPT, serum creatinine, total cholesterol, triacylglycerol, low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL)] were measured using an Automated Biochemical analyzer. Automated high sensitivity C-reactive protein (hs-CRP) measurements concentration was determined with particle-enhanced immunonephelometry using an Immage analyzer [23]. The MTHFR 677C→T gene polymorphism was determined based on previous studies [24, 25].

Hyperhomocysteinemia was defined as a plasma homocysteine concentration ≥ 10 µmol/L based on the cutoff point of the Nutrition Committee of the American Heart Association [26]. Folate and vitamin B_{12} deficiencies were defined as serum concentrations lower than 6 ng/mL and 100 pg/mL, respectively [27]. Borderline vitamin B_6 deficiency was defined as plasma PLP concentration < 30 nmol/L [28], and vitamin B_6 deficiency was defined as PLP < 20 nmol/L [29]. This study was approved by the Institutional Review Board of Chung Shan Medical University and each subject signed the informed consent form.

Statistical analyses

Data were analyzed with SigmaStat statistical software (version 2.03; Jandel Scientific, San Rafael, CA). Differences in subjects' demographic data and hematological measurements were analyzed by Student's *t*-test or Mann-Whitney rank sum test between the two groups. For categorical response variables, differences between two groups were assessed by chi-square test or Fisher's exact test. Multiple linear regression analyses with either SBP or DBP as a dependent variable were used to determine the association of the MTHFR 677C→T genotypes, plasma homocysteine, and B vitamins with blood pressure after adjustment for age, gender, or potential confounders for hypertension. Adjusted odds ratios (ORs) with 95% confidence intervals (CI) for hypertension were calculated from the logistic regression model according to the MTHFR 677C→T genotypes, and vitamin B_6 status. Results were considered statistically significant at $p < 0.05$. Values presented in the text are means ± standard deviation (SD).

Results

Table I shows the demographic data and health characteristics of the subjects. Subjects in the HTN group had significantly higher values for BMI, SBP, DBP, LDL, total cholesterol (TC)-to-HDL ratio, triacylglycerol, and low-

Table I: Demographic and health characteristics of subjects[1]

Characteristics	HTN (n = 50)	Non-HTN (n = 123)
Male / Female	31 / 19	70 / 53
Age (years)	60.6 ± 10.8 (62.0)	59.0 ± 8.7 (56.0)
BMI (kg/m^2)	24.8 ± 3.2[a] (24.9)	23.8 ± 3.1[b] (23.7)
SBP (mmHg)	145.3 ± 19.5[a] (150.0)	111.3 ± 9.5[b] (110.0)
DBP (mmHg)	85.6 ± 12.8[a] (89.5)	69.7 ± 8.9[b] (70.0)
Cholesterol (mg/dL)		
Total	188.5 ± 34.2 (182.5)	182.5 ± 34.4 (182.0)
HDL	52.8 ± 13.6[a] (50.5)	60.0 ± 18.0[b] (57.0)
LDL	115.5 ± 26.2[a] (118.1)	102.9 ± 31.8[b] (98.6)
Total cholesterol/HDL ratio	3.8 ± 1.0[a] (3.6)	3.3 ± 1.0[b] (3.2)
Triacylglycerol (mg/dL)	131.3 ± 69.2[a] (109.5)	106.3 ± 55.0[b] (99.0)
Plasma homocysteine (µmol/L)	9.9 ± 2.5 (9.4)	9.5 ± 2.4 (9.3)
Serum folate (ng/mL)	13.2 ± 7.1 (11.7)	12.3 ± 6.3 (10.9)
Serum vitamin B_{12} (pg/mL)	529.6 ± 204.2 (515.0)	514.1 ± 201.4 (486.5)
Plasma PLP (nmol/L)	44.2 ± 57.1[a] (25.9)	69.4 ± 64.9[b] (46.8)
Serum creatinine (mg/dL)	1.0 ± 0.4 (1.0)	1.0 ± 0.2 (0.9)
hs-CRP (mg/dL)	0.3 ± 0.9 (0.1)	0.3 ± (0.8 (0.1)
MTHFR genotypes		
CC (n, %)	19 (38.0)	73 (59.3)
CT (n, %)	27 (54.0)	44 (35.8)
TT (n, %)	4 (8.0)	6 (4.9)
T-allele carriers[2] (n, %)	31 (62.0)[a]	50 (40.7)[b]

[1] Values are means ± SD with the median value in parentheses. Values with different superscript letters ([a], [b]) are significantly different between the two groups; $P < 0.05$.
[2] T-allele carriers were the subjects with 677CT and 677TT genotypes.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PLP, pyridoxal 5'-phosphate; hs-CRP, high-sensitivity C-reactive protein; MTHFR, methylenetetrafolate reductase.

er values for HDL and plasma PLP than subjects in the non-HTN group. 42% of HTN subjects and 36.6% of non-HTN subjects had hyperhomocysteinemia (≥ 10 µmol/L); 14.5% of subjects had folate deficiency (≤ 6 ng/mL), and 35.3% of subjects had vitamin B_6 deficiency (≤ 30 nmol/L). However, no subjects had vitamin B_{12} deficiency (≤ 100 pg/mL). With regard to the distribution of the three variants of the MTHFR 677C→T genotypes, subjects in the HTN group had significantly higher frequency of T-allele carriers than those in the non-HTN group. The genotypes distribution among the subjects in the two groups was consistent with that calculated from the Hardy-Weinberg equilibrium.

The association of MTHFR 677C→T genotypes, plasma homocysteine, and B vitamins concentrations with blood pressure is shown in Table II. The MTHFR 677C→T genotypes had a significantly positive association with DBP (p = 0.04); however, the association became weaker (p = 0.06) after homocysteine and B vitamins were additionally adjusted. Plasma PLP was negatively associated

Table II: The association of MTHFR genotypes, plasma homocysteine, and B vitamins concentrations with systolic and diastolic blood pressure

	Blood pressure (mm Hg) (n = 142)[1] SBP β[2] (P value)	DBP β (P value)
MTHFR 677C→T[3]		
Model 1[4]	5.08 (0.10)	4.22 (0.04)
Model 2[5]	5.72 (0.10)	4.26 (0.06)
Plasma homocysteine (µmol/L)		
Model 1	1.16 (0.14)	0.40 (0.43)
Model 3[6]	0.58 (0.51)	0.15 (0.80)
Serum folate (ng/mL)		
Model 1	–0.47 (0.07)	–0.32 (0.06)
Model 4[7]	–0.54 (0.06)	–0.33 (0.07)
Serum vitamin B-12 (pg/mL)		
Model 1	–0.01 (0.41)	–0.01 (0.10)
Model 4	–0.00 (1.00)	–0.01 (0.31)
Plasma PLP (nmol/L)		
Model 1	–0.06 (0.01)	–0.02 (0.18)
Model 4	–0.07 (0.02)	–0.02 (0.21)

[1]n = 142; data excluded those receiving medication for hypertension (n = 31). [2]β, regression coefficient. [3]MTHFR 677C→T, wild type = 0; T-allele carrier (677C→T plus 677TT genotypes) = 1. [4]Adjusted for age and gender, body mass index and creatinine. [5]As in model 1 and additionally adjusting for plasma homocysteine and B vitamins (including folate, vitamin B_{12} and PLP). [6]As in model 1 and additionally adjusting for T-allele and B vitamins. [7]As in model 1 and additionally adjusting for T-allele, plasma homocysteine, and other two B vitamins.
SBP, systolic blood pressure; DBP, diastolic blood pressure; MTHFR, methylenetetrafolate reductase; PLP, pyridoxal 5'-phosphate.

with SBP (p = 0.02). Although serum folate concentration did not significantly correlate with the level of blood pressure, the p value was close to statistical significance. Plasma homocysteine and vitamin B_{12} had no significant association with blood pressure. In addition, folate, vitamin B_6 and B_{12} concentrations did not correlate with plasma homocysteine concentration (data not shown).

Table III shows the association of the MTHFR 677C→T genotypes, plasma homocysteine, and plasma PLP with the risk of hypertension. Subjects with T-allele exhibited significantly increased risk of hypertension. Among homocysteine and the three B vitamins (folate, vitamin B_6, and vitamin B_{12}), only vitamin B_6 was significantly associated with the risk of hypertension. Subjects with plasma PLP < 30 nmol/L exhibited significantly greater risk of hypertension than subjects with plasma PLP ≥ 30 nmol/L after adjustment for potential confounders. The risk of hypertension was not significant in subjects who had higher PLP concentration (≥ 30 nmol/L), even in those who carried the T-allele. However, the combined presence of low PLP level and MTHFR 677C→T genotypes enhanced the risk of hypertension and the risk magnitude was substantially greater. The associations of serum folate alone or the combined presence of low folate level and MTHFR 677C→T genotypes with the risk of hypertension were not observed (data not shown).

Discussion

Over the past decade, evidence has accumulated implicating B vitamins deficiency and genetic defects, which cause hyperhomocysteinemia, as risk factors for cardiovascular disease [30–32]. Although hypertension has been recognized to be a primary risk factor for cardiovascular disease, few studies have linked homocysteine, B vitamins and/or genetic defects to the risk of hypertension.

Hyperhomocysteinemia has been shown to cause endothelial damage [30, 33] and vascular dysfunction [34, 35], which may lead to hypertension. Several studies have indicated that elevated plasma homocysteine concentration is positively associated with high blood pressure [36–39]. In a large cohort study (Hordaland Homocysteine Study), plasma homocysteine concentration was found to be positively correlated with blood pressure in about 16 000 subjects with no history of hypertension, diabetes, or coronary vascular disease [40], but this association was weak in younger subjects and was not significant in the subjects aged 65 to 74 years [41]. Dalery and colleagues [42] found there was no significant correlation between plasma homocysteine and the blood pressure in health subjects and patients with coronary artery disease. Our sub-

Table III: The odds ratios for hypertension in relation to the MTHFR genotypes and vitamin B_6 after adjustment for potential confounders

	Factors adjusted[1]			Additional factors adjusted[2]		
	OR	95% CI	*P*	OR	95% CI	*P*
MTHFR genotypes						
CC	1.00	–	–	1.00	–	–
CT	2.34	1.14–4.83	0.021	3.34	1.39–7.99	0.007
TT	1.74	0.85–3.56	0.130	1.80	0.75–4.32	0.190
T-allele carriers[3]	2.44	1.20–4.84	0.013	3.22	1.41–7.38	0.006
Plasma PLP (nmol/L)						
> 30 nmol/L	1.00	–	–	1.00	–	–
20–30 nmol/L	3.62	1.54–8.48	0.003	4.88	1.94–12.32	0.001
< 20 nmol/L	5.35	1.97–14.56	0.001	6.18	2.17–17.65	0.001
PLP (nmol/L) + MTHFR 677C→T						
PLP ≥ 30 + CC genotype	1.00	–	–	1.00	–	–
PLP ≥ 30 + T-allele carriers	1.96	0.70–5.45	0.198	2.01	0.71–5.66	0.187
PLP < 30 + CC genotype	3.30	1.13–9.67	0.029	3.37	1.14–9.95	0.028
PLP < 30 + T-allele carriers	14.74	4.42–49.18	< 0.001	16.44	4.71–57.41	< 0.001

[1] Adjusted for age, gender, body mass index, and creatinine.
[2] Adjusted for age, gender, body mass index, creatinine, homocysteine, and/or T-allele carriers and/or other B vitamins.
[3] T-allele carriers were the subjects with 677CT and 677TT genotypes.
MTHFR, methylenetetrafolate reductase; OR, odds ratio; CI, 95% confidence interval; PLP, pyridoxal 5'-phosphate.

jects were also free of any diseases that could lead to hypertension and we did not observe any relationships between plasma homocysteine concentration and blood pressure. Moreover, in the Tehran Homocysteine Survey (2003–2004), no correlation between homocysteine and blood pressure was observed in 1191 healthy subjects [14]. Several other studies also indicated that the association between plasma homocysteine concentration and blood pressure was not significant after age, gender, or potential confounders were adjusted [43–45]. We, therefore, agree with Fakhrzadeh *et al* [14], who proposed that elevated homocysteine concentration is likely a concomitant rather than a precursor of hypertension. However, there is a possibility that the lack of an association between homocysteine and hypertension might be due to a single measurement of homocysteine and this is not a good representation of long-term homocysteine concentration. Clearly, further investigation in a large trial is needed on the relationship between homocysteine and blood pressure.

Heux *et al* [1] indicated that the MTHFR 677C→T variant causes mild hyperhomocysteinemia, which also moderately but significantly increases the risk of hypertension independent of BMI (OR, 1.57, 95% CI, 1.04–2.37). Results of a study by Inamoto *et al* [18] showed that the 677TT genotype was independently associated with DBP in women. However, Nakata *et al* [20] not only found that subjects with the 677C→T or 677TT genotype had significantly lower blood pressure, but also that the risk of hypertension significantly increased in subjects with the 677CC genotype (OR, 1.97; 95% CI, 1.08–3.59). In this study, the MTHFR 677C→T genotypes were significantly associated with DBP, and this association increased the risk of hypertension independent of potential confounders for hypertension, homocysteine, folate, and vitamin B_{12} and B_6 concentrations. The MTHFR 677C→T variant might mediate the risk of hypertension not through elevated homocysteine concentration but more likely through another mechanism to cause vascular disorder.

Homocysteine is metabolized via two pathways: one is remethylation, which requires folate as a cosubstrate and vitamin B_{12} as a cofactor, and the other is transsulfuration, which requires PLP as a coenzyme. Studies have shown that higher folate intake or folic acid supplementation (5 mg/day) is associated with a decreased risk of hypertension and may even prevent isolated systolic hypertension [46]. In this study, however, only vitamin B_6, not folate or vitamin B_{12}, significantly affected SBP and increased the risk of hypertension after adjusting for all the potential confounders. Lower vitamin B_6 status has been reported to be associated with hypertension in rats [47, 48] and humans [49, 50]. In addition, our results showed that plasma PLP significantly affected subjects' SBP level independently of homocysteine and gene mutation. Since there is no consistent evidence implicating the effect of vitamin B_6 deficiency on an increase in fasting plasma homocysteine concentration, it is not likely that lower PLP level mediates the risk of hypertension through increasing plasma homocysteine concentration. Vitamin B_6 has been considered to share the mechanism with calcium by affecting calcium influx to increase SBP [2–4, 51]. However, the relationship between plasma PLP and serum calcium could not be examined since we did not have the data of subjects' serum calcium concentration in this study. A unique aspect of this study was to simultaneously examine the effect of combination of plasma PLP and MTHFR 677C→T genotypes on the risk of hypertension. Lower plasma PLP concentration (< 30 nmol/L) both in subjects with the normal MTHFR 677CC genotype and in those with the defective MTHFR 677C→T genotypes significantly increased the risk of hypertension. It is clear that even a borderline vitamin B_6 deficiency (< 30 nmol/L) can thus contribute to a higher risk of hypertension.

Gender and coronary artery disease (CAD) risk factors were associated with the elevated plasma homocysteine concentration [52]. In general, men have a higher plasma homocysteine concentration than women [53, 54]. Our male subjects also had a significantly higher homocysteine concentration than females (HTN group, 10.5 ± 2.5 vs. 8.8 ± 2.3 µmol/L; non-HTN group, 10.2 ± 2.1 vs. 8.6 ± 2.4 µmol/L). We further analyzed the data after the stratification by gender to minimize the gender effect. The results did not show any significant modification; therefore, male and female data were included in all the statistical analyses. In addition, after we adjusted for gender, the differences were not expected to affect the results of this study.

In conclusion, the evidence presented here suggests that low plasma PLP concentration and the MTHFR 677C→T genotypes might be significant risk factors for hypertension, independent of plasma homocysteine. Therefore, vitamin B_6 status and MTHFR 677C→T genotypes need to be taken into account when the risk of hypertension is assessed.

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