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Effect of *MTHFR* genotypes and hyperhomocysteinemia on patient and graft survival in kidney transplant recipients

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Background. The total homocysteine (tHcy) plasma level, which is partly determined by the *MTHFR* 677C→T genotype, may be associated with vascular disease. We prospectively examined the influence of *MTHFR* genotypes (677C→T, 1298A→C) and tHcy plasma concentration on all cause mortality and graft outcomes of renal transplant recipients.

Methods. Baseline tHcy plasma levels of 189 patients (three groups with either the *MTHFR* 677CC, CT or TT genotype, including 63 patients in each group, were matched for age, gender, body mass index and creatinine clearance at baseline), were obtained between September 1996 and May 1997. Follow-up data (time until return to dialysis therapy, time and cause of death) were collected from April to June 1999. Kaplan-Meier survival estimations were calculated and plotted, the groups (three *MTHFR* 677C→T genotype groups, or three *MTHFR* 1298A→C genotype groups, or two groups with tHcy plasma levels above/below 15 μmol/L) were compared by log-rank test. Age, gender, body mass index (BMI), time since transplantation, serum creatinine, creatinine clearance, combined *MTHFR* 677C→T/1298A→C genotypes, tHcy, folate and vitamin B_{12} plasma levels were evaluated with regard to graft and patient survival in a multivariate Cox-proportional hazard regression model.

Results. During the follow-up period of 2.26 ± 0.66 years, 9 patients died (5 in the TT, 2 in the CT and 2 in the CC genotype group; $P = 0.34$) and 22 returned to dialysis treatment (7 in the TT, 9 in the CT and 6 in the CC genotype group; $P = 0.65$). There was also no influence of *MTHFR* 1298A→C genotypes (AA genotype, 114 patients; AC genotype, 64 patients; CC genotype, 11 patients) on patient or graft survival ($P = 0.7087$ and $P = 0.1633$, respectively). Two of 93 patients with a tHcy plasma level ≤15 μmol/L died, in contrast to 7 of 96 patients in the tHcy >15 μmol/L group, $P = 0.0778$. Two patients in the low tHcy group had to return to dialysis, in contrast to 20 patients in the high tHcy group ($P = 0.0001$). In the multivariate model there was no significant predictor of patient survival, and the serum creatinine was the only predictor of graft survival ($P < 0.0001$).

Conclusions. In summary, our study shows that neither *MTHFR* 677C→T/1298A→C genotypes nor hyperhomocysteinemia are independently associated with patient or graft survival following kidney transplantation.

Key words: total homocysteine, cardiovascular disease, allograft survival, end-stage renal disease, folate, vitamin B_{12}.

Total homocysteine (tHcy) plasma level was shown to be associated with a markedly increased cardiovascular disease morbidity and mortality in patients with renal failure [1]. Total tHcy plasma concentrations are also elevated in a large proportion of renal transplant patients with a prevalence of fasting and post-methionine loading hyperhomocysteinemia of approximately 50 to 60% [2, 3].

An analysis of major predictors of tHcy plasma levels in 189 kidney graft recipients demonstrated that the *MTHFR* 677TT genotype is associated with significantly higher tHcy levels than the *MTHFR* 677CC or CT genotype [2]. In a multiple stepwise regression analysis, creatinine clearance, plasma folate status, and the *MTHFR* 677TT genotype were the most important predictors of tHcy plasma levels in these patients [2]. In a more recent study, the influence of a novel polymorphism in *MTHFR*, 1298A→C, in combination with *MTHFR* 677C→T, was examined in 733 kidney graft recipients [4]. There was a strong influence of the *MTHFR* 677TT/1298AA genotype on tHcy and folate levels in kidney graft recipients, resulting in elevated tHcy and decreased folate plasma levels. Patients with the compound heterozygous genotype (*MTHFR* 677CT/1298AC) also presented significantly lower folate plasma levels than patients with wild-type alleles [4].

To date, no prospective data are available in the kidney transplant population with regard to the influence of different *MTHFR* genotypes and hyperhomocysteinemia on patient and graft survival. We tested the hypothesis that *MTHFR* genotypes, tHcy plasma levels and other determinants of the homocysteine metabolism may affect patient and graft outcome after kidney transplantation.

Table 1. Baseline characteristics of all 189 kidney graft recipients

Age *years*	54.8 ± 12.2
Gender *female/male*	87/102
Time on transplantation *years*	5.7 ± 4.0
BMI kg/m^2	25.7 ± 3.88
Serum creatinine *mg/dL*	1.76 ± 1.18
Creatinine clearance *mL/min*	55.7 ± 20.9
tHcy *μmol/L*	17.6 ± 9.2
Folate *nmol/L*	12.6 ± 6.0
Vitamin B_{12} *pmol/L*	266.8 ± 157.57

MTHFR 677C→T genotype distribution was: CC, 63; CT, 63; TT, 63. Data are given as means ± SD. Abbreviations are: BMI, body mass index; tHcy, total homocysteine.

Table 3. Outcome of 189 kidney graft recipients according to the total homocysteine (tHcy) plasma levels

	tHcy plasma level	
	≤15 μmol/L	>15 μmol/L
Number of patients	93	96
Patients with functioning graft (N = 158)	89	69
Δ Serum creatinine *mg/dL*	+0.14 ± 0.46	+0.18 ± 0.84
Number of patients on dialysis (N = 22)	2	20
Total number of deaths (N = 9)	2	7
Cardiovascular deaths (N = 5)	1	4

Data are given as means ± SD.

METHODS

Patients

Baseline data of 189 kidney transplant patients were collected between September 1996 and May 1997, and reported previously (Table 1) [2]. These patients comprise three groups with either the *MTHFR* 677CC, CT or TT genotype [63 in each group, matched for age, sex, body mass index (BMI) and creatinine clearance]. Follow-up data (serum creatinine levels, time point of the return to dialysis therapy and time and cause of death) were collected from April to June 1999 by review of the charts. Demographic and biochemical characteristics of all 189 patients are given in Table 1.

Laboratory analyses

Total homocysteine, folate and vitamin B_{12} plasma levels were determined as described previously [2]. The normal reference value of fasting tHcy plasma level was defined as less than 15 μmol/L. This cut-off level is currently judged to be appropriate for the diagnosis of hyperhomocysteinemia, although this value may be revised downward in the near future [5]. The creatinine clearance was estimated according to Cockroft and Gault [6]. Identification of the 677C→T transition and the 1298A→C transversion in *MTHFR* was performed according to Frosst et al [7] and Weisberg et al [8] by restriction fragment length polymorphism (RFLP) analysis.

Statistical analysis

Mean values ± SD are given for continuous data and percentages for categorical data.

Kaplan-Meier estimations for patient and graft survival were calculated according to the three *MTHFR* 677C→T genotype groups, the three *MTHFR* 1298A→C genotype groups or the tHcy level dichotomized by a plasma concentration of 15 μmol/L. The groups were compared by log-rank test.

Age, gender, BMI, time since transplantation, serum creatinine, creatinine clearance, combined *MTHFR* 677C→T/1298A→C genotypes, tHcy, folate and vitamin B_{12} plasma levels were evaluated with regard to graft and patient survival in a multivariate Cox-proportional hazard regression model.

RESULTS

Patient and graft outcome according to *MTHFR* 677C→T genotypes and the tHcy plasma concentration are given in Tables 2 and 3.

During the follow-up period of 2.26 ± 0.66 years, 9 patients died (5 in the TT, 2 in the CT and 2 in the CC genotype group; P = 0.34) and 22 returned to dialysis treatment (7 in the TT, 9 in the CT and 6 in the CC genotype group; P = 0.65). The mean serum creatinine in the remaining 158 patients increased from 1.56 ± 0.81 to 1.68 ± 0.79 mg/dL (CC), from 1.37 ± 0.33 to 1.54 ± 0.76 mg/dL (CT), and from 1.42 ± 0.46 to 1.59 ± 0.66 mg/dL (TT), respectively. There was also no influence of *MTHFR* 1298A→C genotypes (AA genotype, 114 patients; AC genotype, 64 patients; CC genotype, 11 patients) on patient or graft survival (P = 0.7087 and P = 0.1633, respectively).

Two of 93 patients with a tHcy plasma level ≤15 μmol/L died, in contrast to 7 of 96 patients in the tHcy >15 μmol/L (P = 0.0778). Two patients in the low tHcy group had to return to dialysis, in contrast to 20 patients in the high tHcy group (P = 0.0001).

Kaplan-Meier estimations of patient and graft survival according to the three *MTHFR* 677C→T genotypes or the tHcy plasma levels dichotomized by the cut-off level for the normal range (≤15 μmol/L) are shown in Figure 1.

For further analysis of the influence of tHcy plasma levels on graft survival and patient survival (dependent variables), age, gender, BMI, time since transplantation, serum creatinine, creatinine clearance, combined *MTHFR* 677A→C/1298C→T genotypes, tHcy, folate and vitamin B_{12} plasma levels were included in a multivariate Cox-proportional hazard regression model as covariates. For patient survival, there was no significant variable in the univariate model. Age, tHcy plasma levels serum creati-

Table 2. Outcome of 189 kidney graft recipients according to the *MTHFR* 677C→T genotype

	MTHFR 677C→T genotype		
	CC	CT	TT
Number of patients	63	63	63
Patients with functioning graft (N = 158)	55	52	51
Δ Serum creatinine *mg/dL*	+0.12 ± 0.78	+0.17 ± 0.62	+0.17 ± 0.53
Number of patients returned to dialysis (N = 22)	6	9	7
Total number of deaths (N = 9)	2	2	5
Cardiovascular deaths (N = 5)	1	1	3

Data are given as means ± SD.

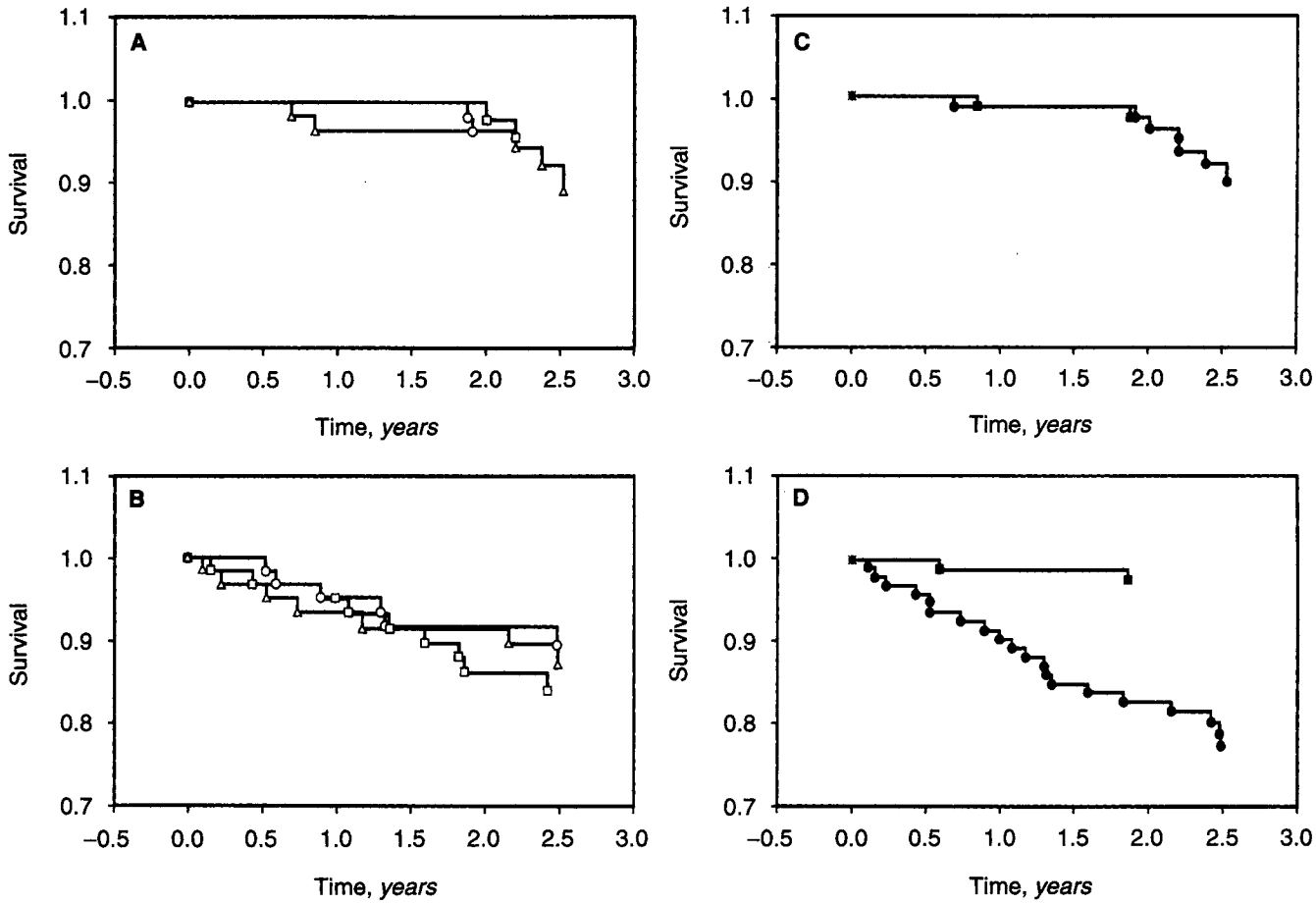

Fig. 1. Kaplan-Meier estimation of patient (*A*) and graft (*B*) survival according to *MTHFR* genotypes. Symbols are: (○) *MTHFR* 677CC; (□) CT; (△) TT. Log-rank test, P = 0.34 and P = 0.65, respectively. Kaplan-Meier estimation of patient (*C*) and graft (*D*) survival according to tHcy plasma levels. Symbols are: tHcy: (■) ≤15 μmol/L; (●) >15 μmol/L. Log-rank test, P = 0.0778 and P = 0.0001, respectively.

nine and creatinine clearance were significantly associated with graft survival in the univariate model. However, the serum creatinine remained the only significant predictor of graft survival in the multivariate model (Table 4).

DISCUSSION

This prospective study clearly shows that neither the *MTHFR* 677C→T nor the 1298A→C genotypes nor the tHcy plasma concentration (above the cut-off level 15 μmol/L) are independent predictors of short-term allograft and patient survival among kidney graft recipients.

In stable kidney graft recipients, hyperhomocysteinemia is present in about 50 to 60% of the patients [2, 3]. An elevated tHcy plasma concentration is suggested to be an important risk factor for cardiovascular disease, and several studies showed higher tHcy plasma concentrations in renal graft recipients suffering from cardiovas-

Table 4. Cox-proportional hazard regression model for graft survival

	P (univariate)	*P* (multivariate)
Age	0.0218	0.9516
Gender	0.1277	0.2933
BMI	0.9144	0.9287
Time on transplantation	0.2084	0.4379
Serum creatinine	<0.0001	<0.0001
Creatinine clearance	<0.0001	0.0854
tHcy	<0.0001	0.1526
Vitamin B_{12}	0.9128	0.4455
Folate	0.4657	0.1182
MTHFR 677/1298 genotype	0.4855	0.1078

cular disease than in patients without cardiovascular disease [9–11]. However, other studies failed to show any association between tHcy plasma concentration and cardiovascular disease morbidity or renal transplant outcomes [12, 13].

Polymorphisms in the gene coding for the enzyme 5,10-methylenetetrahydrofolate reductase (EC 1.5.1.20) have been shown to be associated with decreased enzyme activity, resulting in a decreased formation of 5-methyltetrahydrofolate, which favors the increase of tHcy plasma concentrations [7, 14]. In this context, homozygosity for the *MTHFR* 677C→T transition has been reported to aggravate hyperhomocysteinemia. Moreover, the *MTHFR* 677TT and the compound heterozygous 677CT/1298AC genotype are associated with low folate plasma levels in kidney graft recipients [2, 4].

Only little information is available concerning the impact of *MTHFR* genotypes on graft and patient survival. Interestingly, the *MTHFR* 677C→T polymorphism of the kidney donor had no influence on tHcy levels in a previous study [2]. This finding was supported by the study of Liangos et al, who showed that neither the recipient nor the donor genotype had any influence on kidney graft survival [15].

We here report the results of the first prospective study aimed to examine the impact of *MTHFR* 677A→C/1298A→C genotypes and tHcy plasma concentration on all-cause mortality, cardiovascular mortality and graft survival in 189 kidney graft recipients. No influence of several factors involved in homocysteine metabolism was observed on patient and graft outcomes in this study during a mean follow-up period of 2.3 years. One cannot rule out that *MTHFR* genotypes or tHcy plasma levels may become significant predictors of kidney graft recipients outcome during a longer observation period. An association of tHcy level with graft outcome (that is, a return to dialysis therapy) was only observed in univariate modeling. By contrast, in the multivariate analysis the serum creatinine was the only significant predictor of graft outcome, confirming that hyperhomocysteinemia in kidney graft recipients is mainly due to impaired renal function, which in turn is the most important predictor of graft survival [2].

The strength of the present study is that three groups of patients with the different *MTHFR* 677C→T genotypes were matched at baseline for age, gender, BMI and creatinine clearance. A potential limitation of the study is that other cardiovascular risk factors, like hypertension, hyperglycemia or hyperlipidemia, were not considered.

In summary, our prospective study shows that neither *MTHFR* 677C→T nor 1298A→C polymorphism have any short-term influence on patient or graft survival in kidney transplant patients. Similarly, tHcy plasma concentration has no influence on patient survival. Total Hcy plasma level is associated with graft survival in univariate models. However, a multivariate analysis shows that the elevation of tHcy plasma level reflects the baseline impairment of renal function. Thus, at present it is not clear whether tHcy plasma concentration-lowering interventions may have any impact on patient or graft survival following kidney transplantation.

NOTE ADDED IN PROOF

Ducloux et al examined the association of tHcy plasma levels with 30 cardiovascular disease events among 207 stable renal transplant recipients during a follow-up period of 21.2 ± 1.9 months. They observed an increased relative risk for the occurrence of a cardiovascular disease event of 1.06 (95% CI, 1.04 to 1.09) per μmol increase of plasma tHcy concentration [16].

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REFERENCES

1. Bostom AG, Culleton BF: Hyperhomocysteinemia in chronic renal disease. *J Am Soc Nephrol* 10:891–900, 1999
2. Födinger M, Wölfl G, Fischer G, Rasoul-Rockenschaub S, Schmid R, Hörl WH, Sunder-Plassmann G: Effect of *MTHFR* 677C→T on plasma total homocysteine levels in renal graft recipients. *Kidney Int* 55:1072–1080, 1999
3. Bostom AG, Gohh RY, Tsai MY, Hopkins-Garcia BJ, Nadeau MR, Bianchi LA, Jacques PF, Rosenberg IH, Selhub J: Excess prevalence of fasting and postmethionine-loading hyperhomocysteinemia in stable renal transplant recipients. *Arterioscler Thromb Vasc Biol* 17:1894–1900, 1997
4. Födinger M, Buchmayer H, Heinz G, Papagiannopoulos M, Kletzmayr J, Rasoul-Rockenschaub S, Hörl WH, Sunder-Plassmann G: Effect of *MTHFR* 1298A→C and *MTHFR* 677C→T genotypes on total homocysteine, folate, and vitamin B_{12} plasma concentrations in kidney graft recipients. *J Am Soc Nephrol* 11:1918–1925, 2000
5. American Society of Human Genetics/American College of Medical Genetics Test and Transfer Committee Working Group: Measurement and use of total plasma homocysteine. *Am J Hum Genet* 63:1541–1543, 1998
6. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41, 1976
7. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews

RG, BOERS GJ, DEN HEIJER M, KLUIJTMANS LA, VAN DEN HEUVEL LP, ROZEN RA: A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. (letter) *Nat Genet* 10:111–113, 1995

8. WEISBERG I, TRAN P, CHRISTENSEN B, SIBANI S, ROZEN R: A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* 64:169–172, 1998
9. ARNADOTTIR M, HULTBERG B, VLADOV V, NILSSON-EHLE P, THYSELL H: Hyperhomocysteinemia in cyclosporine-treated renal transplant recipients. *Transplantation* 61:509–512, 1996
10. DUCLOUX D, RUEDIN C, GIBEY R, VAUTRIN P, BRESSON-VAUTRIN C, REBIBOU JM, CHALOPIN JM: Prevalence, determinants, and clinical significance of hyperhomocyst(e)inaemia in renal-transplant recipients. *Nephrol Dial Transplant* 13:2890–2893, 1998
11. MASSY ZA, CHADEFAUX-VEKEMANS B, CHEVALIER A, BADER CA, DRÜEKE TB, LEGENDRE C, LACOUR B, KAMOUN P, KREIS H: Hyperhomocysteinaemia: A significant risk factor for cardiovascular disease in renal transplant recipients. *Nephrol Dial Transplant* 9:1103–1108, 1994
12. DIMENY E, HULTBERG B, WAHLBERG J, FELLSTROM B, ARNADOTTIR M: Serum total homocysteine concentration does not predict outcome in renal transplant recipients. *Clin Transplant* 12:563–568, 1998
13. BRAUN WE, PROTIVA DA, GIFFORD RW JR, STRAFFON RA: Hyperhomocysteinemia and other coronary risk factors in 20-year renal transplant recipients (level 5A) with and without coronary heart disease. *Transplant Proc* 31:1280–1282, 1999
14. VAN DER PUT NM, GABREELS F, STEVENS EM, SMEITINK JA, TRIJBELS FJ, ESKES TK, VAN DEN HEUVEL LP, BLOM HJ: A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural-tube defects? *Am J Hum Genet* 62:1044–1051, 1998
15. LIANGOS O, KREUTZ R, BEIGE J, OFFERMANN G, DISTLER A, SHARMA AM: Methylenetetrahydrofolate-reductase gene C677T variant and kidney-transplant survival. *Nephrol Dial Transplant* 13:2351–2354, 1998
16. DUCLOUX D, MOTTE G, CHALLIER B, GIBEY R, CHALOPIN JM: Serum total homocysteine and cardiovascular disease occurrence in chronic, stable renal transplant recipients: a prospective study. *J Am Soc Nephrol* 11:134–137, 2000