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MTHFR C677T Polymorphism as a Risk Factor for Vascular Calcification in Chronic Hemodialysis Patients

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Polymorphism of 5,10-methylenetetrahydrofolate reductase (*MTHFR*) C677T is one of the suggested risk factors for atherosclerosis. However, few studies have reported on the relationship between *MTHFR* C677T polymorphism and vascular calcification (VC) in chronic hemodialysis patients. We investigated the relationship between the *MTHFR* C677T polymorphism and VC in 152 chronic hemodialysis patients. Patients with a TT genotype exhibited significantly higher VC scores than patients expressing CC and CT ($P = 0.002$). The prevalence of peripheral vascular disease increased with the incidence of *MTHFR* C677T mutations for all patients, and the incidence of cerebrovascular accidents also increased with the presence of mutations for young patients (≤ 60 yr) ($P < 0.05$). Patients with CT and TT genotypes had adjusted odds ratios for VC of 1.39 and 1.58, respectively ($P < 0.05$). In summary, these data suggest that the *MTHFR* C677T polymorphism affects the degree of VC in chronic hemodialysis patients.

Key Words: Methylenetetrahydrofolate Reductase (*MTHFR*); Renal Dialysis; Homocysteine; Vascular Calcification

Vascular calcification (VC) is a common manifestation of end-stage renal disease (ESRD) (1, 2). The presence of VC is associated with aortic stiffness and is predictive of subsequent cardiovascular disease (CVD) and increased mortality (2). The exact pathophysiology of VC in ESRD patients is unclear. Recent studies have reported that VC is an active process regulated by various genes and proteins (2). The 5,10-methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism is associated with decreasing enzyme activity and increasing homocysteine (Hcy) levels (3). Elevated plasma Hcy is one of the suggested risk factors for atherosclerosis due to endothelial dysfunction and oxidative stress (4). There is evidence for an association between increased plasma Hcy and the *MTHFR* polymorphism with an increased risk for developing CVD (5, 6). However, few studies have reported on the relationship between the *MTHFR* C677T polymorphism and VC in patients on chronic hemodialysis. Therefore, the goal of this study was to evaluate the degree of VC (7) and analyze the association with the *MTHFR* C677T polymorphism.

After obtaining approval from the Institutional Review Board of Bundang CHA General Hospital, we recruited 152 patients. Inclusion criteria were ages 20-90 yr and the diagnosis of ESRD in patients that received chronic hemodialysis treatments for more than 3 months. Patients with acute infectious diseases or unstable vital signs were excluded. All patients provided written informed consent.

VC was evaluated by examining plain radiography of the pelvis and hands (7). The total final scores ranged from 0 to 8. Arterial stiffness was assessed using a commercially available device (VP-2000, Colin Corporation, Komaki, Japan) that measures the pulse wave velocity (PWV). Pulse wave forms were obtained from the carotid and femoral artery sites.

Genomic DNA was extracted from peripheral blood leukocytes using Puregene DNA extraction kits (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocols. The *MTHFR* C677T genotypes were identified as previously described (8).

A routine clinical workup of all patients within 1 month of enrollment included fasting blood samples before the mid-week dialysis. Hcy levels were measured by chemiluminescence immunoassay. The level of total intact parathyroid hormone (iPTH) was evaluated by electrochemiluminescence immunoassay.

Statistical comparison between the groups was performed using the independent samples t-test, ANOVA, and chi-square tests. Predictive factors for VC were examined using logistic regression analysis. *P* values less than 0.05 were considered statistically significant.

In terms of patients' genotypes, 28.9% had CC, 47.4% had CT, and 23.7% had TT (Table 1). The patients with the TT genotype had higher VC scores than patients with the CC or CT genotype ($P = 0.002$). The TT genotype tended to be associated with a higher PWV, pulse pressure (PP), and prevalence of CVD; however, the differences were not statistically significant. Only the preva-

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Table 1. Clinical characteristics according to *MTHFR* C677T genotypes

Genotypes	No. of cases (%)	Age (yr)	Hcy (μmol/L)	VC Score (0-8)	PWV (cm/s)	CVD (%)	IHD (%)	CVA (%)	PVD (%)
All patients									
CC	44 (28.9)	61.61 ± 12.4*	16.74 ± 5.5	0.59 ± 1.5	1,592.93 ± 394.1	38.6	38.6	31.8	2.3
CT	72 (47.4)	54.24 ± 14.1	20.17 ± 8.3	1.32 ± 2.1	1,503.38 ± 407.0	41.7	41.7	29.2	12.5
TT	36 (23.7)	56.17 ± 13.6	18.91 ± 5.7	2.33 ± 2.7†	1,613.79 ± 425.0	55.6	55.6	44.4	22.2
P value		0.018	0.051	0.002	0.432	0.142	0.142	0.268	0.006
Patients younger than 60 yr old									
CC	18 (20.5)	49.72 ± 8.8	17.25 ± 5.5	0.33 ± 1.0	1,573.10 ± 441.0	27.8	11.1	22.2	1.2
CT	46 (52.3)	45.89 ± 10.0	20.24 ± 9.2	0.91 ± 1.6	1,443.59 ± 404.8	28.3	17.4	19.6	6.5
TT	24 (27.3)	48.46 ± 8.3	18.92 ± 5.1	2.29 ± 2.8†	1,595.36 ± 384.5	35.2	25.0	50.0	25.0
P value		0.278	0.381	0.004	0.351	0.057	0.245	0.031	0.006
Patients older than 60 yr old									
CC	26 (40.6)	69.85 ± 6.5	16.39 ± 5.6	0.77 ± 1.7	1,611.35 ± 360.9	46.2	26.9	38.5	3.8
CT	26 (40.6)	69.00 ± 6.5	20.05 ± 6.5	2.04 ± 2.7	1,613.51 ± 398.0	65.4	30.8	46.2	23.1
TT	12 (18.8)	71.58 ± 7.3	18.91 ± 7.5	2.42 ± 2.5	1,654.76 ± 527.6	58.3	41.7	33.3	16.7
P value		0.505	0.124	0.069	0.499	0.331	0.390	0.915	0.152

*$P < 0.05$ vs CT and TT genotype by one-way ANOVA with LSD post hoc comparison; †$P < 0.05$ vs CC and CT genotype by one-way ANOVA with LSD post hoc comparison. Results are expressed as means ± SD or number of observations (percentage). CVA, cerebrovascular accidents; CVD, cardiovascular disease including ischemic heart disease, cerebrovascular accident, and peripheral vascular disease; Hcy, homocysteine; IHD, ischemic heart disease; MTHFR, 5,10-methylenetetrahydrofolate reductase; PVD, peripheral vascular disease; PWV, pulse wave velocity; VC Score, vascular calcification score.

lence of peripheral vascular disease (PVD) was significantly increased in patients with a T allele ($P = 0.006$). The study group was divided into two subgroups by 60 yr of age, based on a median age of 56.5 yr; the mean ages of the subgroups were 47.38 ± 9.4 and 69.83 ± 6.2 yr. In young patients (≤ 60 yr), *MTHFR* C677T mutations were associated with higher VC scores ($P = 0.004$). In young patients, *MTHFR* C677T mutations were a significant predictive factor for cerebrovascular accident (CVA) ($P = 0.031$) and PVD ($P = 0.006$), except ischemic heart disease. The *MTHFR* C677T polymorphism was not associated with plasma Hcy levels.

A total of 57 (37.5%) patients presented with VC. Extensive VC scores (> 3) were observed in 25 (16.4%) of all patients. Table 2 provides data indicating that the existence of VC was associated with a higher frequency of CVD and diabetes ($P = 0.001$). Patients with VC had significantly higher systolic blood pressures, PWVs, and PPs ($P < 0.05$). The mean albumin levels were significantly lower in patients with VC. The frequencies of the *MTHFR* C677T mutation significantly differed between patients with or without VC ($P = 0.003$). However, the plasma Hcy levels did not significantly differ between the subgroups.

To identify predictors of VC, multiple logistic regression analysis was performed using the existence of VC as the dependent variable and 10 selected variables, including age and gender, as the independent variables. Age, diabetes, and *MTHFR* C677T mutations were determined to be independent predictors of the existence of VC (Table 3). The adjusted odds ratio (OR) for the TT genotype for the risk of extensive VC score (> 3) was 1.66 (95% confidence interval [CI]; 1.41-19.47, $P = 0.013$) for all patients, and 2.41 (95% CI; 1.70-73.29, $P = 0.012$) for patients younger than 60 years of age (data not shown).

The results of this study suggest that there is a strong relationship between the incidence of *MTHFR* C677T mutations and VC in patients with ESRD on chronic hemodialysis. Compared to patients with the CC genotype, patients with CT and TT genotypes had adjusted ORs for VC of 1.39 and 1.58, respectively ($P = 0.042$ and 0.032). In the subgroup analysis, the correlation of the *MTHFR* C677T mutation and VC persisted in young patients (≤ 60 yr) (Table 1). A similar trend of increased VC scores, although not significant, was observed for the older patient group (> 60 yr). These results suggest that the harmful effect of having a mutant T allele at nucleotide position 677 may be diluted with age, which is another strong risk factor for VC.

The possible association between a genetic polymorphism and the development of VC has been of recent interest to investigators (8). A better understanding of the pathogenesis contributing to the increased VC in ESRD patients can provide a better perspective on the high cardiovascular mortality, which is partially explained by the traditional risk factors. Several recent reports have suggested that the *MTHFR* C677T polymorphism (9) is associated with the development of CVD in patients with ESRD. This is the first study to statistically evaluate the relationship between the extent of VC and *MTHFR* C677T polymorphism.

The mediator of vascular injury has been presumed to be plasma Hcy levels (10), which are increased in patients with mutant T allele (5). However, in this study, plasma Hcy levels were not associated with the *MTHFR* C677T polymorphism and VC. There are several possible explanations for these findings. Several randomized trials focused on reducing Hcy levels in the general population (11) or in patients with ESRD (12) failed to improve outcome. Perhaps, lifetime exposure to increased Hcy levels cannot be remedied by a few years of partial restoration of Hcy levels to normal. Alternatively, high Hcy levels may simply mark rather than mediate the injury caused by impaired MTHFR activity. Recently, the levels of tissue 5-methyl-tetrahydrofolate (5-MTHF), rather than plasma or tissue levels of Hcy,

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Table 2. Clinical characteristics according to the existence of vascular calcification

Characteristics	All patients	According to vascular calcification		
		Patients without VC	Patients with VC	*P*
No.	152	95	57	
Male (%)	54.6	56.8	50.9	0.476
Age (yr)	56.83 ± 13.8	56.13 ± 14.0	60.40 ± 12.3	0.852
Duration of hemodialysis (months)	44.74 ± 49.6	43.46 ± 50.3	51.16 ± 46.16	0.480
High flux dialyzer (%)	37.1	35.8	39.3	0.669
Body mass index (kg/m^2)	22.53 ± 3.5	22.64 ± 3.5	21.98 ± 3.4	0.424
Current smoker (%)	10.1	12.0	6.8	0.968
History of cardiovascular disease (%)	44.1	34.7	63.6	**0.001**
Hypertension (%)	92.1	89.5	96.5	0.112
Diabetes mellitus (%)	53.3	40.0	75.4	**< 0.001**
Systolic blood pressure (mmHg)	142.28 ± 23.9	140.35 ± 24.20	151.86 ± 20.2	**0.044**
Diastolic blood pressure (mmHg)	80.20 ± 13.2	80.6 ± 13.2	78.14 ± 13.5	0.437
Pulse pressure (mmHg)	62.22 ± 17.2	59.73 ± 16.8	74.00 ± 14.1	**< 0.001**
Carotid to femoral PWV (cm/s)	1,554.47 ± 408.1	1,500.89 ± 380.4	1,811.11 ± 448.4	**0.002**
MTHFR C677T genotype, No. (%)				**0.003**
CC	44 (28.9)	36 (37.9)	8 (14.0)	
CT	72 (47.4)	41 (43.2)	31 (54.4)	
TT	36 (23.7)	18 (18.9)	18 (31.6)	
Hemoglobin (g/dL)	10.06 ± 1.4	10.12 ± 1.0	9.76 ± 2.4	0.255
Reticulocyte (%)	0.87 ± 0.5	0.85 ± 0.5	0.95 ± 0.6	0.408
Serum calcium (mg/dL)	9.00 ± 0.9	9.01 ± 0.8	8.92 ± 1.0	0.665
Serum phosphorus (mg/dL)	4.76 ± 1.5	4.76 ± 1.6	4.75 ± 1.3	0.967
Calcium X phosphorus product (mg^2/dL^2)	42.83 ± 14.7	42.93 ± 15.8	42.36 ± 12.2	0.861
Intact parathyroid hormone (pg/mL)	174.97 ± 207.6	188.22 ± 216.0	111.35 ± 148.8	0.092
Serum creatinine (mg/dL)	9.79 ± 3.7	10.03 ± 3.8	8.62 ± 2.9	0.089
Serum albumin (pg/mL)	3.74 ± 0.4	3.77 ± 0.4	3.56 ± 0.4	**0.042**
Total cholesterol (mg/dL)	134.63 ± 37.8	136.13 ± 38.1	126.52 ± 35.5	0.264
LDL cholesterol (mg/dL)	68.03 ± 33.9	69.69 ± 35.3	59.09 ± 23.4	0.169
HDL cholesterol (mg/dL)	38.55 ± 13.9	37.78 ± 13.1	42.55 ± 15.8	0.118
Triglyceride (mg/dL)	113.74 ± 101.1	119.05 ± 107.3	84.87 ± 49.0	0.137
C-reactive protein (mg/dL)	0.43 ± 0.8	0.38 ± 0.8	0.71 ± 1.0	0.138
KT/V	1.43 ± 0.4	1.43 ± 0.6	1.42 ± 0.7	0.862
Homocysteine (μM/L)	18.9 ± 7.1	18.72 ± 7.3	19.99 ± 6.0	0.454
Folate (ng/mL)	46.73 ± 35.6	46.63 ± 34.6	47.30 ± 41.7	0.937
Medications				
ACE inhibitor/ARBs (%)	79.8	76.8	85.1	0.261
B-blocker (%)	38.8	37.8	40.4	0.770
Calcium channel blocker (%)	65.1	62.2	70.2	0.360
Multi-vitamin supplement* (%)	86.0	85.4	87.2	0.769
Statin† (%)	49.2	46.9	53.2	0.495

Statistically significant values $P < 0.05$ in bold. *Multi-vitamin supplement contained 10 mg pyridoxine HCl, 1.5 mg thiamine nitrate, 6 μg cyanocobalamine, 1.7 mg riboflavin, 60 mg ascorbic acid, 1,000 μg folic acid, 300 μg biotin, 20 mg nicotinamide, and ca. 10 mg pantothenic acid; †Cholesterol-lowering HMG-CoA reductase inhibitor. Results are expressed as means ± SD or number of observations (percentage). ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MTHFR, 5,10-methylenetetrahydrofolate reductase; PWV, pulse wave velocity; VC, vascular calcification.

have been suggested to play a role in regulating endothelial function (13).

VC was present in 37.5% of the study participants, which is inconsistent with the rate of 74.8% reported in a study evaluating chronic hemodialysis patients using the same VC scoring method (7, 14). In this study, mean patient age was 56.83 ± 13.8 yr, which is lower than the mean patient age reported in the previous study (14). Unfortunately, there are limited data available on VC in Asian patients assessed using a simple radiogdraphy calcification score.

There was no significant relationship between *MTHFR* C677T polymorphism and CVD in this study. In the ESRD population, evidence for an association of the *MTHFR* C677T mutation with CVD is also inconsistent (9). Since ESRD patients carry a heavy cardiovascular disease burden, these conflicting results may reflect confounding by various risk factors. Thus, in our study, the mean age of the patients with the CC genotype was greater than those of the patients with CT or TT. In addition, methodological limitations constrain the interpretation of the findings from our small-sized cross-sectional study. The prevalence of PVD increased with the incidence of *MTHFR* C677T mutation for all patients, and the CVA also significantly increased for the young

Table 3. Factors associated with vascular calcification in logistic regression analysis

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age (yr)	0.029 (1.00-1.05)	**0.026**	0.073 (1.07-1.02)	**0.004**
Sex (female/male)	-0.240 (0.40-1.52)	0.475	-	-
Diabetes mellitus (present/absent)	1.528 (2.22-9.55)	**0.000**	2.520 (3.59-43.06)	**0.006**
Pulse pressure (mmHg)	0.031 (1.01-1.05)	**0.007**	-	-
Intact parathyroid hormone (pg/mL)	-0.002 (0.99-1.00)	**0.035**	-	-
Serum creatinine (mg/dL)	-0.040 (0.87-1.04)	0.370	-	-
Serum albumin (pg/mL)	-0.426 (0.31-1.35)	0.252	-	-
Calcium X phosphorus product (mg^2/dL^2)	0.000 (0.97-1.02)	0.981	-	-
Homocysteine (μM/L)	0.043 (0.99-1.09)	0.089	-	-
MTHFR C677T genotype*			-	-
CT	1.224 (1.38-8.34)	**0.007**	1.394 (1.04-15.62)	**0.042**
TT	1.504 (1.64-12.31)	**0.003**	1.580 (1.14-20.50)	**0.032**

Statistically significant values $P < 0.05$ in bold. *CC, CT, and TT genotypes were assigned the values of 0, 1, and 2, respectively. MTHFR, 5,10-methylenetetrahydrofolate reductase; CI, confidence interval; OR, odds ratio.

patients (≤ 60 yr). Further studies using a larger population are required to confirm these findings.

The *MTHFR* 677TT genotype was not associated with the PWV in this study. It is possible that the association of the *MTHFR* genotype with the PWV was attenuated by several therapeutic interventions such as correction of uremia (15), hypertension (16), hyperhomocysteinemia (17), or the use of rennin-angiotensin-aldosterone system antagonists (18), statins (19) and/or beta blockers (20).

This study has several limitations. It was a small-sized cross-sectional study. No variability was determined by repeated measures of the Hcy or PWV parameters. However, individual investigators, who were blind to the clinical data, independently reported the VC and PWV measurements. Dietary calcium intake was not quantified, and the 25-hydroxy vitamin D levels were not determined, which would have been useful for assessing VC in the ESRD population.

In conclusion, the present study indicates that the *MTHFR* C677T mutation is an important factor influencing VC in chronic hemodialysis patients. However, further large-scale studies are required to fully characterize the relationship between the extent of VC and *MTHFR* C677T mutational status.

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