



Hyperhomocysteinemia and MTHFR C677T and A1298C Polymorphisms are Associated With Chronic Allograft Nephropathy in Renal Transplant Recipients

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ABSTRACT

Hyperhomocysteine has been reported to be an important risk factor for the development of atherosclerosis. Identification of risk factors, such as hyperhomocysteinemia, is crucial for a better understanding of the events that lead to degenerative processes in the vascular system and for a correct understanding of the potential role of methylene-tetrahydrofolate reductase enzymes (MTHFR) to help in the treatment of vascular disease observed in chronic allograft nephropathy (CAN). In this study we analyzed the plasma homocysteine concentrations and MTHFR C677T and A1298C polymorphism frequencies among 110 renal transplant recipients (53 with CAN and 57 with normal renal function). All recipients had undergone renal transplantation at least 12 months prior to this investigation to establish a possible correlation with the posttransplant outcome. Plasma homocysteine concentrations were measured by liquid chromatography-tandem mass spectrometry and MTHFR polymorphisms were investigated by the PCR-RFLP technique. The results demonstrated that in renal transplant recipients, hyperhomocysteinemia in addition to the presence of the allelic variants for both MTHFR polymorphisms (677T/1298C) might play a role as an additional risk factor for CAN. We understand that analysis of these polymorphisms might have a role in the CAN process. Therefore, studies to evaluate their presence in renal transplant patients may be extremely useful to individualize immunosuppressive protocols to inhibit or retard the progression of CAN.

CHRONIC ALLOGRAFT NEPHROPATHY (CAN) remains the main cause of loss of renal function in patients undergoing renal transplantation.¹ Recently, vascular disease in renal transplant patients has been suggested to have a similar pathophysiology as vascular aging and atherosclerotic processes.^{2,3} Therefore, genes whose enzymes are linked to these processes, such as methylenetetrahydrofolate reductase (MTHFR) may be important in the pathogenesis of CAN.⁴⁻⁶ The MTHFR enzyme participates in the metabolic degradation of homocysteine, an amino acid derived from protein catabolism. Avoidance of hyperhomocysteinemia is important since it seems to be linked to premature arteriosclerosis and other endothelium degenerative events.^{3,7} A moderate increase of total homocysteine (tHcy) plasma concentration is commonly associated with a switch of cytosine to thymine at nucleotide 677, the region defining the MTHFR gene, resulting in the substitution of alanine for valine within the protein.³ Another MTHFR

gene polymorphism, a substitution of adenine for cytosine at the 1298 nucleotide has also been associated with decreased enzymatic activity⁸ glutamic acid for alanine. In this study, in addition to homocystein levels, we also analyze the frequencies of MTHFR C677T and A1298C polymorphisms as well as homocysteine levels seeking to identify

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genetic biomarkers causing susceptibility to the development of CAN.

SUBJECTS AND METHODS

All patients included in this retrospective cross-sectional study were recruited from September 2002 to March 2004. Inclusion criteria included a time since transplantation of at least 12 months and written informed consent. The study protocol was approved by the National Ethics Committee. The cohort included 110 renal transplant recipients: 40 women, 70 men of mean age $42.0 \pm$ SD 12.4 years and time since transplantation of $6.0 \pm$ 3.9 years. Patients were classified according to their values of 24-hour proteinuria and serum creatinine (sCr): Group I recipients displayed renal function (NRF) with persistent sCr measurements ≤ 1.5 mg/dL and proteinuria lower than 500 mg/24 hours during the follow-up period. The group included 26 women, and 31 men of mean age $41.3 \pm$ SD 13.2 years and time since transplantation of $6.5 \pm$ 4.2 years. Group II recipients displayed CAN with persistent sCr > 1.5 mg/dL and proteinuria ≥ 500 mg/24 hours. This group included 14 women and 39 men of mean age $42.8 \pm$ 11.6 years and time since transplantation of $5.5 \pm$ 3.5 years.

Blood was collected after overnight fasting into tubes containing EDTA, immediately put on ice, and centrifuged within 10 minutes at 18°C at 3000 rpm. The supernates were stored in aliquots at -80°C until the assay. Plasma tHcy levels were determined by liquid chromatography-tandem mass spectrometry (LS-MS/MS).^{9,10} Hyperhomocysteinemia was defined as tHcy values above 15 $\mu\text{mol/L}$.¹¹

Genomic DNA was isolated from peripheral blood according to the method described by Abdel-Rahman et al.¹² MTHFR (C677T, A1298C) Polymorphisms were identified by restriction fragment length polymorphism analysis.¹³ Statistical evaluations performed using the Minitab for Windows - Release 12.22 computer program included mean values \pm SD for continuous data and percentages for categorical data. The prevalences of the different genotypes were compared by tests for two independent proportions by normal approximations. Differences in tHcy concentrations were analyzed by the Mann-Whitney test. Univariate logistic regression was used to assess the risk of CAN among the hyperhomocysteinemic and among genotype combinations of allelic variants 677CT/1298AC; 677CT/1298CC or 677TT/1298AC genotypes versus other genotype combinations, employing odds ratios (ORs) and 95% confidence

limits (CL). *P* less than .005 was considered to indicate statistical significance.

RESULTS

The allelic frequencies of MTHFR 677T and MTHFR 1298C among CAN patients were, 0.41 and 0.35 versus 0.38 and 0.25 among patients with NRF, respectively. The most frequently observed genotypes in both groups were 677CT/1298AA (29.1%) and 677CT/1298AC (32.7%). There were no significant differences in the allelic and genotype distributions between the CAN versus NRF groups of patients. Hyperhomocysteinemia was observed in 48 (90.5%) patients with CAN versus 44 (72%) NRF patients (*P* = .026). Furthermore, the mean levels of plasma tHcy were significantly higher in CAN than NRF patients, namely, 37.0 $\mu\text{mol/L}$ (9.0–183.0) versus 24.3 $\mu\text{mol/L}$ (6.3–170.0; *P* < .0001). The distribution of the eight genotype combinations in the two groups and tHcy plasma levels are shown in Table 1.

The presence of the allelic variants for both MTHFR polymorphisms (677CT/1298AC; 677CT/1298CC and 677TT/1298AC genotypes) was associated with an elevated level of homocysteinemia (> 15.0 $\mu\text{mol/L}$; hyperhomocysteinemia) significantly more frequently in the CAN group (*P* = .005). Logistic regression analysis also revealed a significant risk associated with CAN (ORs = 2.97; 95% CL 1.26 to 6.98).

DISCUSSION

Only a few studies have addressed the effect of MTHFR genotypes on the Hcy metabolism in renal transplant recipients.^{4,5,14–16} These data have documented that MTHFR 677TT/1298AA and 677CT/1298AC genotypes influence Hcy concentrations in kidney graft recipients.⁴ Our study demonstrates that among renal transplant recipients, hyperhomocysteinemia was associated with the presence of the allelic variants of both MTHFR polymorphisms (677T/1298C) and seemed to play a role as an additional risk factor for CAN. The 92/110 (83.6%) incidence of hyperhomocysteinemia patients observed here was similar to that previously reported (90%) in renal transplant recipients.⁵

Table 1. MTHFR (C677T and A1298C) Genotype and Total Homocysteine Plasma Levels in Renal Transplanted Patients With Chronic Allograft Nephropathy and Normal Renal Function

MTHFR Genotype	Number of Subjects with CAN and (NRF)	Median of tHcy Plasma in CAN and (NRF) Patients	Number of Subjects With tHcy Level < 15 $\mu\text{mol/L}$ with CAN and (NRF)	Number of Subjects With tHcy Level > 15 $\mu\text{mol/L}$ with CAN and (NRF)
CC/AA	4 (9)	28.1 (19.1)	0 (2)	4 (7)
CC/AC	7 (7)	23.2 (16.4)	1 (3)	6 (4)
CC/CC	3 (2)	21.7 (19.2)	1 (0)	2 (2)
CT/AA	14 (18)	41.3 (23.0)	2 (2)	12 (16)
CT/AC	20 (16)	33.5 (20.0)**	1 (5)	19 (11)
CT/CC	1 (0)	32.3 (0)	0 (0)	1 (0)
TT/AA	2 (4)	87.7 (77.8)	0 (0)	2 (4)
TT/AC	2 (1)	81.8 (13.7)	0 (1)	2 (0)
Total	53 (57)	37.0 (24.3)*	5 (13)	48 (44)**

CAN: chronic allograft nephropathy; NRF: normal renal function.

Patients with NRF given in parentheses.

**P* < .0001.

***P* < .05.

In summary, to our knowledge, these data are the first to suggest that elevated Hcy levels in addition to the presence of the allelic variants 677T/1298C are significant predictors of CAN. Thus, it would be of great relevance to intensify studies in this area to identify possible genetic biomarkers involved in the development of CAN.

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