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Associations between *MTHFR* 1793G>A and plasma total homocysteine, folate, and vitamin B_{12} in kidney transplant recipients

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Background. Currently, no evidence is available on the putative associations between a novel single nucleotide polymorphism of the 5,10-methylenetetrahydrofolate reductase gene *MTHFR* 1793G>A and plasma levels of vitamin B_{12}, folate, or total homocysteine (tHcy).

Methods. In a cross-sectional study of 730 kidney allograft recipients, patients were categorized by *MTHFR* 1793G>A genotype. In univariate and multivariate linear regression models that allowed the outcome variables vitamin B_{12}, folate, and tHcy plasma levels to follow a gamma distribution, we tested for possible associations of allelic variants of *MTHFR* 1793G>A and these three dependent variables. As hypothesized in previous work, we specifically evaluated possible effect modification between the *MTHFR* 1793G>A and 1298A>C mutations on these outcomes.

Results. The allele frequency for *MTHFR* 1793G>A was 0.052. Heterozygosity ($N = 72$) or homozygosity ($N = 2$) for *MTHFR* 1793G>A was not independently associated with plasma levels of vitamin B_{12} ($P = 0.33$) or tHcy ($P = 0.70$), but a borderline association with higher folate concentrations was detected (Δfolate = 1.91 nmol/L) (95% CI −0.03 to 3.86 nmol/L) ($P = 0.05$). Further, we found strong and significant positive interactions between the *MTHFR* 1793G>A and 1298A>C mutations on vitamin B_{12} concentrations.

Conclusion. Higher folate concentrations in kidney transplant recipients with *MTHFR* 1793GA or 1793AA and markedly higher concentrations of vitamin B_{12} in patients with combined *MTHFR* 1793G>A and 1298A>C mutations may contribute to the survival advantage that has been postulated for such patients showing these genotypes.

Key words: *MTHFR*, genetic polymorphism, mutation, vitamin B_{12}, folate, homocysteine, kidney transplants.

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Elevated plasma total homocysteine (tHcy) is an established risk factor for various cardiovascular outcomes as well as for neural tube defects [1, 2]. The relevance of tHcy as a risk factor is even more pronounced in patients with chronic kidney disease, or on renal replacement therapy, due to an altered Hcy metabolism in such patients [3]. Mutations in specific genes encoding enzymes are involved in these metabolic abnormalities. It has been hypothesized for several of these mutations to be related to increases in tHcy plasma levels, either directly, or via changes in other factors involved in the Hcy metabolism, such as folate or vitamin B_{12}. While there is wide agreement that the mutation at nucleotide position 677 in the 5,10-methylenetetrahydrofolate reductase gene (*MTHFR* 677C>T) is related to hyperhomocysteinemia [4], such effects could not be demonstrated for other single nucleotide polymorphisms (SNPs) in the MTHFR encoding gene (e.g., 1298A>C) or those encoding other enzymes such as the reduced folate carrier 1 gene *(RFC1* 80G>A) [5, 6], the glutamate carboxypeptidase II gene (*GCP2* 1561C>T) [5, 6], the methionine synthase reductase gene (*MTRR* 66A>G) [7], and the transcobalamin II gene (*TCN2* 776C>G) [8, 9] among end-stage renal disease (ESRD) or kidney transplant patients.

Recently, another polymorphism, *MTHFR* 1793G>A, was first described [10]. To date, it is unknown whether *MTHFR* 1793G>A affects the functional activity of the enzyme. Such information would be of great interest in light of a recent study that examined patterns of co-occurrence of three polymorphisms of *MTHFR* (677C>T, 1298A>C, and 1793G>A) in a large sample of kidney transplant recipients [11]. In that study, higher prevalences than expected of dual, triple and quadruple mutations of *MTHFR* were found (1298AC/1793GA, 1298CC/1793GA, and 1298CC/1793AA) ($P < 0.001$)

Table 1. Frequencies of *MTHFR* 1298A>C and 1793G>A allelic variants (observed and expected cell counts)

	Observed (expected)	*MTHFR* 1793 GG	GA	AA	Total
MTHFR 1298	AA	319 (288.5)	2 (31.7)	0 (0.9)	321
	AC	288 (306.4)	53 (33.6)	0 (0.9)	341
	CC	49 (61.1)	17 (6.7)	2 (0.2)	68
	Total	656	72	2	730

This table corresponds directly with Table 3 in reference [11]. The one patient that was excluded from all association studies was in *MTHFR* 1298AA and 1793GG.

(Table 1). Based on these findings, the hypothesis was raised that a mutually stabilizing effect might be operational that yielded a survival benefit for carriers of these allele combinations [11]. This effect could be mediated via beneficial changes in the tHcy, folate, or vitamin B_{12} concentrations.

The present study was designed to address these questions. We specifically aimed to test the hypothesis whether allelic variants of *MTHFR* 1793G>A were independently associated with vitamin B_{12}, folate, or tHcy plasma levels in kidney transplant recipients. Further, we sought to test for interaction between the *MTHFR* 1298A>C and 1793G>A allelic variants on these outcomes.

METHODS

Study population

We conducted a cross-sectional study of 733 kidney allograft recipients who received routine follow-up at a large academic transplant center. The detailed rules for study inclusion have been published elsewhere [12]. Material for full genetic analysis was unavailable for three individuals, which left a final study population of 730 patients. None of the patients was prescribed routine folic acid or vitamin B supplementation. However, one patient had excessively high vitamin B_{12} concentrations, possibly indicating self-medication. We decided a priori to exclude this patient from all association studies (Note: including this patient influenced the regression results substantially, as found by post hoc analyses that included the patient). All patients provided written informed consent according to the Declaration of Helsinki and the Austrian Law on Gene Technology.

Biochemical methods

Fasting citrated blood was immediately placed on ice and centrifuged at 2000 $\times$ *g* at 4°C (20 minutes) within 60 minutes. Plasma aliquots and 500 μL of citrated blood for isolation of DNA were snap frozen and stored at −70°C. Plasma concentrations of tHcy (free plus protein-bound Hcy) [13] were determined by automated high-performance liquid chromatography (HPLC) with reversed-phase separation and fluorescence detection using tri-*n*-butylphosphine as a reducing agent. Intra-assay variability was between 1.4% and 1.7% and interassay variability was between 1.5% and 1.9% for tHcy concentrations of 15.9 and 6.9 μmol/L, respectively. Folate and vitamin B_{12} plasma levels were measured with a radioassay (SimulTRAC-SNB) (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA). Interassay variability was 4% to 5% for folate measurements and 4% to 6% for vitamin B_{12} levels. The creatinine clearance was calculated using the equation of Cockcroft and Gault and standardized to a body surface area of 1.73 m^2 [14].

Genotyping

Identification of the *MTHFR* 677C>T transition and the *MTHFR* 1298A>C transversion was conducted using restriction fragment length polymorphism (RFLP) analysis and the polymerase chain reaction (PCR) primers and restriction enzymes *Hinf*I and *Fnu4*HI published by Frosst et al [15] and Weisberg et al [16]. We selected the RFLP system of Weisberg et al since this test system allowed for a clear descrimination beween *MTHFR* 1298A>C and the silent polymorphism *MTHFR* 1317T>C.

The 1793G>A mutation was analyzed in PCR products that had been amplified with the primers published by Rady et al [10]. *MTHFR* 1793G>A abolishes the restriction site for *Bsrb*I. After cleavage with *Bsrb*I samples without mutation show two fragments of 233 and 77 bp, whereas samples with mutations exhibit an uncleaved PCR fragment of 310 bp. Heterozygous samples show all three fragments of 310, 233, and 77 bp, respectively. *Bsrb*I-treated PCR products were analyzed by electrophoresis through 6% polyacrylamide gels (Novex, San Diego, CA, USA) followed by Sybr Green staining (Nucleic Acid Gel Stain, Molecular Probe, Eugene, OR, USA). All samples showing the homozygous mutant genotype were retested in an independent RFLP analysis.

Statistical analyses

The SAS for Windows (release 8.2) statistical software was used for all analyses (SAS Institute Inc., Cary, NC, USA). We described important patient characteristics using mean values and standard deviations, or medians and the 25th/75th percentiles for continuous variables, and percentages for categorical variables. As the distributions of plasma vitamin B_{12}, folate levels, and tHcy were found to be skewed to the right, and therefore nonnormally distributed, we used a special case of general linear models for all advanced analyses: the link function was chosen to be linear and spread was determined to follow a gamma distribution (in SAS, PROC GENMOD,

Table 2. Characteristics of 729 kidney graft recipients

Variable	*MTHFR* 1793GG	*MTHFR* 1793GA/AA
Mean (SD) or counts (%)	$N = 655$	$N = 74$
Age *years*	52.0 (±13.6)	50.0 (±12.5)
Male gender	399 (60.9%)	39 (52.7%)
Body mass index kg/m^2	25.3 (±4.2)	25.5 (±5.0)
Estimated glomerular filtration rate $mL/min/1.73\ m^2$	56.1 (±19.8)	55.3 (±22.3)
Years since transplantation	5.1 (±4.1)	4.2 (±4.0)
Plasma vitamin B_{12} [median (25th/75th percentile)] *pmol/L*	217 (168/303)	240 (184/340)
Plasma folate [median (25th/75th percentile)] *nmol/L*	12.9 (9.9/16.8)	14.0 (11.3/16.9)
Plasma total homocysteine [median (25th/75th percentile)] *μmol/L*	15.1 (12.0/19.9)	13.4 (11.0/18.0)
Likely underlying renal disease		
Diabetic nephropathy	42 (6.4%)	6 (8.1%)
Glomerulonephritis	219 (33.4%)	28 (37.8%)
Interstitial nephritis	111 (17.0%)	10 (13.5%)
Polycystic kidney disease	88 (13.4%)	11 (14.9%)
Miscellaneous other kidney disease, defined	57 (8.7%)	6 (8.1%)
Kidney disease, not otherwise defined	138 (21.1%)	13 (17.6%)

All statistical comparisons between *MTHFR* 1793GG and 1793GA/AA were not significant ($P > 0.05$). For detailed comparisons of folate, vitamin B_{12}, and total homocysteine by *MTHFR* 1793 genotype, see Tables 3 and 4.

LINK = IDENTITY, DIST = GAMMA). Such an approach provides unbiased estimates of effect for positively skewed data without the need to conduct data transformation (e.g., to a logarithmic scale) [17, 18]. Like in previous work, we used such models both for univariate and multivariate analyses [5, 7, 8]. Three sets of analyses were conducted, the outcomes being plasma levels of vitamin B_{12}, folate, and tHcy concentration, respectively. The results reflect the absolute difference in concentration of the respective dependent variable, and asymmetric 95% CI are given to reflect precision and the likely range of the true parameter given the data [19]. Consistent with similar studies conducted previously, we did not adjust for multiple testing, due to the hypothesis generating nature of this work [5, 7, 9]. Independent covariates included patient age (in decades), gender, time since kidney transplantation (<2 vs. ≥2 months), and categories of underlying kidney disease (diabetic nephropathy, glomerulonephritis, interstitial nephritis, polycystic kidney disease, miscellaneous other defined, and kidney disease not otherwise defined). We calculated each patient's body mass index (BMI) (categorized in quintiles) as the weight in kilograms divided by the squared height in meters, and defined quintiles of the plasma levels of folate and vitamin B_{12}. For each patient, we staged renal function in accordance with the Dialysis Outcomes Quality Initiative (K/DOQI) guidelines (>90, 60 to 90, 30 to 60, and <30 mL/min/1.73 m^2) [20]. We created dummy covariates for the genotypes of *MTHFR* 677C>T and 1298A>C. A single dummy covariate was created for either heterozygosity or homozygosity of *MTHFR* 1793G>A, since only two patients were homozygous for that variant. In accordance with our a priori hypothesis, interactions between *MTHFR* 1793G>A and *MTHFR* 1298A>C genotypes were also tested for significance. All multivariate models adjusted for age, gender, BMI, estimated glomerular filtration rate (GFR), time since transplantation, underlying kidney disease, *MTHFR* 677C>T and *MTHFR* 1298A>C genotype, plasma levels of vitamin B_{12} (in models of plasma folate and tHcy), and plasma levels of folate (in models of plasma vitamin B_{12} and tHcy).

RESULTS

Study population

The allele frequency of *MTHFR* 1793G>A among all 730 patients was 0.052. Seventy-two patients (9.9%) were heterozygous and two patients (0.3%) were homozygous. Six hundred and fifty-six patients (89.9%) were found to have no mutation (the one patient who was later dropped from the association studies was in this wild-type group) [11]. The assumption of a Hardy-Weinberg equilibrium was not violated for this mutation ($P = 0.98$) [21]. The distribution of combinations of *MTHFR* 1793G>A and 1298A>C genotypes is shown in Table 1 [11]. The prevalence of *MTHFR* 677C>T and 1298A>C as well as association studies of these genotypes with the three outcomes of interest have been published in detail elsewhere [5, 11, 12].

The mean age in the sample of 729 kidney transplant recipients who were used for the association studies was 51.8 years, 60% were male, and more than half of the patients were obese or overweight (i.e., BMI >25 kg/m^2). Approximately 50% ($N = 364$) of the patients had elevated tHcy (>15 μmol/L), only one patient (0.1%) was below reference for plasma folate (<3.4 nmol/L), and 65 patients (8.9%) had vitamin B_{12} levels below the reference threshold (<118 pmol/L). Table 2 lists important patient characteristics stratified by *MTHFR* 1793 genotype. None of these characteristics differed between the groups (all $P > 0.05$). Immunosuppressive treatment

Table 3. Plasma vitamin B_{12}, folate and total homocysteine (tHcy) concentrations of 729 kidney transplant recipients by *MTHFR* 1793 genotype (mean ± SD)

	MTHFR 1793G>A genotype		
	GG	GA	AA
Number	655	72	2
Plasma vitamin B_{12} *pmol/L*	255 ± 157	274 ± 153	226 ± 41
Plasma folate *nmol/L*	14.4 ± 9.8	18.3 ± 27.3	20.8 ± 8.0
Plasma tHcy *μmol/L*	17.2 ± 8.4	16.5 ± 11.5	14.8 ± 3.7

in this study population has been described in detail elsewhere [22]; there were no differences between the two *MTHFR* 1793 groups.

Effect of *MTHFR* 1793G>A genotype on plasma vitamin B_{12} concentrations

Mean plasma concentrations of vitamin B_{12} stratified by *MTHFR* 1793G>A genotype are shown in Table 3. Univariate analyses did not reveal any associations between *MTHFR* 1793GA/AA vs. the wild-type and plasma vitamin B_{12}. Similarly, after multivariate adjustment for age, gender, BMI, estimated GFR, time since transplantation, underlying kidney disease, *MTHFR* 677C>T genotype, and plasma levels of folate, there were no differences in vitamin B_{12} concentrations by *MTHFR* 1793 genotype (Table 4). When testing for effect modification between *MTHFR* 1298A>C and *MTHFR* 1793G>A, we found statistically significant interactions between these mutations. The 53 patients who carried the *MTHFR* allelic combination 1298AC and 1793GA or 1793AA (see Table 1) had vitamin B_{12} concentrations that were 171 pmol/L higher (95% CI 74 to 268 pmol/L) ($P < 0.001$) and the 19 patients with *MTHFR* 1298CC and 1793GA or 1793AA had vitamin B_{12} concentrations that were 117 pmol/L higher (95% CI 6 to 228 pmol/L) ($P = 0.04$) compared to patients who showed wild-type alleles (Table 5). These findings remained robust whether or not we excluded folate from these models.

Effect of *MTHFR* 1793G>A genotype on plasma folate concentrations

Mean plasma levels of folate stratified by *MTHFR* 1793G>A genotypes are shown in Table 3, and it appeared that folate concentrations were higher in patients who were heterozygous or homozygous for the mutant allele. From univariate linear regression analyses, we found that patients with the 1793GA or AA genotype had folate concentrations that were 3.91 nmol/L higher, on average, than patients with wild-type alleles (95% CI 1.89 to 5.94 nmol/L) ($P < 0.001$). Multivariate regression adjustment (for included variables see Table 4) attenuated this finding and the difference was of borderline significance (1.91 nmol/L) (95% CI −0.03 to 3.86 nmol/L) ($P = 0.05$). No interactions between *MTHFR* 1298A>C and *MTHFR* 1793G>A were found. Excluding vitamin B_{12} plasma level from the multivariate regression analysis did not change these results.

Effect of *MTHFR* 1793G>A genotype on plasma tHcy concentrations

Mean tHcy plasma levels sorted by *MTHFR* 1793G>A genotype are indicated in Table 3. When testing whether *MTHFR* 1793GA/AA was associated with tHcy plasma levels compared to *MTHFR* 1793GG in regression models, we did not detect any significant associations in univariate or in multivariate analyses (Table 4). There was no detectable interaction between *MTHFR* 1298A>C and *MTHFR* 1793G>A. These results did not change materially whether or not we included vitamin B_{12} and/or folate in the multivariate models.

DISCUSSION

The present report constitutes the first study of the putative influence of a novel polymorphism, *MTHFR* 1793G>A, on important biochemical parameters of Hcy metabolism. In a large population of kidney transplant recipients, we found that patients carrying the *MTHFR* 1793G>A mutation had approximately 4 nmol/L higher plasma folate concentrations, on average, when compared to individuals with the corresponding wild-type alleles. Furthermore, patients who had both the *MTHFR* 1793G>A *and* the *MTHFR* 1298A>C mutation exhibited markedly higher plasma vitamin B_{12} levels compared to patients without these mutations.

Our finding of higher folate concentrations in patients with mutations in *MTHFR* 1793G>A may have implications for the prognosis of these patients. Different folate derivatives (e.g., 5,10-methylenetetrahydrofolate; 5-formiminotetrahydrofolate; 5,10-methenyltetrahdrofolate; 10-formyltetrahydrofolate; and 5-methyltetrahydrofolate) are essential coenzymes for the conversion of serine to glycine, the catabolism of histidine, the synthesis of purine and thymidylate, as well methionine synthesis. The synthesis of methionine and thus remethylation of Hcy is accomplished via 5-methyltetrahydrofolate that represents the principal folate derivative present in human serum [23]. Activated methionine (S-adenoslymethionine), in turn, acts as a methyl donor for numerous methyltransferases present in human cells (examples for methylated products are lipids, myelin basic protein, dopamine, DNA) [24]. Clearly, if folate status is low, reduced acitivity of folate enzymes can result in pathology (e.g., anemia) via reduction of DNA biosynthesis and cell division [24, 25]. In this context, it is important to mention that we used a competitive protein binding assay (SimulTRAC-SNB) which includes the pteroylglutamic acid form of folate (PGA) as a standard and tracer at an binding pH where

Table 4. Estimated univariate and multivariate linear associations between *MTHFR* 1793 genotype and plasma vitamin B_{12}, folate, and total homocysteine (tHcy) levels

Outcome modeled by *MTHFR* genotype	Univariate results			Multivariate results[a]		
	Estimate	95% CI	*P* value	Estimate	95% CI	*P* value
Plasma vitamin B_{12}	(ΔB_{12} in pmol/L)			(ΔB_{12} in pmol/L)		
1793GG	—	(referent)	—	—	(referent)	—
1793GA/AA	18	(−14; 51)	0.27	17	(−17; 51)	0.33
Plasma folate	(delta folate in nmol/L)			(Δfolate in nmol/L)		
1793GG	—	(referent)	—	—	(referent)	—
1793GA/AA	3.91	(1.89; 5.94)	<0.001	1.91	(−0.03; 3.86)	0.05
Plasma tHcy	(ΔtHcy in μmol/L)			(ΔtHcy in μmol/L)		
1793GG	—	(referent)	—	—	(referent)	—
1793GA/AA	−0.67	(−2.31; 0.97)	0.42	−0.24	(−1.45; 0.98)	0.70

[a]All multivariate models simultaneously accounted for age, gender, body mass index, estimated glomerular filtration rate, time since transplantation, underlying kidney disease, *MTHFR* 677C>T and *MTHFR* 1298A>C. Plasma levels of vitamin B_{12} were additionally included in models of plasma folate and tHcy. Plasma levels of folate were additionally included in models of plasma vitamin B_{12} and tHcy.

Table 5. Interaction of *MTHFR* 1793G>A and 1298A>C on vitamin B_{12} concentrations

Genotype	Estimate (ΔB_{12} in pmol/L)	95% CI	*P* value
1793GG and 1298AA (wild-type)	—	(referent)	—
1793GG and 1298AC	−14	(−36; 9)	0.23
1793GG and 1298CC	6	(−37; 48)	0.80
1793GA/AA and 1298AC	171	(74; 268)	<0.001
1793GA/AA and 1298CC	117	(6; 228)	0.04

Multivariate results. *MTHFR* 1793GA/AA and 1298AA not estimated (small cell count) ($N = 2$).

both 5-methyltetrahydrofolate in the sample and PGA have equal affinity for the milk folate-binding protein. Therefore, the higher folate levels observed among patients with the *MTHFR* 1793G>A mutation most likely represent 5-methyltetrahydrofolate concentrations and thus constitute the active folate derivative involved in methionine synthesis.

While *MTHFR* 1793G>A was associated with higher folate concentrations in this study, there was no association with tHcy plasma concentrations. However, mean tHcy plasma concentrations were 17.2 μmol/L, 16.5 μmol/L, and 14.8 μmol/L in patients with the *MTHFR* 1793GG (wild-type), 1793GA, and 1793AA genotype suggesting a tendency toward lower tHcy concentrations in patients with the *MTHFR* 1793G>A mutation who had higher folate levels. Furthermore, while an increased tHcy plasma concentration is a sensitive marker of folate and vitamin B_{12} deficiency [26], tHcy concentrations could be more tightly balanced when folate and vitamin B_{12} supplies are adequate. In kidney transplant recipients, tHcy has been shown to be associated with adverse outcomes such as cardiovascular events, mortality, and possibly increased allograft failure [22, 27]. To our knowledge, associations between folate and such outcomes have not yet been reported.

Most interestingly, we found that patients who tested positive for *MTHFR* 1793G>A as well as *MTHFR* 1298A>C (double or triple mutation combination) had higher plasma vitamin B_{12} concentrations when compared with patients who did not carry these allelic variants. Vitamin B_{12} concentrations in patients with the *MTHFR* 1298AC/1793GA and the *MTHFR* 1298CC/1793GA or 1793AA genotypes were 171 pmol/L and 117 pmol/L higher, respectively (Table 5). It is well established that folate and vitamin B_{12} metabolism are closely linked by the reaction that transfers a methyl group from 5-methyltetrahydrofolate to vitamin B_{12} [28]. This linkage has been observed among patients with folate deficiency who can exhibit low serum vitamin B_{12} levels in about one third of cases [29]. Conversely, in cases of cobalamin deficiency, folate levels can be low [30].

The mechanisms by which *MTHFR* 1793G>A, and *MTHFR* 1793G>A together with *MTHFR* 1298A>C, result in increased folate and vitamin B_{12} concentrations, respectively, are unclear. In this context, our group previously observed that combinations of mutant *MTHFR* 1298 and 1793 alleles are more prevalent among the same 730 kidney transplant patients than could be expected under an assumption of statistical independence [11]. We identified 53 patients (expected 33.6) with the *MTHFR* 1298AC/1793GA genotype, and 17 patients (expected 6.7) with the *MTHFR* 1298CC/1793GA genotype, numbers that were greater than the expected cell counts (Table 1). Furthermore, the number of patients with double homozygosity for *MTHFR* 1793G>A and *MTHFR* 1298A>C (*MTHFR* 1298CC/1793AA genotype) ($N = 2$) was greater than expected (Table 1). Our observation of a higher prevalence of these genotype combinations suggested a selection or survival advantage for individuals showing these genotype combinations, which might be related to the higher concentrations of vitamin B_{12} described in the present study.

The present study has certain limitations. Findings from a population of renal transplant patients may not be representative of other populations. The cross-sectional design makes it possible that time-related biases are operational, most important survival bias. Certain genotype combinations might affect eligibility for transplantation

or patient survival so that such patients are less likely to be represented in the analysis. However, such a bias would solely influence prevalence estimates of various genotypes, but not necessarily associations between allelic variants and folate or tHcy plasma levels. We did not assess dietary intake of folate, which should not be an issue, because none of the patients received folic acid or multivitamin supplements and so dietary folate intake can be assumed to be within a relatively narrow range. Finally, we neither used a microbiologic assay for estimation of folate supply, nor was red blood cell folate measured.

From this first study of the associations between the novel polymorphism *MTHFR* 1793G>A and important factors of homocysteine metabolism, we found that any presence of this polymorphism was associated with higher folate concentration in kidney transplant patients. Further, we found evidence for an interaction between *MTHFR* 1793G>A and 1298A>C. Patients who had both the *MTHFR* 1793G>A and the 1298A>C mutant allelic variant of that gene showed significantly higher vitamin B_{12} concentrations. While higher concentrations of folate and vitamin B_{12} might play a role in a possible survival/selection advantage in these patients, the clinically more relevant parameter of tHcy was unaffected by these genotypes. Confirmation of these findings in independent populations as well as studies of these genotypes and long-term outcomes are warranted.

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