Methylenetetrahydrofolate reductase (MTHFR) polymorphism A1298C (Glu429Ala) predicts decline in renal function over time in the African-American Study of Kidney Disease and Hypertension (AASK) Trial and Veterans Affairs Hypertension Cohort (VAHC)

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Abstract

Background. Hyperhomocysteinemia is associated with increased venous thrombosis and cardiovascular disease (CVD). Mutations in the human methylenetetrahydrofolate reductase (MTHFR) gene have been associated with increased homocysteine levels and risks of CVD in various populations including those with kidney disease. Here, we evaluated the influence of MTHFR variants on progressive loss of kidney function.

Methods. We analyzed 821 subjects with hypertensive nephrosclerosis from the longitudinal National Institute of Diabetes and Digestive and Kidney Diseases African-American Study of Kidney Disease and Hypertension (AASK) Trial to determine whether decline in glomerular filtration rate (GFR) over ~4.2 years was predicted by common genetic variation within MTHFR at non-synonymous positions C677T (Ala222Val) and A1298C (Glu429Ala) or by MTHFR haplotypes. The effect on GFR decline was then supported by a study of 1333 subjects from the San Diego Veterans Affairs Hypertension Cohort (VAHC), followed over ~4.5 years. Linear effect models were utilized to determine both genotype [single-nucleotide polymorphism (SNP)] and genotype (SNP)-by-time interactions.

Results. In AASK, the polymorphism at A1298C predicted the rate of GFR decline: A1298/A1298 major allele homozygosity resulted in a less pronounced decline of GFR, with a significant SNP-by-time interaction. An independent follow-up study in the San Diego VAHC subjects supports that A1298/A1298 homozygotes have the greatest estimated GFR throughout the study. Haplotype analysis with C677T yielded concurring results.

Conclusion. We conclude that the MTHFR-coding polymorphism at A1298C is associated with renal decline in African-Americans with hypertensive nephrosclerosis and is supported by a veteran cohort with a primary care diagnosis of hypertension. Further investigation is needed to confirm such findings and to determine what molecular mechanism may contribute to this association.

Keywords: AASK; glomerular filtration rate; hypertension; kidney disease; MTHFR

Introduction

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme for intracellular folate homeostasis and metabolism which irreversibly catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF), a cosubstrate for homocysteine remethylation to methionine. Mutations in the MTHFR gene, found on human chromosome 1p36.3, have been associated with increased homocysteine levels [1, 2], which are in turn linked to increased venous thrombosis and cardiovascular disease (CVD) [3–5]. Two common nonsynonymous single-nucleotide polymorphisms (SNPs) have been noted in the MTHFR gene, which functionally alter the protein product [6]. C677T, found in Exon 4, (rs1801133, Ala222Val) results in a reduced-activity thermolabile variant, which has decreased stability and specificity of action [7] and is associated with decreased MTHFR activity and increased homocysteine levels [1]. A1298C (rs1801131, Glu429Ala), found in Exon 7, also reduces MTHFR activity, though seemingly less severely than C677T [8]. There may also be a functional interaction between these two polymorphisms which affect MTHFR enzyme activity [8]. These polymorphisms have previously been associated with a number of diseases...
including neural tube defects [1, 8], malignancies [9, 10], peripheral vascular disease [11] and CVD [7, 12].

Hyperhomocysteinemia is a long recognized problem in kidney disease patients, particularly in those with end-stage renal disease (ESRD) on dialysis, and thus may play a role in their increased CVD risk [13]. Combined as a risk factor with fibrinogen, homocysteine may explain almost 40% of the attributable mortality in chronic kidney disease (CKD) [14]. MTHFR polymorphisms have also been associated with CVD in ESRD subjects [15, 16] and increased risk of diabetic nephropathy [17, 18]. Whether common MTHFR variation is associated with accelerated chronic decline in renal function has not been systematically studied in a large at risk cohort.

Our study aims to evaluate two longitudinal hypertensive cohorts, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored multicenter randomized trial African-American Study of Kidney Disease and Hypertension (AASK) [19] and a local San Diego Veterans Affairs Hypertension Cohort (VAHC) [20] to determine if common MTHFR polymorphisms predict decline in renal function over time [by glomerular filtration rate (GFR) or estimated glomerular filtration rate (eGFR) slope].

Materials and methods

Subjects

AASK study subjects. The first cohort included subjects from the AASK study, a 21 center randomized controlled prospective trial which has been described previously [19, 21]. Briefly, participants were 18- to 70-year-old self-identified African-Americans with hypertension and a clinical diagnosis of hypertensive renal disease, documented by an initial GFR (by [125I]-iothalamate clearance) between 20 and 65 mL/min/1.73 m², urine protein:creatinine ratio (UPCR) <2.5 g/g and no other identifiable causes of renal insufficiency, particularly excluding subjects with diabetes mellitus. Based on a 3 × 2 factorial design, participants were randomized to one of two goal blood pressure (BP) ranges (‘usual’ mean arterial pressure goal of 102–107 mmHg or a lower mean arterial pressure goal of 101–106 mmHg or a lower mean arterial pressure goal of ≤92 mmHg) and to double-blinded treatment with one of three antihypertensive drug classes (40% to beta-blockade with metoprolol, 50–200 mg/day; 40% to calcium channel blockade with amlodipine, 5–10 mg/day).

The first cohort included subjects from the AASK cohort and buccal cells in the VAHC group, was typed by an immobilized probe approach [31]. Reproducibility of genotyping was verified with 50 blinded replicate samples. Both SNPs evaluated in this study were in Hardy–Weinberg equilibrium (Table 1; P > 0.05) and were in partial linkage disequilibrium (for African-American subjects in each cohort D’ = 0.99, whereas r² = 0.029 in VAHC and r² = 0.024 in AASK), as determined by the Hill algorithm [32]. Minor allele frequency of A1298C and C677T were 15.7 and 11.3%, respectively in AASK and 16.6 and 12.9%, respectively in African-American subjects in VAHC. Online supplemental Table 1 shows the minor allele frequencies for each ethnic group in VAHC. Excluded subjects with incident or prevalent ESRD in VAHC had similar MTHFR A1298C and C677T distributions of genotypes compared to the study cohort.

Haplotype inference. Two common variants were genotyped at MTHFR, allowing the inference of haplotypes from unphased diploid genotypes by the HAP imperfect phylogeny method (version 3.0; http://research.calit2.net/hap/Instructions.htm) [33]. Three common haplotypes (C677T/A1298, 677T/A1298 and C677/A1298) were observed in the AASK study and VAHC study, shown in Online Supplemental Table 2, with the fourth haplotype (1298C/677T) absent in AASK and <1% in VAHC (0% in African-Americans). Due to the multithetic nature, in the VAHC cohort, haplotype inference were performed in white and African-American subjects only and then combined for analysis. The other ethnic groups were too small for accurate inference to be determined. Haplotype analyses were determined by subjects with greater than or equal to one copy of each haplotype.

Statistical analyses

Descriptive statistics. Means ± standard deviations are reported except as noted. All variables were normally distributed with the exception of UPCR in the AASK cohort, thus values are log transformed for analysis of association with the genetic polymorphisms.

Progression of GFR loss in AASK and VAHC using mixed models. Linear mixed effects models (PROC MIXED) in SAS 9.2 (Cary, NC) [34] were utilized to assess the influence of the MTHFR polymorphisms on longitudinal GFR [23] (R. M. Salem, M. M. Fung, V. Bhattachar, M. S. Lipkowitz et al., in preparation). This mixed model approach tests for both SNP main and SNP-by-time interaction effects and can be applied to unstructured and unbalanced longitudinal data [35], particularly to the repeated measurements taken at unequal time intervals of the VAHC study, which utilizes...
Table 1. Demographic characteristics at the start of the study for subjects from each of the two cohorts

<table>
<thead>
<tr>
<th></th>
<th>AASK</th>
<th>VAHC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>821</td>
<td>1333</td>
</tr>
<tr>
<td><strong>Means</strong></td>
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<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.9 ± 10.7</td>
<td>64.0 ± 12.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.0 ± 6.5</td>
<td>30.2 ± 5.9</td>
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<tr>
<td>SBP (mmHg)</td>
<td>150.0 ± 23.8</td>
<td>140.5 ± 19.4</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>95.8 ± 14.3</td>
<td>76.3 ± 12.8</td>
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<tr>
<td>Pulse (mmHg)</td>
<td>72.0 ± 12.2</td>
<td>72.7 ± 14.1</td>
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<td>Serum creatinine (mg/dl)</td>
<td>2.0 ± 0.71</td>
<td>1.09 ± 0.31</td>
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<tr>
<td>Baseline GFR (mL/min/1.73m²)</td>
<td>47.6 ± 13.5 (iothalamate)</td>
<td>80.0 ± 23.8 (eGFR by MDRD)</td>
</tr>
<tr>
<td>GFR slope over the study (mL/min/1.73m²/year)</td>
<td>-1.92 ± 2.4</td>
<td>-1.86 ± 1.4</td>
</tr>
<tr>
<td>Follow-up—months (years)</td>
<td>51.1 ± 15.3 (4.3 ± 1.3)</td>
<td>53.9 ± 22.8 (4.5 ± 1.9)</td>
</tr>
<tr>
<td><strong>Percentages (N)</strong></td>
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<tr>
<td>Ethnicity</td>
<td>100% African-American</td>
<td>68.2% Caucasian (909)</td>
</tr>
<tr>
<td>Smokers (never/current/past)</td>
<td>42.9/26.8/29.9% (352/220/245)</td>
<td>25.5/20.0/54.5% (330/259/705)</td>
</tr>
<tr>
<td>Males/females</td>
<td>60.1/39.8% (494/327)</td>
<td>95.3/4.7% (1270/63)</td>
</tr>
<tr>
<td><strong>Genotypes (N)</strong></td>
<td></td>
<td></td>
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<tr>
<td>C677T: CC, CT, TT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C677T: CC, CT, TT</td>
<td>645, 164, 11 (χ² = 0.025, P = 0.88)</td>
<td>649, 551, 131 (χ² = 1.01, P = 0.31)</td>
</tr>
<tr>
<td>C677T: CC, CT, TT</td>
<td>(HWE χ² = 0.021, P = 0.89)</td>
<td>(HWE χ² = 0.74, P = 0.39)</td>
</tr>
</tbody>
</table>

*Means reported ± standard deviations. Randomized BP goal and anti hypertensive regimen are only available in the clinical trial AASK. HWE, Hardy–Weinberg equilibrium; DBP, Diastolic blood pressure.

Observational information in the EMR [34, 36, 37]. As noted, GFR in AASK was determined by renal clearance of [125I]-iothalamate assessed at baseline twice, at 3 and 6 months and then every 6 months thereafter, for a mean of 4.3 ± 1.3 years. Model 1 of the AASK cohort includes age at the start of the study, sex and randomization BP goal and antihypertensive regimen as covariates. In VAHC, the GFR was estimated by the MDRD equation and Model 1 includes age at date of enrollment, sex and ethnicity as covariates. Additionally, principal components were calculated using 77 additional SNPs from unrelated loci in order to adjust for possible admixture due to an ethnically diverse sampling in the VAHC cohort. Four principal components were then used to capture differences due to ethnicity and were entered as covariates.

Joint modeling. In AASK, to address the potential confounding effects of reaching ESRD death, loss to follow-up or decrease in 50% of GFR (n = 192) on the longitudinal GFR change, joint modeling of longitudinal and time to event data was implemented [34]. In VAHC, participants with incident or prevalent ESRD were excluded from this analysis due to the EMR nature of the cohort and difficulty in discerning precisely when ESRD was reached, thus only death (n = 210) or loss to follow-up were included in the joint modeling. All VAHC subjects were noninformatively censored at their last serum creatinine and eGFR measurement. In brief for both cohorts, a flexible joint model framework has been formulated [38], allowing a broad range of dependencies between the longitudinal responses and event endpoints through a latent bivariate Gaussian process without specifying a class variable [39]. The joint model combines longitudinal and time to event information, allowing the covariance structure to be adjusted for censoring and event data [34, 38].

Plots/figures. To illustrate the influence of the MTHFR A1298C variant on longitudinal GFR decline, chronic longitudinal GFR was plotted by SNP genotype over time (Figures 1 and 2). For demonstrative purposes, the longitudinal GFR profile by SNP genotype was generated using an adaptive regression cubic spline approach [40], with 95% confidence intervals for fitted spline function of the covariate-adjusted GFR values (from the joint model). The haplotype figures (Figures 1b and 2b) show presence of the MTHFR C677T/1298C (one or two copies) versus absence (zero copies) due to the low frequency of two copies (3.0% in AASK and 8.0% in VAHC).

Power analyses. Statistical power was determined using the online instrument G*Power 3 (wwwpsycho.uni-duesseldorf.de/abteilungen/aap/ gpower3/literature)[41]. More than 95% power to detect an effect size of 0.15 would be achieved with a sample size of 800, assuming alpha of 0.025.

Results

Primary studies in NIDDK AASK subjects with hypertensive nephrosclerosis

MTHFR polymorphism contributes to change in GFR over time. This genomic study cohort of 821 AASK study participants genotyped at the MTHFR loci (Table 1) had a mean age of 53.9 ± 10.7 years and initial GFR of 47.6 ± 13.5 mL/min/1.73 m². Table 2 reports the results of association testing of MTHFR genetic variants on chronic GFR decline.

MTHFR A1298C affected change in GFR: heterozygotes A1298/1298C and 1298C/1298C minor allele homozygosity predicted a greater decline of GFR with a significant SNP-by-time interaction (P = 0.021 in Model 1 which includes enrollment age, sex and BP and medication randomization groups as covariates; Table 2). Slope estimates are shown in...
Online Supplemental Table 2, with subjects with A1298/A1298 having a decline per week of 0.034 mL/min/1.73 m² versus 0.048 and 0.042 mL/min/1.73 m² in heterozygotes and 1298C/1298C subjects, respectively. The association was also not attenuated in a fully adjusted model which included sex, baseline age and UPCR, randomization group for BP control and medication, a moving average of SBP over the study period and history of CVD or stroke (P = 0.029, results not shown). Furthermore, when adjusting the models additionally for baseline GFR, results were not significantly altered. A joint analysis of this linear mixed model and time to event analysis accounting for death, dialysis, loss to follow-up and decrease of GFR by 50%, shown in Figure 1a, continued to show this association, P = 0.023 (with the Model 1 covariates). C677T alone did not show an individual effect, but haplotypes shown in Online Supplemental Table 3 suggest a potential interaction between C677 and the 1298C, such that the presence of the C677/1298C haplotype was associated with increased decline in GFR over time (P = 0.013 in Model 1; Figure 1b). No independent main effects of either of the polymorphisms or haplotypes were observed.

Neither of the MTHFR polymorphisms (A1298C or C677T) associated with drug or BP-goal randomization groups, sex, age, GFR or BMI at the start of the study. Baseline UPCR ratio (log transformed for normalization) was associated with MTHFR A1298C such that those with major allele homozygosity had the least amount of UPCR at enrollment (P = 0.014 adjusted for sex and age and P = 0.022 adjusted for sex, age, initial GFR and randomized drug and BP goal; results not shown).

Follow-up study: San Diego VAHC. The supportive follow-up study consisted of 1333 veterans genotyped at the two common non-synonymous MTHFR loci as described in Table 1. In total, 95.3% were male and 68.2% self identified as Caucasian.

In VAHC, MTHFR A1298C predicted differences in eGFR over a mean follow-up of 4.5 ± 1.9 years, adjusted in Model 1 by sex, ethnicity and baseline age, though in a main SNP effect and not an SNP-by-time effect (Table 2). Slope estimates for the SNP-by-time effect, similar to those seen in AASK, are shown in Online Supplemental Table 2. Subjects homozygous for the minor allele (1298C/1298C) had a lower eGFR compared to heterozygotes (A1298/A1298, light gray line) and homozygotes for the wild-type allele (A1298/A1298, dark gray line) had greater declines, showing the significant SNP-by-time interaction, P = 0.023 (main SNP effect P = 0.65), with adjustment for age, sex, drug and BP goal randomization groups. Below the graph indicates the number of GFR measurements that are included by week at each time point. (b) Haplotype. The influence of MTHFR haplotype 677C/1298C on longitudinal GFR decline are plotted for the AASK Study. Adjusted GFR values from the linear mixed effects model are plotted using an adaptive regression cubic spline with 95% confidence intervals (dashed lines) for fitted curves. Haplotype 677C/1298C had a haplotype-by-time interaction on GFR (by iothalamate clearance) as determined by linear mixed model analysis, such that those with at least one copy (dark gray line) had accelerated decline (P = 0.013; main haplotype effect P = 0.36) compared to those with no copies of the haplotype (solid black line).
analysis adjusting for censoring (loss to follow-up) and death, yielded similar results, with a main SNP effect for eGFR \( (P = 0.0041, \text{Model 1, shown in Figure 2a}) \). To adjust for the potential confounding effect of admixture, these analyses were further adjusted for four principal components, which capture ethnic differences within the VAHC cohort; results were statistically similar (for the main SNP effect, Model 1 \( P = 0.0055 \)). However, given the main effect of the association, further adjustment for baseline eGFR reduced the significance of the association with the SNP.

Haplotype analyses (shown in Online Supplemental Table 3) of combined white and African-American subjects revealed a similar effect, such that subjects with presence of the 1298C allele, in conjunction with the C677 allele had a lower eGFR \( (P = 0.047, \text{Figure 2b}) \) adjusted for age, sex and ethnicity. No polymorphism or haplotype was associated SNP-by-time with eGFR decline.

Consistent with the main effect noted, eGFR at baseline was associated with the A1298C polymorphism, such that the homozygotes with the minor allele (1298C/1298C) had a lower eGFR at the start of the study \( (P = 0.008 \text{ after age, sex and ethnicity adjustment}) \) than those with homozygote major alleles (A1298/A1298). The variant was not associated with sex or BMI in the VAHC study, though the participants differed in age at the date of consent \( (P < 0.001) \), with A1298/A1298 homozygotes being younger \( (62.7 \pm 12.3 \text{ years}) \) than A1298/1298C \( (65.8 \pm 12.7 \text{ years}) \) or 1298C/1298C \( (65.9 \pm 12.9 \text{ years}) \) subjects.

Discussion

Overview

We present here evidence that the MTHFR polymorphism A1298C is associated with decline of GFR and GFR in two different populations, African-Americans with hypertensive nephrosclerosis and ethnically mixed veterans with hypertension, respectively. Previous studies have indicated that this gene is associated with a number of morbid conditions including cardiovascular and cerebrovascular disease. This is the first study to our knowledge that evaluates two common polymorphisms within the gene and associates them with longitudinal continuous renal function decline from either change in iothalamate-derived GFR or eGFR over time.

MTHFR genetic variation and the kidney

Few studies have evaluated MTHFR polymorphisms associations with renal disease. Consistent with our study, two case–control studies showed increased incidence of ESRD in subjects with 1298C/1298C \[16, 42\]. A study that divided
patients by diagnostic categories of renal failure found that the C677T polymorphism alone and in combination with the A1298C polymorphism was associated with hypertensive nephrosclerosis [43]. One study of subjects with diabetes noted that the C677T mutation may have increased risk of diabetic nephropathy, though the A1298C polymorphism showed no association [18]. Two other studies reported the same association with C677T, though did not evaluate the A1298C polymorphism [44, 45]. The AASK trial excluded subjects with diabetes at the start although ~14% developed diabetes by the end of the study and 38.4% of the VAHC cohort had diabetes by the end of the study. Thus, our study was not powered to study such an association.

In renal failure, associations of these MTHFR polymorphisms and CVD have had mixed results [15, 46, 47]. One case–control study failed to find an association with these polymorphisms and CVD has had mixed results [15, 46, 47]. One recent genome wide association study of BP with >34 000 subjects implicated a MTHFR polymorphism (rs17367504, in partial LD with A1298C and C677T with $r^2 = 0.36$ and 0.064, respectively, from http://hapmap.ncbi.nlm.nih.gov/, which is located in an intron [49]), and a pharmacogenetic study suggested that the C677T polymorphism may influence response to an ACE inhibitor [50]. Thus, there may be unknown mechanisms in which MTHFR gene influences BP and drug response which may be either the cause or the result of decline in renal function either directly or indirectly.

The two common genetic mutations studied here, C677T and A1298C, have both been noted to decrease MTHFR enzyme activity [8]. Increased plasma homocysteine levels have then been associated with the C677T polymorphism alone and in combination with the A1298C mutation [8, 51]. As these polymorphisms [52] and elevated homocysteine levels have been associated with CVD morbidity and mortality, it was hypothesized that the pathophysiologic mechanism might be mediated through the elevated homocysteine levels. Yet effectively lowering the homocysteine levels with combination vitamin treatment, such as folate, B6 and B12, has not been shown to reduce mortality in advanced renal failure patients [53], who may have too large of a burden of disease to be successfully modified. Additionally, it has been suggested that homocysteine may be a marker of vascular disease rather than a causative agent [54]. A recent report notes that the C677T polymorphism may be a marker of 5-MTHF, the circulating metabolite of folic acid participating in homocysteine metabolism which appears to be the key regulator of endothelial nitric oxide synthase coupling and nitric oxide bioavailability in human vessels [55]. This may then encourage formation of oxygen-free radicals, contributing to elevated vascular oxidative stress, leading to a number of alterations in endothelial function and thrombogenicity and ultimately resulting in atherothrombosis [56]. Thus, plasma homocysteine, often associated with cerebrovascular and CVD, may be an indirect marker of 5-MTHF rather than a primary regulator of endothelial function. Consistent with this hypothesis are reports that elevated homocysteine levels may not be predictive of GFR progression in patients with CKD [57] nor their CVD mortality [58].

It is uncertain whether the MTHFR A1298C polymorphism also increases homocysteine levels. Previous studies have noted conflicting reports [16, 42, 48]. We were unable to evaluate plasma homocysteine levels since they were not measured at the start of the AASK trial and unavailable in the VAHC (an entirely observational cohort, utilizing the clinical EMR).

Most studies have focused on the C677T polymorphism, which is in incomplete linkage disequilibrium with A1298C [6, 59]. The C677T polymorphism has been hypothesized to have greater effects on MTHFR activity and influence on phenotypic outcomes than A1298C because of its location within the catalytic region. The C677T polymorphism is in Exon 4, within the N-terminal catalytic domain of the enzyme, whereas the A1298C polymorphism is in Exon 7, within the C-terminal regulatory domain [8]. The A1298C polymorphism may thus affect enzyme regulation, possibly by influencing S-adenosylmethionine, which is an allosteric inhibitor of MTHFR that binds in the C-terminal region [60].

**Advantages and limitations of this study**

Advantages of this study include use of the AASK trial with an independent supporting follow-up study. This AASK trial had accurately measured GFR by different

<table>
<thead>
<tr>
<th>Position</th>
<th>RefSNP</th>
<th>Minor allele frequency by cohort</th>
<th>SNP effect</th>
<th>SNP-by-time effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR variant</td>
<td>C677T</td>
<td>rs1801133</td>
<td>AASK: T (0.11); VAHC: T (0.31)</td>
<td>0.54</td>
</tr>
<tr>
<td>A1298C</td>
<td>rs1801131</td>
<td>AASK: C (0.16); VAHC: C (0.29)</td>
<td>0.0065&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0041</td>
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</table>

<sup>a</sup>Effects of MTHFR polymorphisms and haplotypes are shown. In NIDDK AASK subjects, slope is chronic (after 3 months in the trial), determined by iothalamate clearance, with Model 1 results adjusted for sex, baseline age and randomized BP goal and antihypertensive treatment as covariates. In VAHC, the GFR was estimated by the MDRD equation, and Model 1 includes age at date of enrollment, sex and ethnicity as covariates. Bold P-values denote significance after Bonferroni correction.

<sup>b</sup>The joint model for VAHC did not converge, and thus results are not reported.

<sup>c</sup>After additional adjustment from principal component analysis of admixture in VAHC, P = 0.0055. The Joint Model combines the linear mixed model and time to event analysis, allowing estimation of the longitudinal analysis adjusted for time to event information and considers the same covariates as Model 1.

In AASK, this includes time to death, dialysis, loss to follow-up or decrease of GFR by 50% and in VAHC includes the time to death or loss to follow-up.
techniques at multiple time points with very close follow-up. We were able to demonstrate the importance of the MTHFR gene by supportive results in a diverse observational cohort, VAHC, with detailed data from an EMR (further discussed below).

Our study also has limitations. In genetic studies, we must reduce the possibility of false-positive findings. Thus, we reduced the target P-value (by Bonferroni correction P = 0.025) and performed haplotype analyses, which continued to yield significant GFR predictions, with results that were congruent across the mixed model approach.

An exact replication of the AASK trial is unlikely, given the scope of its duration, size, intensity, complexity and expense. Though randomized clinical trials are considered the gold standard for clinical research, detailed comparison with observational studies via meta-analysis provides evidence that well-designed observational studies can produce valid and similar results [61, 62]. The VAHC has many characteristics which make it an ideal study population: primary care ascertainment basis, detailed and comprehensive EMR, nearly complete picture of medical use from a single provider, and the ability to track subjects longitudinally. Though, a limitation is that accuracy and completeness is reliant upon the EMR. Advanced statistical methods (including linear mixed models; see Methods) were employed to best utilize and transform the ambulatory setting data into models which may emulate a clinical trial.

The VAHC cohort consists of all veteran subjects, who are primarily male and with heterogeneous biogeographic ancestry. A principal component analysis was performed in order to adjust for potential confounding effect of admixture. This population was generally elderly (at 64.0 ± 12.7 years at the start of the study) with a corresponding modest decrease in eGFR (at 80.0 ± 23.8 mL/min/1.73 m²), though not so low as GFR in subjects with nephrosclerosis from the AASK study (Table 1).

Because the VAHC cohort is based upon the EMR, iothalamate clearance results, which were utilized in the AASK study, were not available. Rather, eGFR was determined by the MDRD equation [29], using ambulatory serum creatinine measurements all performed and reported by the same San Diego Veterans Affairs clinical laboratory. Such eGFR values are most accurate in subjects with GFR <60 mL/min/1.73 m², tending to underestimate GFR in healthy individuals [29]. Though our sample included healthy subjects as well as those with CKD Stages 1 through 4 [30], imprecision of eGFR determination by underestimation in healthy individuals would be expected to bias the results toward the null, in contrast to the significant findings in our analyses. In AASK, eGFR and iothalamate clearance yielded similar results [63]. Further adjusting the results of the MTHFR A1298C association for baseline GFR in AASK did not significantly change the results. However, in VAHC given that a main SNP effect is noted, adjustment for baseline eGFR reduced the significance of the result. In longitudinal analyses, inclusion of the main effect as a covariate has been shown to result in an attenuation of a true signal and lead to an erroneous result [64]. Additionally, MTHFR A1298C was associated with UPCR in AASK as a potential confounder to the association with GFR decline over time. However, UPCR was available in few VAHC subjects at baseline and thus, we were unable to confirm the association or statistically adjust for it. As proteinuria is an important risk factor for kidney disease progression that may provide a potential link to the genotype, future study is needed to investigate this relationship.

Though MTHFR genetic variation at the A1298C locus influenced GFR progression in both cohorts, an SNP-by-time interaction is noted that in the AASK cohort, whereas a main SNP effect was noted in the VAHC cohort (Figures 1 and 2; Table 2). One potential reason may be that the influence of the variant may change over time as the kidney disease progresses. The VAHC cohort, all with both hypertension and CKD, had a lower mean GFR at the start of the study and greater progression with more variability throughout the study, thus illustrating an SNP-by-time interaction (Figure 1a). Whereas the VAHC cohort, all with hypertension but only partially with CKD, had higher GFR at the start of the study illustrated only the main SNP effect (Figure 2a). The two cohorts studied here also differ in other substantive ways (Table 1): entry age (AASK less than VAHC), entry level of hypertension (AASK greater than VAHC), sex (more females in AASK than VAHC) and perhaps most importantly, biogeographic ancestry (the AASK cohort entirely African-American compared with the multiethnic VAHC). African-Americans with ‘hypertensive nephrosclerosis’ may be victims of more specific genetic predispositions to progressive azotemia, arising from genetic susceptibility variation at the MYH9 [65]/ApolI [66] loci. Treatment of BP also differed between the cohorts such that the AASK participants had well controlled BP monitored by the trial regularly and VAHC patients received standard care by their primary care physicians.

Conclusions and perspectives

Our study indicates that the MTHFR-coding polymorphism at A1298C is associated with renal decline. This was shown in African-Americans with hypertensive nephrosclerosis and supported by a veteran cohort with a primary care diagnosis of hypertension. Though polymorphisms from this gene appear to be associated with homocysteine levels and have been associated with cardiovascular risk factors including hypertension and here renal function, results should be confirmed in other populations and the precise pathophysiological mechanism will require further investigation.

Supplementary data

Supplementary Tables 1–3 are available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. At the time of research, M.M.F. had grant support from Forest Laboratories; currently she owns stock and is employed by Amgen. No other conflicts of interest. The results presented in this paper have not been published previously in whole or part, except in abstract form. This paper was presented as a poster at the American Society of Nephrology Renal Week 2009.

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