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

So-Young Lee, Hoe-Young Kim, Kyung Mi Park, Stephen Yong Gu Lee, Seong Geun Hong, Hyung-Jong Kim, and Dong Ho Yang

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-
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]; 1.41-1.94, *P* = 0.013) for all patients, and 2.41 (95% CI; 1.70-7.39, *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.

### 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 | 38 (23.7) | 56.17 ± 13.6 | 18.91 ± 5.7 | 2.33 ± 2.7* | 1.613.79 ± 425.0 | 56.6 | 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 | 27.9 | 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 | 28.2 | 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 | 1.611.36 ± 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.

The level 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]; 1.41-1.94, *P* = 0.013) for all patients, and 2.41 (95% CI; 1.70-7.39, *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.
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 radiography calcification score.

There was no significant relationship between \textit{MTHFR} C677T polymorphism and CVD in this study. In the ESRD population, evidence for an association of the \textit{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 \textit{MTHFR} C677T mutation for all patients, and the CVA also significantly increased for the young

### 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     | 0.069 |
| Body mass index (kg/m²)          | 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 ± 24.2                      | 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 |
| \textit{MTHFR} C677T genotype, No. (%) |              |                                     |          |
| CC                               | 44 (28.9)    | 36 (23.7)                           | 8 (14.0) | 0.003 |
| CT                               | 72 (47.4)    | 41 (24.3)                           | 31 (42.4) | 0.255 |
| TT                               | 36 (23.7)    | 18 (12.3)                           | 18 (31.6) | 0.408 |
| Hemoglobin (g/dL)                | 10.06 ± 1.4  | 10.12 ± 1.0                         | 9.76 ± 2.4 | 0.665 |
| Reticulocyte (%)                 | 0.87 ± 0.5   | 0.85 ± 0.5                          | 0.95 ± 0.6 | 0.976 |
| Serum calcium (mg/dL)            | 9.00 ± 0.9   | 9.01 ± 0.8                          | 8.92 ± 1.0 | 0.118 |
| Serum phosphorus (mg/dL)         | 4.76 ± 1.5   | 4.76 ± 1.6                          | 4.75 ± 1.3 | 0.044 |
| Calcium X phosphorus product (mg/2/dL²) | 42.83 ± 14.7 | 42.93 ± 15.8                      | 42.36 ± 12.2 | 0.089 |
| Intact parathyroid hormone (pg/mL) | 174.97 ± 207.6 | 188.22 ± 216.0                  | 113.35 ± 148.8 | 0.092 |
| Serum creatinine (mg/dL)         | 9.79 ± 3.7   | 10.03 ± 3.8                         | 8.62 ± 2.9 | 0.042 |
| Serum albumin (mg/dL)            | 3.74 ± 0.4   | 3.77 ± 0.4                          | 3.56 ± 0.4 | 0.264 |
| Total cholesterol (mg/dL)        | 134.63 ± 37.8| 136.13 ± 38.1                      | 126.52 ± 35.5 | 0.169 |
| LDL cholesterol (mg/dL)          | 68.03 ± 33.9 | 69.69 ± 35.3                       | 59.09 ± 23.4 | 0.118 |
| HDL cholesterol (mg/dL)          | 38.55 ± 13.9 | 37.78 ± 13.1                       | 42.55 ± 15.8 | 0.044 |
| Triglyceride (mg/dL)             | 113.74 ± 101.1| 119.05 ± 107.3                    | 84.87 ± 49.0 | 0.044 |
| 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 (mg/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 nitrile, 6 µg cyanocobalamin, 1.7 mg riboflavin, 60 mg ascorbic acid, 1,000 µg folic acid, 300 µg biotin, 20 mg nicotinamide, and ca. 10 mg pantothentic 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.

DOI: 10.3346/jkms.2011.26.3.461
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.

REFERENCES

1. Braun J, Oldendorf M, Moshage W, Heidler R, Zeiter E, Luft FC. Electron beam computed tomography in the evaluation of cardio calcification in chronic dialysis patients. Am J Kidney Dis 1996; 27: 394-401.
2. London GM, Marchais SJ, Guérin AP, Métivier F. Arteriosclerosis, vascular calcifications and cardiovascular disease in uremia. Curr Opin Nephrol Hypertens 2005; 14: 1265-71.
3. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111-3.
4. Spark JJ, Laws P, Fitridge R. The incidence of hyperhomocystinaemia in vascular patients. Eur J Vasc Endovasc Surg 2003; 26: 558-61.
5. Wald DS, Wald NJ, Mocro M, Folic acid, homocysteine, and cardiovascular disease: judging causality in the face of inconclusive trial evidence. BMJ 2006; 333: 1114-7.
6. Kh Randall pour N, Willis G, Meyer FI, Armon MP, Loke YK, Wright AI, Finglas PM, Jennings BA. Peripherial arterial disease and methylenetetrahydrofolate reductase (MTHFR) C677T mutations: a case-control study and meta-analysis. J Vasc Surg 2009; 49: 711-8.
7. Adragão T, Pires A, Birne R, Curto JD, Lucas C, Gonçalves M, Negrão AP. A plain X-ray vascular calcification score is associated with arterial stiffness and mortality in dialysis patients. Nephrol Dial Transplant 2009; 24: 997-1002.
8. Cozzolino M, Biondi ML, Galassi A, Cusi D, Brancaccio D, Gallieni M. Vascular calcification and cardiovascular outcome in dialysis patients: the role of gene polymorphisms. Blood Purif 2010; 29: 347-51.
9. Jamison RL, Shih MC, Humphries DE, Guarino PD, Kaufman JS, Goldfarb DS, Warren SR, Gazzano JM, Lavori P; Veterans Affairs Site Investigators. Effect of the MTHFR C677T and A1298C polymorphisms on survival in patients with advanced CKD and ESRD: a prospective study. Am J Kidney Dis 2009; 53: 779-89.
10. Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg H. The kidney and homocysteine metabolism. J Am Soc Nephrol 2001; 12: 2181-9.
11. Ebhing M, Bleie O, Ueland PM, Nordrehaug JE, Nilsen DW, Vollset SE, Refsum H, Pedersen EK, Nygård O. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. JAMA 2006; 296: 755-801.
12. Jamison RL, Hartigan P, Kaufman JS, Goldfarb DS, Warren SR, Guarino PD, Gazzano JM; Veterans Affairs Site Investigators. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. JAMA 2007; 298: 1163-70.
13. Antoniades C, Shirodaria C, Leeson P, Baarholm OA, Van-Assche T, Cunningham C, Pillai R, Ratnaguru C, Tousoulis D, Stefanadis C, Refsum H, Channon K. MTHFR 677 C>T Polymorphism reveals functional importance for 5-methyltetrahydrofolate, not homocysteine, in regulation of vascular redox state and endothelial function in human atherosclerosis. Circulation 2009; 119: 2507-15.
14. Adragão T, Pires A, Lucas C, Birne R, Magalhaes L, Gonçalves M, Negrão AP. The MTHFR Gene and Vascular Calcifications.
AP. A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. Nephrol Dial Transplant 2004; 19: 1480-8.

15. Annuk M, Zilmer M, Lind L, Linde T, Fellström B. Oxidative stress and endothelial function in chronic renal failure. J Am Soc Nephrol 2001; 12: 2747-52.

16. Agabiti-Rosei E, Heagerty AM, Rizzoni D. Effects of antihypertensive treatment on small artery remodelling. J Hypertens 2009; 27: 1107-14.

17. Levy D, Hwang SI, Kayalar A, Benjamin EJ, Vasan RS, Parise H, Larson MG, Wang TJ, Selhub J, Jacques PF, Vita JA, Keyes MJ, Mitchell GF. Associations of plasma natriuretic peptide, adrenomedullin, and homocysteine levels with alterations in arterial stiffness: the Framingham Heart Study. Circulation 2007; 115: 3079-85.

18. Ichihara A, Hayashi M, Kaneshiro Y, Takemitsu T, Homma K, Kanno Y, Yoshizawa M, Furukawa T, Takenaka T, Saruta T. Low doses of losartan and trandolapril improve arterial stiffness in hemodialysis patients. Am J Kidney Dis 2005; 45: 866-74.

19. Ferrier KE, Muhlmann MH, Baguet JP, Cameron JD, Jennings GL, Dart AM, Kingwell BA. Intensive cholesterol reduction lowers blood pressure and large artery stiffness in isolated systolic hypertension. J Am Coll Cardiol 2002; 39: 1020-5.

20. Mahmud A, Feely J. Beta-blockers reduce aortic stiffness in hypertension but nebivolol, not atenolol, reduces wave reflection. Am J Hypertens 2008; 21: 663-7.