Hyperhomocysteinaemia and MTHFR C677T gene polymorphism in renal transplant recipients

A J Szabó, T Tulassay, B Melegh, T Szabó, A Szabó, Á Vannay, A Fekete, Z Süveges, G S Reusz

Abstract

***Aim*—To study the effect of folate treatment on hyperhomocysteinaemia and the effect of 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphism on total homocysteine and folate concentrations after renal transplantation.**

***Methods*—A total of 30 transplanted children and adolescents were investigated for total homocysteine and folate serum concentrations before and after folate treatment, as well as for the presence of the MTHFR C677T polymorphism.**

***Results*—The allele frequency of C677T polymorphism in the MTHFR gene in the study population (0.33) was not different to that in controls (0.38). Before folate treatment the homocysteine concentration was raised in all groups; following folate supplementation it was significantly decreased in the CC and CT groups, but not in the TT group. In patients with CC genotype, serum homocysteine correlated with serum creatinine and cholesterol, and time since transplantation before treatment.**

***Conclusion*—Folate supplementation appears to be an effective strategy to normalise total homocysteine concentration in renal transplanted children and adolescents.**

(*Arch Dis Child* 2001;**85**:47–49)

Keywords: renal transplantation; homocysteine; folate; MTHFR gene polymorphism

First Department of Pediatrics, Semmelweis University, Budapest Bókay 53, 1083-Hungary
A J Szabó
A Szabó
Á Vannay
A Fekete
Z Süveges
G S Reusz

Research Laboratory of the Hungarian Academy of Sciences
T Tulassay

Department of Pediatrics, Medical University, Pécs, Hungary
B Melegh

Heim Pál Children's Hospital, Budapest, Hungary
T Szabó

Correspondence: Dr A J Szabó, H-1083 Budapest, Bókay u. 53, Hungary
szabat@gyer1.sote.hu

Accepted 30 January 2001

Cardiovascular morbidity in renal transplant recipients is high and remains the leading cause of mortality in these patients. Recently, hyperhomocysteinaemia has been implicated as an independent risk factor for the development of arteriosclerotic lesions.[1]

Serum and total plasma homocysteine concentrations are increased in incipient renal failure and any further decline in renal function.[2] Recently, it has been reported that treatment with cyclosporin A also induces an increase in homocysteine concentration.[3]

Supplementation with folate has been shown to represent a new therapeutic approach for lowering homocysteine concentration.[4] A common genetic variant C677T of the gene encoding for the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) was recently described.[5] This polymorphism has also been shown to correlate with raised total plasma homocysteine concentrations.[6]

The aims of the present study were therefore: (1) to measure total homocysteine concentration before and after folate treatment; (2) to investigate the prevalence of C677T MTHFR gene polymorphism; and (3) to examine the relation between C677T MTHFR gene polymorphism and the effect of folate treatment in kidney transplanted children and adolescents.

Patients and methods

Thirty patients (15 girls; age range 9.4–28.1 years; mean (SD) BMI 20.2 (4.4) kg/cm^2; mean (SD) serum creatinine 142 (30) μmol/l) participated in the study.

Patients had been on haemodialysis for a mean of 2.5 years (range 0.5–5.5 years) prior to transplantation. The mean age of the grafts was 5.9 years at the time of study (range 1.1–12.9 years). Basic immunosuppression consisted of combined cyclosporin A (3.6 (0.8) mg/kg/day, trough level 100–250 ng/ml) and low dose (0.12 (0.2) mg/kg/day) methylprednisolone therapy. Ten patients were on triple therapy, receiving additional azathioprine (1.8 (0.4) mg/kg/day). All patients had stable renal function; no rejection episodes had occurred for at least six months prior to this study.

Total homocysteine concentrations (free and protein bound) were determined by fluorescence polarisation immunoassay using the IMX analyser (Abbott Diagnostics Division). Serum concentrations of folate and vitamin B_{12} were measured by multiparticular enzyme immunoassay with the AXSYM analyser (Abbott Diagnostics Division). Blood cyclosporin A trough concentrations were measured by radioimmunoassay (Cyclo-Trac, Incstar, Stillwater). After the baseline measurement oral folate supplementation was started at a dose of 9 mg/day. Following six weeks of supplementation the same parameters were measured again.

Identification of C677T polymorphism in the MTHFR gene was performed as described by Frosst and colleagues.[5] One hundred blood samples from healthy Hungarian subjects were used as controls.

For statistical analysis the χ^2 test, analysis of variance, and Pearson's correlation were performed using the Graphpad Prism 2.01 software package.

The study conformed to the Helsinki declaration and was approved by the local ethics committee.

Results

The allele frequency of the C677T transition was 0.33, which is similar to that previously reported in renal transplant donors (0.34), recipients (0.35),[7] and in our control patients (0.38). However, the genotype distribution in

Table 1 Serum concentrations of total homocysteine, folate, and vitamin B_{12} in renal transplant recipients

MTHFR C677T gene polymorphism	*Before folate treatment*	*After folate treatment*	*p value*
Homocysteine (µmol/l)			
CC	16.3 (7.7)	9.7 (3.3)	<0.01
CT	20.1 (18.4)	10.3 (3.3)	<0.05
TT	19.6 (11.7)	10.8 (4.4)	NS
Folate (ng/ml)			
CC	8.2 (4.3)	17 (3.6)	<0.01
CT	5.3 (4.8)	16.4 (4.8)	<0.01
TT	7.8 (3.2)	15 (4.9)	<0.05
Vitamin B_{12} (pg/ml)			
CC	388 (187)	402 (202)	NS
CT	285 (175)	323 (191)	NS
TT	283 (107)	358 (129)	NS

Data are presented as mean (SD).
NS, non-significant.

the study group (CC 50%; CT 33.3%; TT 16.6%) was different compared to controls (CC 66%; CT 30%; TT 4%). In the case of TT genotype this difference was statistically significant ($p < 0.05$).

Within the three groups with different genotype there was no significant difference in any of the investigated parameters. MTHFR gene polymorphism had no influence on graft function. Genotype distribution was not significantly different between subgroups of patients with different aetiologies for end stage renal failure.

Table 1 shows serum concentrations of homocysteine, folate, and vitamin B_{12} in the renal transplant patients before and after folate treatment according to MTHFR C677T genotype. Before folate treatment the mean homocysteine concentration was raised in all groups, whereas the concentration of folate was in the normal range. Following folate treatment, folate concentration significantly increased in all groups, and total homocysteine decreased significantly in the CC and CT groups. In patients with TT genotype, total homocysteine decreased to normal, but this decrease was not statistically significant. There were no differences in vitamin B_{12} concentration according to MTHFR genotype or folate treatment. Table 2 shows the prevalence of borderline, moderate, or severe hyperhomocysteinaemia and borderline, moderate, or severe deficiency of folate or vitamin B_{12}.

In renal transplant recipients with CC genotype, homocysteine concentration correlated with serum creatinine ($r = 0.77$, $p < 0.01$), time since transplantation ($r = 0.60$, $p < 0.05$), and serum cholesterol ($r = 0.74$, $p < 0.01$) prior to folate treatment. Following administration of folate these correlations were no longer present. There was no correlation between serum homocysteine and any parameters measured in both groups with the T allele.

Table 2 Prevalence of no, borderline, moderate, or severe hyperhomocysteinaemia, and folate and vitamin B_{12} deficiency in renal transplant recipients

	Before treatment			*After treatment*		
MTHFR C677T gene polymorphism	*No*	*Borderline*	*Moderate or severe*	*No*	*Borderline*	*Moderate or severe*
Homocysteine (µmol/l)	<12	12–15	>15	<12	12–15	>15
CC	4 (26.6)	3 (20)	8 (53.3)	12 (80)	1 (6.6)	2 (13.3)
CT	3 (30)	2 (20)	5 (50)	6 (60)	4 (40)	0 (0)
TT	1 (20)	1 (20)	3 (60)	3 (60)	1 (20)	1 (20)
Folate (ng/ml)	>5.3	3.7–5.3	<3.7	>5.3	3.7–5.3	<3.7
CC	8 (53.3)	7 (46.6)	0 (0)	15 (100)	0 (0)	0 (0)
CT	2 (20)	2 (20)	6 (60)	9 (90)	1 (10)	0 (0)
TT	3 (60)	2 (40)	0 (0)	5 (100)	0 (0)	0 (0)
Vitamin B_{12} (pg/ml)	>187	157–187	<157	>187	157–187	<157
CC	14 (93.3)	1 (6.6)	0 (0)	15 (100)	0 (0)	0 (0)
CT	6 (60)	3 (30)	1 (10)	10 (100)	0 (0)	0 (0)
TT	4 (80)	0 (0)	1 (20)	5 (100)	0 (0)	0 (0)

Percentages are in brackets.

Discussion

In accordance with studies in adult patients, our results showed an increased homocysteine concentration children and adolescents who had undergone transplantation. It is well known that treatment with folate lowers serum homocysteine in renal transplant recipients or patients on dialysis.[4] Daily oral administration of 9 mg folate was highly effective in correcting the hyperhomocysteinaemia of our patients. This supplementation seems to be an effective and safe strategy that leads to normalisation of homocysteine concentration in the majority of these subjects; this may reduce cardiovascular morbidity and mortality after renal transplantation.

The metabolic pathway of homocysteine requires the enzyme MTHFR for conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the active form of folate. Recent studies clearly showed that homozygosity for the C677T transition in the MTHFR gene of patients on dialysis or after renal transplantation results in a further increase of homocysteine concentrations.[8] However, the polymorphism of the MTHFR gene does not seem to be a clinically important determinant of renal graft survival.[7]

In our study the allelic frequency of MTHFR gene polymorphism in renal transplant recipients was comparable to that found in other investigations,[6] and did not differ from that in the healthy population. There was no correlation between primary renal disease and the MTHFR genotype. We could not show the influence of MTHFR gene polymorphism on homocysteine or folate concentrations, although homocysteine concentrations were slightly higher, and folate concentrations lower in patients with the T allele. The lack of correlation between homocysteine and folate concentrations and MTHFR C to T transition might be a result of the relatively small number of patients included in the study. Interestingly, the percentage of patients with moderate or severe hyperhomocysteinaemia or folate deficiency did not differ between the groups with respect to MTHFR genotype.

Our findings also suggest that MTHFR polymorphism has no influence on the effect of folate administration. The role of impaired renal function and serum cyclosporin A and folate concentration as a determinant of homocysteine concentrations has been previously confirmed.[3] In our study population there was a significant effect of serum creatinine, cholesterol, and time after transplantation on homocysteine concentration in renal graft recipients with CC genotype. In subjects with one or two mutated alleles there was no

correlation between homocysteine concentration and the observed parameters.

Hyperhomocysteinaemia has been described as an additional risk factor for cardiovascular disease in renal transplant patients. This may be a result of the fact that homocysteine induces endothelial activation of factor V, reduces protein C activator production by arterial and venous endothelial cells, and induces endothelial cell injury in vitro.

In summary, we believe this to be the first investigation into homocysteine concentration, treatment of hyperhomocysteinaemia, and prevalence of MTHFR polymorphism in renal transplanted children and adolescents. The increased total homocysteine in our patients was reduced by oral folate administration. MTHFR genotype had no significant influence on the treatment of hyperhomocysteinaemia. Based on these results, folate supplementation appears to be an effective strategy for normalisation of total homocysteine concentrations in renal transplanted children and adolescents.

Parts of the study were supported by the OTKA F 029782/029779 and FKFP 0134/01, ETT 157-299/200, and OMFB TeT 5/98. Attila J Szabo is a research fellow, supported by the Magyary postdoctorial fellowship.

1 Stampfer MJ, Malinow MR, Willet WC, *et al*. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA* 1992;**268**:877–81.
2 Soria C, Chadefaux B, Coude M, *et al*. Concentrations of total homocysteine in plasma in chronic renal failure. *Clin Chem* 1990;**36**:2137–8.
3 Armadottir M, Hultberg B, Vladov V, *et al*. Hyperhomocysteinemia in cyclosporione-treated renal transplant recipients. *Transplantation* 1996;**61**:509–12.
4 Schröder CH, de Boer AW, Giesen AM, *et al*. Treatment of hyperhomocysteinemia in children on dialysis by folic acid. *Pediatr Nephrol* 1999;**13**:583–5.
5 Frosst P, Blom HJ, Milos R, *et al*. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;**10**:111–13.
6 Födinger M, Wölfl G, Fischer G, *et al*. Effect of MTHFR 677C>T on plasma total homocysteine levels in renal graft recipients. *Kidney Int* 1999;**55**:1072–80.
7 Liangos O, Kreutz R, Beige J, *et al*. Methylenetetrahydrofolate-reductase gene C677T variant and kidney-transplant survival. *Nephrol Dial Transplant* 1998;**13**:2351–4.
8 Födinfer M, Mannhalter C, Wölfl G, *et al*. Mutation (677 C to T) in the methylenetetrahydrofolate reductase gene aggravates hyperhomocysteinemia in hemodialysis patients. *Kidney Int* 1997;**52**:517–23.