Brief Report

Methylenetetrahydrofolate-reductase gene C677T variant and kidney-transplant survival

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Abstract

Background. Hyperhomocysteinaemia, a risk factor for atherosclerosis, is common in haemodialysis and renal-transplant patients. As atherosclerotic lesions in hyperhomocysteinaemia resemble those of chronic allograft injury, we examined the hypothesis that the C677T variant of the methylenetetrahydrofolate reductase (MTHFR) gene, which is linked to elevated plasma homocysteine levels in patients with renal failure, determines renal allograft survival.

Methods. DNA was prospectively collected from 336 patients undergoing renal transplantation in our clinic between 1988 and 1994 and their corresponding donors. Patient and allograft survival was analysed by blinded review of all case records over a follow-up period of 36 months. Additionally, we recruited 83 patients surviving with a functional kidney allograft for at least 10 years (mean: 156, range 120–240 months). MTHFR-C677T genotype was determined by a PCR-RFLP technique. The influence of genotype on transplant survival was analysed by Kaplan–Meyer life-table analysis and two-tailed global log-rank testing.

Results. Frequency of the MTHFR-C677T allele in the cohort group was identical in recipients (0.35) and donors (0.34), and comparable to that in the long-term allograft survivors (0.37). Furthermore, life-table analysis revealed a similar allograft survival over 36 months between the genotype groups (CC 74%, CT 69%, TT 75%). Other risk factors including donor and recipient age, hypertension, body-mass index, and number of rejection therapies were evenly distributed between the different genotype groups.

Conclusions. These findings do not support the hypothesis that the C677T variant of the MTHFR gene is an important determinant of renal-transplant survival.

Key words: gene mutation; homocysteine; MTHFR gene; kidney transplantation; renal failure; cardiovascular disease

Introduction

Chronic allograft rejection, characterized by progressive loss of function and sclerotic vascular lesions in the transplant biopsy, is the most important determinant of long-term kidney transplant survival [1,2]. Furthermore, cardiovascular morbidity in renal-allograft recipients is high and remains the leading cause of premature mortality in these patients [3,4]. It has been known for nearly two decades that homocysteine (Hcy), a small sulphur-containing amino acid, is markedly elevated in patients on maintenance haemodialysis [5] and in renal transplant recipients [6]. Recently, hyperhomocysteinaemia has been implicated as an independent risk factor for the development of atherosclerotic lesions both in patients with normal [7,8] and impaired renal function [9,10]. Interestingly, the vascular lesions found in patients suffering from homocysteinuria or other conditions with elevated plasma Hcy are characterized by fibromuscular thickening of small arterial vessels [11] and resemble those found in chronic allograft injury [1,12,13]. Clearly this raises the question of whether elevated plasma Hcy adversely influences long-term renal graft survival by promoting vascular sclerosis in the kidney allograft.

In 1995, a common genetic variant of the gene encoding for methylenetetrahydrofolate-reductase (MTHFR), a cytosolic flavoprotein involved in the enzymatic remethylation and therefore elimination of Hcy, was described [14]. This variant, consisting of a cytosine (C) to thymidine (T) transition at nucleotide position 677 leading to the exchange of a highly conserved alanine to valine in the mature protein, has been associated with reduced activity and increased thermolability of this enzyme in lymphocyte extracts [15]. The presence of this mutation has also been shown to correlate with elevated total plasma Hcy.
both in patients with normal [14,16] or impaired [17] kidney function. Recently several authors have reported an association between this genetic variant and the development of coronary vascular disease [14,15,18]. It is therefore conceivable that this genotype may also be an important determinant of hyperhomocysteinaemia in patients undergoing transplantation for end-stage renal failure, and may thereby contribute to the development of atherosclerotic lesions, including those found in the renal allograft.

The aim of the present study was therefore to examine the relationship between the MTHFR-C677T genotype and renal allograft survival in kidney transplant recipients. As MTHFR is highly expressed in the kidney [19], and impairment of intra-renal Hcy metabolism may contribute to the development of hyperhomocysteinaemia, we also studied the effect of the donor MTHFR-C677T genotype on transplant survival.

Subjects and methods

Study population

Cohort study

DNA was prospectively collected from all Caucasian patients (n = 336; 134 female, age: 43.5 ± 13.2 years) receiving first time cadaveric kidney transplantation between 1988 and 1994 at our clinic. Consent for collection of DNA samples for genetic analyses and permission to review the medical records regarding outcome variables was obtained from all patients. Genomic DNA was extracted from all recipients and their respective donors. The clinical course for the first 36 months following transplantation was retrospectively analysed by blinded review of all case records with regard to graft loss as previously reported [20,21]. The number of antihypertensive drugs administered (excluding diuretics) was used as a surrogate measure for the severity of arterial hypertension (1.15 ± 1.1 drugs/patient).

Long-term survivors

In order to examine the relationship between MTHFR genotype and long-term allograft survival, we also identified 124 patients who underwent kidney transplantation at the Renal Transplantation Centre of our hospital and are currently surviving with a functional graft of at least 10-years duration. Of these, 117 were identified as currently living in Berlin, and 83 of these agreed to participate in this study. Following informed consent for genetic studies, DNA samples were obtained from these patients.

Genotyping

MTHFR genotype was determined as previously described [22]. In brief, recipient and donor DNA was extracted from peripheral leukocytes by a selective preparation method (Quiagen, Hilden, Germany). Reagent concentrations in the 10 µl PCR reaction were 600 nmol/l each for sense (5'-CAAG CCA CCC CGA AGC-3') and reverse (5'-AGG ACG GTG CGG TGA GAG TG-3') primers, 200 µmol/l deoxy-nucleotide triphosphates, 1.5 mmol/l MgCl₂ and 0.375 units of Taq DNA-polymerase. Samples were amplified for 35 cycles consisting of denaturation at 94°C for 15 s, annealing at 58°C for 45 s and extension at 72°C for 40 s, followed by a final extension step at 72°C for 5 min. The resulting amplification product (246 bp) was digested with Hinf I restriction endonuclease (New England Biolabs, Schwalbach/Ts., Germany), according to the manufacturer’s instructions. Following digestion, restriction fragments (175 bp and 71 bp) were size fractionated on 2% agarose gels. Genotype analysis was carried out by two independent investigators (OL and RK) who were unaware of the clinical data. Wherever there was any ambiguity, the PCR reaction, Hinf I digestion, and scoring were repeated.

Statistical analysis

In the cohort study, the relationship between MTHFR genotype and allograft survival was examined using Kaplan–Meier life-table analysis and two-tailed global log-rank testing as previously reported [20,21]. Patient deaths were also counted as graft loss. Based on a two-by-two contingency table estimating a χ²-value corresponding to an odds ratio of two for transplant loss associated with the TT genotype, our cohort study had a statistical power of 0.86. The relationship between MTHFR genotype and long-term allograft survival was analysed by comparing the genotype distribution and allelic frequencies between the long-term survivors and the subset of patients in the cohort study whose allograft survival was less than 36 months (n = 108). Data are reported as mean ± SD.

Results

Cohort study

The allelic frequency for the C677T mutation was almost identical between the recipients (qT = 0.35) and donors (qT = 0.34), and similar to that previously reported in healthy Caucasians (qT = 0.38) [14]. Allelic distribution was in Hardy–Weinberg equilibrium in all groups. Genotypic distribution was not significantly different between subgroups of recipients with different aetiologies for end-stage renal failure, of which diabetic nephropathy (11.3%, qT = 0.03) and chronic glomerulonephritis (31.5%, qT = 0.32) comprised the largest groups. The 36-month cumulative graft survival according to recipient genotype was 74% (CC), 69% (CT), and 75% (TT) respectively, with no statistically significant difference between the groups (P > 0.1).

Life-table analysis revealed virtually identical allograft survival in all groups regardless of recipient or donor genotype (Figure 1). Risk factors influencing graft survival (hypertension, recipient and donor age, body mass index, and number of rejection therapies) were likewise similar between the genotype groups (Table 1).

Long-term survivors

Genotype distribution in long-term renal allograft survivors with an average graft survival of 156 months
Fig. 1. Relationship between graft survival and recipient (left panel) and donor (right panel) MTHFR-C677T genotype.

Table 1. Patient characteristics by genotype in renal-transplant recipients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gender (m/f)</th>
<th>Recipient age (kg/m²)</th>
<th>Donor age (kg/m²)</th>
<th>Recipient BMI (kg/m²)</th>
<th>Antihypertensive drugs (n)</th>
<th>Rejection therapies (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (336)</td>
<td>104/132</td>
<td>43.5 ± 13.2</td>
<td>41.7 ± 16.2</td>
<td>22.9 ± 4.2</td>
<td>1.2 ± 1.1</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td>CC (140)</td>
<td>89/51</td>
<td>43.3 ± 13.7</td>
<td>41.0 ± 16.6</td>
<td>22.7 ± 4.5</td>
<td>1.2 ± 1.2</td>
<td>0.8 ± 0.9</td>
</tr>
<tr>
<td>CT (160)</td>
<td>93/67</td>
<td>44.1 ± 12.4</td>
<td>42.7 ± 15.9</td>
<td>23.3 ± 3.9</td>
<td>1.1 ± 1.1</td>
<td>0.7 ± 0.8</td>
</tr>
<tr>
<td>TT (36)</td>
<td>22/14</td>
<td>42.9 ± 14.4</td>
<td>42.7 ± 14.8</td>
<td>22.1 ± 3.8</td>
<td>1.3 ± 1.0</td>
<td>0.8 ± 1.0</td>
</tr>
</tbody>
</table>

Mean ± SD.

(range 120–240) (CC = 30, CT = 44, TT = 9, qT = 0.37) was similar to that in the control group who had an allograft survival of less than 36 months (CC = 43, CT = 54, TT = 11, qT = 0.35), and comparable both to the donor and recipient genotype distribution in the cohort study.

Discussion

Our results clearly show that the MTHFR-C677T variant encoding for a thermolabile isoform of this enzyme is not a clinically important determinant of short or long-term renal allograft survival. Thus lifestyle analysis revealed a similar transplant survival over the 36-month follow-up period regardless of donor or recipient genotype. Furthermore the virtually identical frequency of the T allele in long-term survivors suggests that this variant is not an important predictor of long-term allograft or patient survival in patients undergoing renal transplantation for end-stage renal failure. The similar distribution of the MTHFR-C677T genotype in donors and recipients, and the fact that this distribution was similar to that previously reported in healthy Caucasian controls [14], also demonstrates that the presence of the TT genotype is not a risk factor for the development of end-stage kidney disease.

The rationale for studying the relationship between the MTHFR-C677T genotype and renal allograft survival was provided by the following: (i) non-immunological factors are believed to play an important role in the development and progression of chronic allograft injury [2,23]. (ii) The histopathological changes in chronic transplant rejection are characterized by wall thickening of small arterial vessels due to smooth muscle proliferation and increased synthesis of extracellular matrix [13,24], changes that could be accelerated by Hcy which is known to have a dose dependent effect on vascular smooth muscle cell proliferation in vitro brought about by induction of cyclin synthesis [25]. (iii) Hyperhomocysteinaemia is a well-established risk factor to the development of atherosclerotic lesions in a variety of vascular beds both in patients with normal [7,8,26] and impaired renal function [9,10]. Finally, (iv) plasma total homocysteine levels are known to be elevated in patients with renal failure [5,27] and are in part, determined by the MTHFR-C677T genotype [17]. Our findings, however, do not support this line of reasoning and do not provide evidence for a role of this genetic variant of the MTHFR gene as a marker of renal allograft survival.
It is, however, important to note that the lack of a relationship between the MTHFR-C677T genotype and renal allograft survival does not rule out a role for hyperhomocysteinemia as a determinant of chronic transplant rejection. By design, plasma Hcy levels were not measured in our patients and thus, we do not know whether MTHFR-C677T genotype is an important determinant of plasma Hcy levels in these renal transplant recipients. Other factors including folate, vitamin B₆ or B₁₂ status could be more important determinants of plasma Hcy levels than the MTHFR-C677T genotype [19,28]. In fact, there is some evidence that the effect of MTHFR-C677T genotype on plasma Hcy levels is more pronounced in patients with suboptimal levels of folate [16]. It can also not be ruled out that other determinants of chronic transplant rejection including delayed acute-rejection episodes, hypertension, proteinuria, infection, delayed graft function, or obesity may have stronger effects on transplant survival [23,29,30], thereby masking any effect attributable to the MTHFR-C677T genotype in this setting.

Nonetheless, identification of additional non-immunological factors contributing to accelerated chronic graft rejection remains an important issue both from the basic research and the clinical point of view, as knowledge about the underlying mechanisms could open up new arenas for therapeutic intervention. Our study, however, does not support the hypothesis that the C677T mutation of the MTHFR gene represents an important risk factor for chronic rejection in patients undergoing renal transplantation for endstage-kidney failure.

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References


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