An MTHFR variant, homocysteine, and cardiovascular comorbidity in renal disease

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Background. It is unclear whether total serum homocysteine (tHcy) and the C677T mutation of methylenetetrahydrofolate reductase (MTHFR) are associated with cardiovascular disease (CVD) in patients with end-stage renal disease (ESRD).

Methods. A cross-sectional sample of 459 patients with ESRD on chronic dialysis was assessed to determine whether tHcy and the C677T mutation are associated with CVD prevalence in multiple logistic regression. As CVD mortality is high, we examined the relationship between homozygosity and duration of dialysis.

Results. Mean tHcy was higher in patients without a history of CVD (34.4 μmol /L versus 30.0 μmol /L, P = 0.02). In multivariate models, CVD was negatively associated with tHcy and positively associated with TT-genotype, male gender, and body mass index. Mean tHcy levels were higher among those with the TT-genotype compared to those with the CC-genotype when adjusted for age, folate, creatinine, and albumin (37.9 μmol versus 31.9 μmol, P = 0.005). Among whites, prevalence of the TT-genotype was higher in those having undergone less than one year of dialysis (P = 0.002).

Conclusions. C677T genotype of MTHFR is associated with CVD in ESRD and may be a more meaningful marker than tHcy for abnormal homocysteine metabolism in ESRD. Prospective data from on-going clinical trials are needed to improve our understanding of these findings. Screening for this polymorphism may help guide prevention measures.

Keywords: homocysteine, end-stage renal disease, cardiovascular disease, methylenetetrahydrofolate reductase.

Hyperhomocysteinemia is under intense investigation as a readily modifiable risk factor for cardiovascular disease in the general population and in those with renal impairment. Despite homocysteine's role in vascular pathophysiology in vitro and animal studies, clinical and epidemiologic evidence is mixed and the lack of clinical trials leaves the question of causality open. There is less evidence concerning the relationship between tHcy (total serum homocysteine[1]) and cardiovascular risk in the subset of patients with ESRD than there is in the general population.

A common variant of a key enzyme in homocysteine metabolism, the C677T mutation (also known as the thermolabile variant) of methylenetetrahydrofolate reductase (MTHFR), located on chromosome 21, is emerging as a possible genetic contributor to hyperhomocysteinemia and cardiovascular disease. The C677T point mutation of MTHFR results in a valine substitution for alanine [2]. With two copies of this variant (TT) serum activity of MTHFR is reduced to 30% while serum activity of heterozygotes (CT) is 65% of that of wild-type (CC) individuals [2]. Epidemiologic evidence supports an influence of C677T genotype on homocysteine metabolism for those with relatively reduced folate levels. [3-5] and mildly and less consistently for the general population [3, 6]. The C677T mutation may also influence tHcy levels in ESRD, especially in those with low folate levels[7, 8]. The role of the C677T mutant allele in increasing the risk for vascular disease was unconfirmed in large studies [3, 9], a recent meta-analysis [6] and was not found in a study of 545 Japanese hemodialysis patients[7]. While it appears that the C677T mutation of the MTHFR gene may influence tHcy levels, especially in folate depletion, its role in hyperhomocysteinemia and cardiovascular disease is unclear.

Patients with ESRD have levels of tHcy that are three times those of the general population [10]. Despite a strong, positive relationship between serum creatinine and tHcy[11], the mechanism of hyperhomocysteinemia in renal failure appears to operate through the inhibitory effect of putative uremic factors on enzymes in the folate-homocysteine pathway [12]. It appears to be unrelated to reduced renal clearance as renal failure progresses [13]. Because patients with ESRD have a crude mortality rate of 24%, half of which is due to cardiovascular disease [14], improved understanding of tHcy and cardiovascular risk may lead to development of treatment and prevention strategies.

We undertook an investigation of tHcy and the C677T mutation of MTHFR in the pathogenesis of cardiovascular disease. This report is a cross-sectional analysis of base-line entry data from a randomized prospective clinical trial of tHcy reduction in ESRD patients. The questions addressed in this report are:

1. Is tHcy associated with cardiovascular disease in this population?
2. What factors influence tHcy level?
3. Is the C677T variant of MTHFR associated with elevated tHcy, cardiovascular disease, and survival on dialysis?

Methods

Study Population

This analysis represents the findings of the baseline entry exam to a clinical trial, after informed consent and prior to initiation of any study procedures or treatments. The clinical trial is a randomized, standard-therapy controlled study of high-dose folic acid for the

reduction of tHcy with clinical outcomes as endpoints. We studied 459 prevalent patients with ESRD undergoing chronic dialysis treatment (430 hemodialysis and 29 peritoneal dialysis) at 10 affiliated non-profit outpatient units in Northern California from March 1998 to May 1999. Adult patients undergoing hemodialysis or peritoneal dialysis and were able to participate in the consent process were eligible for the study. Patients who were undergoing intra-dialytic parenteral nutrition, anticipating a living-related kidney transplant, receiving an anti-seizure medication, or were terminally ill were excluded from the study. A written consent was obtained from every patient after a full explanation of the study, which was approved by the Administrative Panel on Human Subjects in Medical Research of Stanford University. At the time these data were gathered, the participants had not received instructions to change their pattern of folic acid or multivitamin use. Cardiovascular disease was defined as being present if there was a history of coronary artery intervention, myocardial infarction, stroke, transient ischemic attack, carotid end-arterectomy, or other clinical evidence of cardiovascular disease as documented in hospital discharge summaries or history and physical examination upon admission to the dialysis unit.

Baseline clinical variables were collected at the time of enrollment. These included diabetes status, smoking history, duration of renal replacement therapy, prescribed medication use including dose and formulation of all vitamin supplements, height, and dry weight (the prescribed, post-dialysis weight). Race was assigned from one of the following categories: African-American, Asian/Pacific Islander, other/multi-racial, or white. Patients were assigned a separate ethnic status that included Hispanic or non-Hispanic. Patients from four facilities were further assessed for compliance with prescribed vitamin use and for over-the-counter

vitamin supplement intake. For those on hemodialysis the following were collected: vascular access history, pre and post-dialysis systolic and diastolic blood pressure (averaged over 9 treatments). In addition, the most recently monthly dialysis adequacy parameters were collected for all hemodialysis subjects. Adequacy parameters included Kt/V, a unit-less measure of dialysis dose, and normalized protein catabolic rate. This kinetic modeling was single-pool, variable-volume urea using pre and post dialysis blood urea nitrogen assessment. The pre-dialysis blood sample was taken prior to the initiation of dialysis and administration of saline or heparin. The post-dialysis sample was taken from the arterial port closest to the patient using the slow flow technique (within 15-30 seconds with the blood flow rate decreased to between 50-100 ml/min).

Biochemical Assays

All patients had pre-dialysis study blood samples drawn in conjunction with routine monthly specimens. THcy concentrations were determined by high-performance liquid chromatography (HPLC) of serum specimens by Quest Diagnostics, San Diego, CA. Normal values by this method are 2.8 –13.5 μmol/L. Serum folate was determined using an ion capture assay which remains linear up to 20 ng/ml. Specimens with levels higher than 20 ng/ml were diluted. The normal range for serum folate was 3.1-12.4 ng/mL. Vitamin B12 determination was performed using standard clinical diagnostic techniques by Quest Diagnostics. The normal range for vitamin B12 was 200-1100 pg/mL. Total cholesterol, albumin, urea, creatinine, pre-albumin, phosphorus, glucose, and triglyceride determinations were performed at Satellite Laboratory Services, Inc. as part of the routine monthly care. Identification of the C677T translocation was performed using *Hin*fI digestion and polymerase chain reaction (PCR) amplification from specimens of peripheral blood

lymphocytes as described by Frosst [2]. PCR analysis was performed in the Molecular Diagnosis Laboratory, Department of Pathology, Stanford University Medical Center.

Statistical Methods

Correlations between continuous variables were tested with Pearson's correlation coefficient. Chi-square tests and analysis of variance were used to test relationships concerning dichotomous variables. Student's t-test was used for testing involving means of distributions. The distribution of genotypes was assessed for conformation to the Hardy-Weinberg equilibrium by Chi-square analysis as described by Emery [15]. Logistic regression was used to identify independent predictors of cardiovascular disease and multiple linear regression was used to identify independent relationships to tHcy. All data analysis was performed using SAS Systems version 6.1.2. Results are reported as mean ± standard deviation, except where otherwise indicated.

Results

Subjects

Mean age of the patients (230 males and 229 females) was 60.4 ± 15.1 years. The racial/ethnic groups represented were 186 non-Hispanic white, 137 Hispanic (126 of which were Hispanic-white), 71 Asian/Pacific Islanders, 54 African-American, 11 other/multi-racial. Etiology of ESRD was: diabetes type I (5.4%), diabetes type II (35.4%), hypertension/large vessel disease/cardiac (24.6%), glomerulonephritis (18.1%), secondary glomerulonephritis (3.1%), interstitial/pyelonephritis (5.2%), cystic/hereditary/congenital (4.6%), neoplasms (0.4%), miscellaneous (2.0%), and etiology uncertain or unknown (2.2%).

The clinical characteristics of the cohort with respect to the presence of cardiovascular disease are described in Table 1. Mean tHcy was significantly higher in patients without a history of cardiovascular disease (35.2 μmol /L ± 24.0) as compared to those with a history of cardiovascular disease (30.4 μmol /L ± 12.9, P = 0.02). Patients with cardiovascular disease were of older age, smaller size, and higher normalized protein catabolic rate (nPCR). Duration of dialysis, vitamin B12 levels, folate levels, prealbumin, cholesterol, creatinine, phosphorus, and glucose were not significantly different. 156 patients had at least one cardiovascular procedure or event including coronary artery bypass graft, percutaneous transluminal coronary angioplasty, previous myocardial infarction, stroke, or carotid end-arterectomy. By individual event: coronary artery bypass graft (54); previous myocardial infarction (54); percutaneous coronary intervention (24); heart valve replacement (11); stroke (37); carotid end-arterectomy (9); and at least one limb amputation (50). There were 50 patients with at least one limb or partial limb amputation. THcy was higher in males than in females, (34.9 ± 25.0 μmol /L versus 32.2± 16.0 μmol /L, P = 0.17) although this was not a statistically significant finding. Diabetics and non-diabetics had similar tHcy levels.

Characteristics by C677T mutation of MTHFR genotype

There were 59 patients with two copies (TT) of the allele, 184 with one copy (CT), and 216 with the wild-type allele (CC). This distribution did not vary significantly from the expected frequencies (49.7 TT, 202.6 CT, 206.7 CC) of these genotypes based on a Hardy-Weinberg equilibrium (Chi-sq = 3.89 d.f. 2, P = 0.14). There were very few copies of the thermolabile variant in non-white ethnic groups, three Asian patients were homozygous for the mutation as well as one African-American patient. There were 6 patients with CT genotype in the other/multi-racial group and 5 with CC genotype. Hispanics as a group had a higher

representation of heterozygotes and homozygotes than non-Hispanic whites. TT-genotype comprised 23.4 % for Hispanics and 12.4% for non-Hispanic whites (Chi-sq 9.0, d.f. 2, P = 0.01).

Table 2 shows the clinical characteristics of the three genotypes. The genotypes were similar with respect to age, age at initiation of dialysis, duration of dialysis, folate, and albumin. When adjusted for age, folate, creatinine, and albumin, tHcy levels were significantly higher in the TT group than in the CC group (39.4 μmol /L in TT versus 31.4 μmol /L in CC at the <0.005 level by Bonferroni T test for multiple comparisons). The genotypes differed with respect to creatinine, and percent male. The TT genotype had a significantly higher prevalence of cardiovascular disease than either the CT or CC genotypes, (52.5%, versus 29.9%, and 32.4%, respectively, P = 0.05 by Bonferroni t-test for multiple comparisons). This relationship persisted after adjustment for age, race, and gender. The effect of the TT genotype under conditions of below-median folate status is illustrated in Figure 1. The study group was divided in two groups based on folate levels (above and below the median level of 17.6 mg/L).

Concomitant vitamin and folate use

Of the 459 patients, 80% were prescribed a supplement containing folic acid, vitamin B6, and vitamin B12. At four facilities representing 206 patients we obtained further concomitant vitamin information. Of these 206, 80% of patients prescribed a folic acid-containing supplement were at least partially compliant (taken at least once in past 2 weeks) and 18% of patients not prescribed a folic-acid containing supplement were taking at least one folic-acid containing supplement. Brand name, dose, and actual dosing frequency were obtained on all

206 and the mean total daily amounts were: folic acid 0.67 mg, vitamin B6 7.27 mg, and vitamin B12 5.52 g.

Prediction of tHCY

Univariate analysis of tHcy is shown in Table 3, which presents the Pearson correlation coefficients of several clinical variables. tHcy had a strongly positive correlation to creatinine, albumin, and a strongly negative correlation to age, folate, and daily intake of folic acid, vitamin B6, and vitamin B12. There were milder associations between tHcy and duration of dialysis, body mass index, vitamin B12 level, and prealbumin.

In multiple linear models for prediction of tHcy values, homozygosity for the C677T mutation, albumin, creatinine, and an interaction term for folate and genotype were strong, independent predictors. Body mass index, vitamin B12 level, and folate level were weaker independent predictors. Age, gender, and actual daily intake amounts of folic acid, vitamin B6, and B12 dropped out of models.

Cardiovascular disease

The presence of cardiovascular disease was independently predicted by gender (female protective), body mass index (negatively), pre-dialysis diastolic blood pressure (negatively) tHCY (negatively), and the TT-genotype in a model which removed age, race, cholesterol, albumin, diabetes, CT-genotype, past and current smoking, and prealbumin by backward selection. In addition, a yes/no term for concomitant vitamin intake was examined and was eliminated by backward selection from models. (see Table 4).

Relationship of MTHFR status to duration of dialysis

The mutant allele frequency and the percentage of homozygotes were lower in those with duration of dialysis greater than one year (see Figure 2). This was statistically significant for non-Hispanic whites (25% compared to 8%, P = 0.002). Hispanic ethnicity had an overall frequency of the TT-genotype of 23.4%. Among those with less than one year duration of dialysis the TT-genotype prevalence was 36% compared to 20% for those with greater than one year duration of dialysis (P=0.13).

Discussion

In this cross-sectional analysis of 459 dialysis patients, patients with a history of cardiovascular disease had lower levels of tHcy than those without a history of cardiovascular disease. This negative relationship persisted in multivariate analysis when controlling for predictors of cardiovascular disease, such as age, gender, and body mass index. However, the TT-genotype was associated with prevalent cardiovascular disease even after adjustment for other cardiovascular risk factors including tHcy and was also associated with duration of dialysis in non-Hispanic whites. Consistent with other investigations in the general population and ESRD, we found an association of the TT-genotype and tHcy among those in the lower-half of the folate distribution.

There are several reasonable explanations for the inverse association between tHcy and cardiovascular disease and for the association of the TT genotype with cardiovascular disease that we observed in this cross-sectional analysis. First, a single determination of tHcy in ESRD, as measured in a cross-sectional study, may not represent lifetime tHcy exposure. During the years prior to the development of ESRD, tHcy was likely lower than the tHcy measured after dialysis was initiated. Historical tHcy determinations are required to assess

the relationship to evolving cardiovascular disease. However, the presence of genetic polymorphisms, such as the C677T variant of MTHFR, remains constant over a lifespan and can suggest a lifetime of abnormal homocysteine metabolism. Our data support the association of the homozygous C677T variant of MTHFR and cardiovascular disease. This implies that C677T genotype may be a better predictor of cardiovascular disease in ESRD than a single measurement of tHcy.

Second, methodological limitations constrain the interpretation of findings from cross-sectional analyses. For studies of ESRD patients this is particularly relevant due to survivor bias from high mortality rates. Survivor bias can potentially exert a strong influence on both cross-sectional studies and clinical trials during the course of dialysis especially when patients are enrolled with varying degrees of duration of dialysis. Ideally, clinical studies include patients from the same duration of dialysis cohort, i.e. recent initiators and attempt to control for differences in residual renal function. Survival to the clinical state of ESRD itself may in and of itself impart a different risk profile, as patients with chronic renal insufficiency (and the most adverse homocysteine metabolism) succumb to cardiovascular disease. Additional methodologic explanations for reversal of association include error of tHcy measurement and cardiovascular disease assessment. Possible specimen collection errors include failure to spin within 30 minutes or a failure to refrigerate. This seems unlikely since the mean levels are very similar to other studies. THcy assessment was done using a commercial HPLC assay that is well established. With respect to using clinical records for the assessment of cardiovascular disease, we are likely overlooking cases of atherosclerotic disease in this

population as most patients would have pathologic findings revealed by further clinical tests or autopsy.

Third, there may be a strong nutritional component driving the relationship between tHcy and comorbidity in ESRD due to the influence of serum albumin and dietary protein intake on tHcy levels. This is supported by similar findings in a recent cross-sectional study of 117 hemodialysis patients[16]. In that study, tHcy levels were dependent on nutritional status, protein intake and serum albumin, and patients with cardiovascular disease had lower tHcy levels as well as a higher prevalence of malnutrition and hypoalbuminemia than those without cardiovascular disease. Furthermore, tHcy rises during treatment of malnourished patients with an amino-acid-containing peritoneal dialysate (1.7 gm methionine/day or half-normal intake) [17]. The presence of chronic inflammation further complicates the relationship by suppressing serum albumin, an important binding site for tHcy, and by contributing to cardiovascular disease [18]. While in the univariate analyses in this study, tHcy correlated with serum albumin, prealbumin and BMI, there was no evidence that those with cardiovascular disease possessed a worse nutritional status than those with cardiovascular disease. Inflammatory markers were not measured in our study. If nutritional or inflammatory status is playing a role in the reverse association between tHcy and cardiovascular disease prevalence, we were unable to detect it in this cross-sectional analysis.

Fourth, the phenomenon of an established risk factor in the general population having a markedly different, if not opposite pattern in ESRD is not unique to our data. Blood pressure, body size, and serum cholesterol are well-established factors in the general population

associated with increasing cardiovascular risk [19, 20] whereas in ESRD, there is a dramatically different picture. Blood pressure tends to have a 'U' shaped relationship with mortality [21] while body size and cholesterol have an inverse relationship [22, 23]. This does not mean that the forces of vascular pathophysiology are different in ESRD, but suggests there are underlying factors responsible for the apparent reversal of relationships manifest when traditional variables are analyzed in ESRD. For example, in a hypothetical model adjusting for malnutrition the direction of the relationship of cholesterol and mortality reverses [24]. It is conceivable that cardiovascular risk factors, as we understand them in the general population, behave quite differently in ESRD due to reverse causation [24]. Similar relationships may apply to tHcy as well whereas a marker that remains constant over a lifespan, such as the TT genotype, would not experience this relationship.

Fifth, should the observation that dialysis patients with prevalent cardiovascular disease had lower levels of tHcy than those without a history of cardiovascular disease represent a refutation of the hypothesis that tHcy plays a role in vascular pathogenesis? While this study is not designed to answer the question, it can lend credence to the assertion that tHcy represents a marker for other factors that are important in vascular pathophysiology, rather than a causative agent. Not all epidemiologic studies in the general population and clinical studies in ESRD have supported an association between homocysteine and cardiovascular disease. Prospective case-cohort analyses from three large-scale community and population studies, The Atherosclerosis Risk in Communities Study [25], a 9-year Finnish study [26], a report from the US Physicians Health Study [27] , as well as a meta-analysis of 36 studies [6] have failed to find an association. Cross-sectional studies in ESRD have reported conflicting

associations [10, 11, 28-30], and case-control studies may inflate an association by selection of non-ESRD controls [31]. A study of 73 ESRD patients followed for 17 months was only able to adjust for one co-variate at a time [32]. Another 17-month study of 167 patients with ESRD on dialysis found the relative risk for cardiovascular disease was 1.01 per 1 μmol /L increase in tHcy, with a 95% confidence interval that included 1.00 [33]. Larger, prospective studies are needed in ESRD to address important questions about the causal role of homocysteine and clinical trials are needed in both ESRD and the general population to establish tHcy as a modifiable risk factor.

Previous studies have shown that the prevalence of the TT genotype remains stable across age groups in the general population[34, 35] and that the superior survival of African-Americans and Asians compared to whites in the US has of yet to be fully explained [36, 37]. Unlike some of these studies in the general population, our results support the assertion that the presence of the TT-genotype impairs survival in ESRD and may be associated with racial differences in survival. This was evidenced by the finding that the prevalence of the TT-genotype is decreased in those with greater than one-year duration of dialysis. A recent study of 545 Japanese hemodialysis patients reported similar findings with respect to survival, despite a clinically and demographically different population[7]. Compared to our sample of US dialysis patients, their patients were younger, had lower levels of comorbidity, and were presumably racially homogeneous. Furthermore, the mean age of their patients with TT genotypes was lower than the other two genotypes. However, our data mirrored these Japanese findings when whites (non-Hispanic and Hispanic) were excluded from the analysis by showing a trend toward older age in the TT genotype. Our findings of decreased

prevalence of the TT genotype after one-year duration of dialysis and the fact that 93% of those with the TT genotype were white, suggests that the TT genotype may be a contributor to mortality in white dialysis patients and may partially explain racial differences in survival in US hemodialysis patients.

This investigation has implications for further study in other ethnic groups. To our knowledge, this study represents the only determination of C677T genotypes of MTHFR in Hispanic dialysis patients residing in the US. The relatively large prevalence of the TT genotype, 23.4%, should be confirmed by other studies. This is especially relevant given the epidemic of type II diabetes and high prevalence of ESRD in this group [38, 39].

The finding that the C677T mutation of MTHFR is associated with cardiovascular disease and possibly, decreased duration of dialysis in white patients, supports the hypothesis that abnormal homocysteine metabolism is a risk factor atherosclerotic disease. Genetic assessment of MTHFR status may offer a more reliable indication of cardiovascular disease risk, and help direct preventive therapy as those with the TT genotype and below-median folate levels may benefit the most from homocysteine reduction. This study also found that tHcy levels were inversely associated with cardiovascular disease. This description of this association is limited due to factors such as survivor bias, confounding by malnutrition or inflammation as seen with other cardiovascular risk factors in ESRD, or the possibility that tHcy is not a causative risk factor in ESRD. THcy levels obtained after the onset of ESRD may not accurately represent the lifetime tHcy exposure and the C677T genotype may be a

more meaningful marker of abnormal homocysteine metabolism in ESRD. Prospective data from on-going clinical trials are needed to improve our understanding of this finding.

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Table 1. Characteristics of study cohort. Number of subjects are in parentheses.

	No Cardiovascular Disease n=303	Cardiovascular Disease n=156	P
Demographics			
Age, years	58.8 ± 16.0	63.6 ± 12.6	0.001
Male, %	42.9	64.1	0.001
Race/ethnicity = white[a], %	66.7	70.5	0.40
Diabetic, %	44.2	48.1	0.43
Duration of dialysis, years	3.9 ± 4.7	3.5 ± 4.0	0.28
Body mass index, kg/m^2	26.6 ± 6.4	24.7 ± 5.3	0.002
Current smokers, %	9.9	12.8	0.34
Past smokers, %	30.4	42.3	0.01
Laboratory Assessments			
Serum folate, nmol/L	20.0 ± 14.1(302)	21.8 ± 15.6(155)	0.22
Vitamin B12, pg/ml	715 ± 635(302)	644 ± 298(153)	0.19
Serum creatinine, mg/dL	9.1 ± 3.2	8.7 ± 2.7	0.18
Serum albumin, mg/dL	3.97 ± 0.36	4.00 ± 0.31	0.43
Pre-albumin, mg/dL	31.3 ± 7.7	32.2 ± 8.2	0.24
Serum cholesterol, mg/dL	178 ± 42.4	174 ± 41.9	0.38
TT genotype C677T variant MTHFR, %	9.2	19.9	0.001
CT genotype C677T variant MTHFR, %	42.6	35.3	0.13
Homocysteine, μmol /L	35.2 ± 24.0	30.4 ± 12.9	0.02
Homocysteine[b], μmol /L	34.6	31.5	0.0001

Hemodialysis patients, n=430			
Systolic blood pressure[c], mmHg	157 ± 21.0	153 ± 19.3	0.06
Diastolic blood pressure[c], mmHg	80.5 ± 12.5	76.3 ± 11.0	0.0009
Kt/V[d], n=428	1.55 ± 0.47	1.63 ± 0.51	0.10
NPCR[e] mg/Kg,n=428	0.93 ± 0.23	1.00 ± 0.30	0.01

[a]Includes both non-Hispanic and Hispanic whites.

[b]Adjusted for age, gender, and body mass index.

[c]Pre-dialysis, averaged over 9 treatments.

[d]Kt/V is a unit-less measure of dialysis dose.

[e]NPCR is normalized protein catabolic rate.

Table 2. Clinical characteristics by C677T variant of MTHFR.

	TT N=59	CT N=184	CC N=216	P
Age, years	63.6 ± 13.4	61.2 ± 14.5	59.0 ± 15.8	0.08
Age initiation ESRD (yr)	60.1 ± 15.7	57.4 ± 16.4	55.1 ± 17.4	0.10
Age initiation ESRD (yr), non-white[a]	48.2 ± 19.6(n=4)	56.8 ± 17.1(n=40)	53.3 ± 17.0(n=103)	0.42
Male, %	62.7*	53.3	44.0	0.02
Race/ethnicity = white[a], %	93.2*	78.2*	52.3	0.0001
Diabetic, %	55.9	45.7	42.6	0.19
Duration dialysis, years	3.5 ± 4.8	3.8 ± 4.5	3.9 ± 4.3	0.81
Serum folate, nmol/L	20.5 ± 12.1	20.3 ± 14.1	20.9 ± 15.8 (214)	0.93
Vitamin B12, pg/L	618 ± 319(56)	658 ± 418(183)	740 ± 674(214)	0.18
Serum creatinine, mg/dL	8.3* ± 3.1	8.7* ± 2.7	9.3 ± 3.2	0.01
Serum albumin, mg/dL	3.96 ± 0.4	3.98 ± 0.3	3.99 ± 0.3	0.83
Homocysteine, μmol /L	37.9 ± 16.8	34.1 ± 43.5	31.9 ± 13.5	0.14
Homocysteine[b], μmol /L	39.4†	34.4	31.4	0.0001
Cardiovascular Disease, %	52.5*‡	29.9	32.4	0.005
Cardiovascular Disease[c],%	49.0*‡	29.0	34.0	0.0001

[a]Includes both non-Hispanic and Hispanic whites.

[b]Adjusted for age, folate, creatinine, albumin.

[c]Adjusted for age, race/ethnicity, gender.

*Comparison with CC genotype significant at the 0.05 level by Bonferroni T test for multiple comparisons.

[†]Comparison with CC genotype significant at the <0.005 level by Bonferroni T test for multiple comparisons.

[‡]Comparison with CT genotype significant at the 0.05 level by Bonferroni T test for multiple comparisons.

Table 3. Correlation of homocysteine with continuous variables. Pearson correlation coefficients for homocysteine.

Variable	Rho	P
Age	-0.20	0.0001
Duration of dialysis	0.12	0.009
Body mass index , kg/m^2	0.19	0.0001
Folate, serum, n=457	-0.21	0.0001
Vitamin B12, serum, n=455	-0.12	0.009
Creatinine	0.33	0.0001
Albumin	0.27	0.0001
Prealbumin	0.12	0.009
Cholesterol	0.04	0.36
Kt/V[a], n=428	-0.18	0.0002
NPCR[b], n=428	0.13	0.008
Folic acid, daily intake, n=206	-0.33	0.0001
Vitamin B6, daily intake, n=206	-0.33	0.0001
Vitamin B12, daily intake, n=206	-0.26	0.0002

[a]Kt/V is a unit-less measure of dialysis dose.

[b]NPCR is normalized protein catabolic rate.

Table 4. Logistic regression for cardiovascular disease prevalence. Age, race, cholesterol, albumin, diabetes, CT-genotype, past and current smoking, and prealbumin were removed by backward selection.

Predictor Variables	Odds Ratio[a]	95% Confidence Limit		
		Lower	Upper	P
Intercept	-	-	-	0.0001
Gender (male = 0)	0.40	0.26	0.62	0.0001
Body mass index	0.94	0.90	0.98	0.003
Blood pressure[b]	0.97	0.95	0.99	0.0009
TT versus not TT	2.39	1.29	4.44	0.006
tHcy	0.98	0.97	1.00	0.04

[a]For one unit incremental increase.

[b]Pre-dialysis diastolic blood pressure.

Figure 1. The relationship between low-folate status and tHCY stratified by genotype. Low-folate was defined as below-median folate level, (less than 17.6 mg/ml). Mean tHCY levels are shown with standard error bars for each genotype. There was no significant differences in tHCY among the genotypes in the above-median folate group. There was an effect of genotype on tHCY in the below-median folate group ($p=0.01$). This effect persisted when adjusting for age, creatinine, and albumin. *Refers to significant difference compared to the CC genotype in the below-median folate group at the 0.05 level using the Bonferroni analysis of multiple comparisons.

Figure 1.

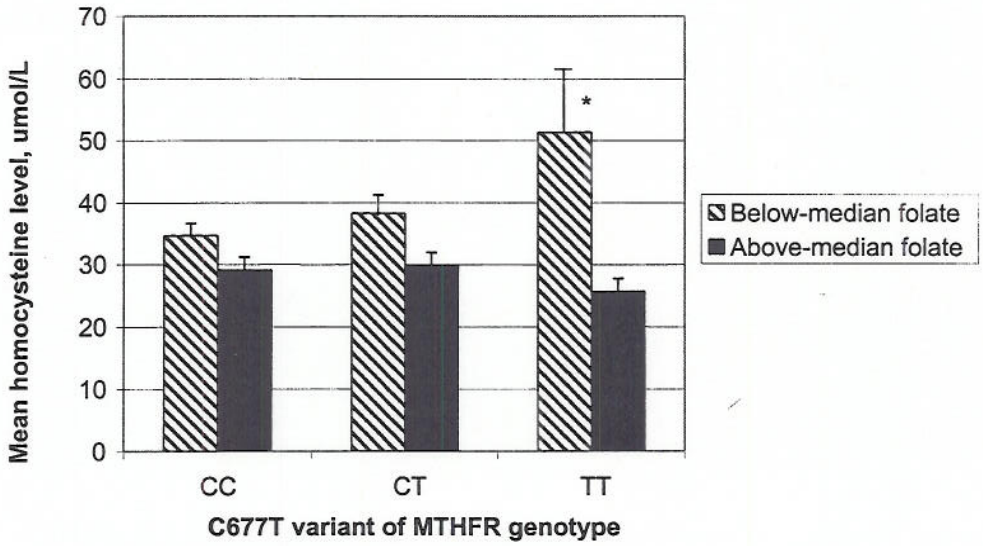
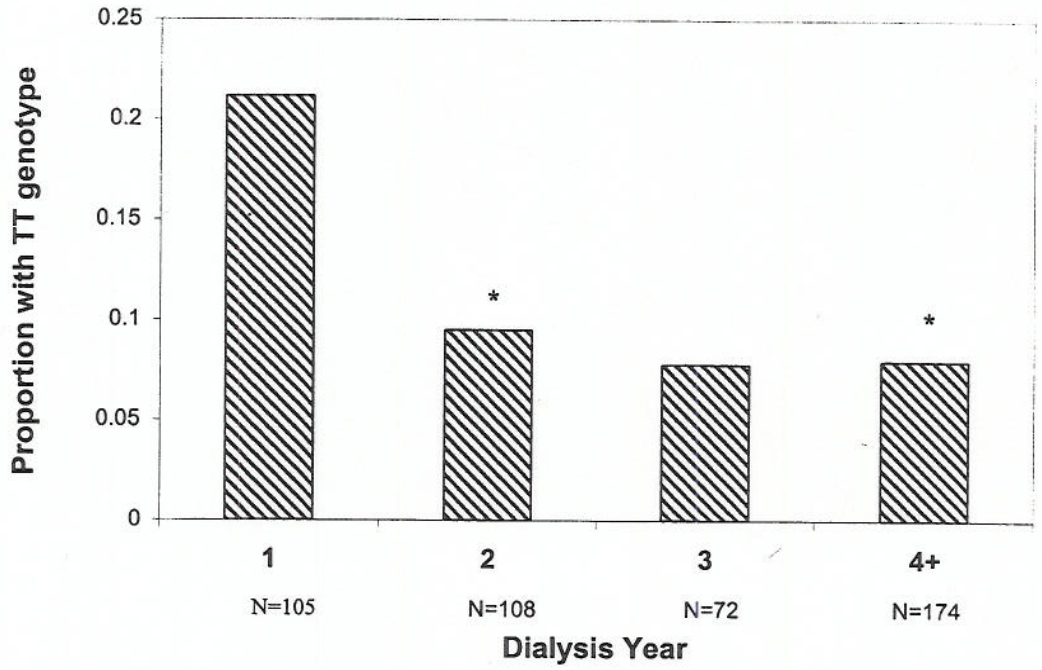

70
60
50
40
30
20
10
0
Mean homocysteine level, umol/L
*
CC
CT
TT
C677T variant of MTHFR genotype
Below-median folate
Above-median folate

Figure 2. Proportion of study patients by dialysis year. Dialysis year is the year-subgroup of duration of dialysis. * refers to those groups identified as statistically different from year 1 ($p = 0.0001$, controlling for age, race/ethnicity, gender, and diabetes).

Figure 2.


Proportion with TT genotype
0.25
0.2
0.15
0.1
0.05
0
*
*
1
2
3
4+
N=105
N=108
N=72
N=174
Dialysis Year