Major determinants of hyperhomocysteinemia in peritoneal dialysis patients

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Major determinants of hyperhomocysteinemia in peritoneal dialysis patients. The mechanisms leading to elevated total homocysteine concentrations in peritoneal dialysis patients are only partially understood. We show that a common polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene (C677T transition) results in increased total homocysteine levels in peritoneal dialysis patients compared to age- and sex-matched healthy individuals. The allelic frequency of the C677T transition in the MTHFR gene in peritoneal dialysis patients (0.29) was comparable to the frequency in healthy individuals (0.34). Separate comparison of the total homocysteine plasma levels between non-carriers of the MTHFR polymorphism (C/C), heterozygous (C/T) and homozygous (T/T) subjects was performed by analysis of covariance in the patient and the control group. In the patient group the mean total homocysteine level was 61.7 ± 40.1 μmol/liter in individuals with the (T/T) genotype, which was significantly higher than the total homocysteine concentration of 23.1 ± 15.8 μmol/liter in (C/T) patients and 22.2 ± 11.1 μmol/liter for non-carriers (P = 0.0001). Vitamin B12 (P = 0.0001), folate (P = 0.0005), serum creatinine (P = 0.016), albumin (P = 0.0157) and dialysis center (P = 0.0173) significantly influenced total homocysteine plasma levels in peritoneal dialysis patients, whereas this was not the case for age, gender, weekly Kt/V, weekly creatinine clearance, residual renal function, duration of dialysis, mode of peritoneal dialysis and vitamin intake. Folate levels in peritoneal dialysis patients were significantly affected by the MTHFR genotype (P = 0.016). Elevated total homocysteine levels in diabetic patients with cardiovascular disease were associated with increased cardiovascular morbidity. In summary, the present study provides evidence that homozgyosity for the C677T transition in the MTHFR gene, low vitamin B12 and low folate levels result in elevated total homocysteine levels in peritoneal dialysis patients.

Hyperhomocysteinemia is frequently observed in patients with chronic renal failure and represents an independent cardiovascular risk factor in these patients [1–5]. Recently, a common C to T mutation at nucleotide position 677 of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene was identified, which may cause elevated total homocysteine plasma levels on the basis of a decreased MTHFR activity and result in lower folate levels in (T/T) homozygous subjects [6–9]. In chronic hemodialysis patients the allelic frequency of 0.35 of the T allele has been shown to be in line with the frequency of 0.34 in individuals without renal failure [10]. The mean total homocysteine plasma level in hemodialysis patients homozygous for the (T/T) allele was 36.4 μmol/liter, which was 43% higher compared to total homocysteine concentrations of 25.4 μmol/liter in patients without mutation and was 3.8 times higher than the mean value of 9.6 μmol/liter in healthy non-carriers [10]. Thus far, the mechanisms underlying hyperhomocysteinemia in peritoneal dialysis patients have only been investigated either in small patient populations [3, 11–15] or in patient populations differing in dialysis procedure or ethnic origin [2, 4, 16].

We studied the influence of the (C677T) polymorphism in the MTHFR gene on total homocysteine levels as well as plasma folate concentrations in 154 patients maintained on peritoneal dialysis treatment at five peritoneal dialysis units in Austria. Additionally, we analyzed the impact of residual renal function, Kt/V, weekly creatinine clearance, age, gender, folate levels, vitamin B12 concentrations, creatinine levels, serum albumin concentrations, dialysis center, duration of dialysis, mode of peritoneal dialysis and vitamin intake on total homocysteine levels. Cardiovascular morbidity related to total homocysteine plasma levels was assessed using an arbitrary atherosclerosis score.

METHODS

Study population

One hundred fifty-four patients with end-stage renal failure treated at five peritoneal dialysis units in Austria were investigated. The age of these patients (69 females, 85 males) was 54.0 ± 15.1 years (mean ± sd). Dialysis treatment was initiated because of end-stage renal disease due to diabetic nephropathy (N = 47), shrunken kidneys of unknown etiology (N = 36), chronic glomerulonephritis (N = 27), chronic interstitial nephritis (N = 16), polycystic kidney disease (N = 10), nephrosclerosis (N = 10) and miscellaneous nephropathies in 8 cases. In 20 of the 154 patients
peritoneal dialysis was initiated because of secondary kidney graft failure. Twenty-seven patients previously treated with hemodialysis had been switched to peritoneal dialysis. The mean duration of peritoneal dialysis treatment was 1.46 ± 1.27 years. One hundred twenty-one patients were maintained on continuous ambulatory peritoneal dialysis (CAPD) and 33 were treated with automated peritoneal dialysis (APD), including 22 patients on continuous cyclic peritoneal dialysis (CCPD) and 11 patients on intermittent peritoneal dialysis (IPD). Twenty-six of the patients received low dose oral vitamin supplementation therapy routinely (consisting of low dose folate and/or low dose vitamin B₁₂ and/or low dose vitamin B₆). To calculate weekly Kt/V and creatinine clearance, each patient was instructed to collect 24-hour dialysate and urine samples at the same time-point.

The prevalence of cardiovascular complications was assessed according to Robinson and colleagues [3]. Coronary artery disease and cerebral artery disease was diagnosed if a stenosis of >50% was envisaged by angiography or duplex-sonography. Two arbitrary vascular disease scores (VDS 1 and VDS 2) were calculated, based on whether or not the patient suffered from arterial occlusive disease in the coronary, cerebral or peripheral vascular system (VDS 1; 1 point for each affected vascular system; possible score, range 0 to 3). The second score (VDS 2) was applied on the basis of the first score including one additional point for infarction/gangrene or vascular surgery or angioplasty in the different vascular systems (possible score, range 0 to 6). The patient characteristics are summarized in Table 1.

Age- and sex-matched healthy subjects (N = 154) with normal kidney function and without clinical evidence of cardiovascular disease were selected from 298 healthy volunteers in whom MTHFR-alleles, total homocysteine, folate and vitamin B₁₂ levels were available.

Written informed consent was given by all patients according to the Declaration of Helsinki and the Austrian Law on Gene Technology.

Biochemical assays
Fasting citrated blood was collected, immediately placed on ice and centrifuged within 60 minutes at 2000 g at 4°C (20 min). Plasma aliquots were snap frozen and stored at -70°C. Citrated blood was kept at -20°C for isolation of DNA. All laboratory investigations were performed at the Department of Laboratory Medicine at the University of Vienna.

Total (that is, free plus protein-bound) plasma homocysteine concentrations were determined by automated high-performance liquid chromatography (HPLC) with reverse-phase separation and fluorescent detection using a commercially available kit (Immundiagnostik, Bensheim, Germany) according to the method originally described by Araki and Sako [17]. Hyperhomocysteinemia was defined as total homocysteine levels above 15 μmol/liter.

Plasma folate (5-methyltetrahydrofolate) and vitamin B₁₂ levels were measured with a radioassay which allowed simultaneous determination of both vitamin concentrations in a single reaction tube (SimulTRAC-SNB, Becton Dickinson, Ontario, Canada). Folate deficiency was considered present at a plasma concentration of less than 3.4 nmol/liter. Vitamin B₁₂ deficiency was defined as a concentration of less than 118 pmol/liter, respectively.

Polymerase chain reaction analysis of MTHFR gene
Identification of the 677 C to T transition in the MTHFR gene was performed as previously described [6]. In brief, 500 μl of citrated blood was frozen twice (−70°C). Cellular DNA was obtained by thawing and boiling for 10 minutes, followed by centrifugation at 12,000 g (10 min). The supernatants were collected and 5 μl of a 1:10 dilution were used in a 50 μl polymerase chain reaction (PCR) reaction containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 1.25 mM MgCl₂, 0.2 mM of each nucleoside triphosphate, 30 pmol of each primer and 1.25 units of AmpliTaq DNA Polymerase (Perkin Elmer Cetus, Norwalk, CT, USA). PCR was performed using the primers described by Frost and colleagues 5′-TGAAGGAGAAGGTGTCGCGGA-3′ and 5′-AGGACGTTGCGGTGAGAGTG-3′ [6]. Thermocycling conditions consisted of 40 cycles of denaturation at 95°C for one minute, annealing at 60°C for one minute and extension at 72°C for one minute, preceded by an initial denaturation step at 95°C for three minutes and followed by a terminal extension of five minutes at 72°C. Two microliters of the 198-bp PCR product were subjected to HinfI digestion (0.5 Units enzyme in a 20 μl digest). The presence of a mutation creates a HinfI recognition sequence that leads to digestion of the 198 bp PCR product into fragments of 175 bp and 23 bp, respectively. Heterozygous subjects show three fragments (198 bp, 175 bp and 23 bp), whereas a homozygous C to T transition results in the production of two fragments of 175 bp and 23 bp. HinfI digests of PCR amplification products were analyzed by electrophoresis through 6% polyacrylamide gels (Novex, San Diego, CA, USA) followed by ethidium bromide staining.

Statistical methods
Comparison of the frequency of the different MTHFR alleles in peritoneal dialysis patients and the control group was performed by chi square test. Since plasma total homocysteine and folate measures were positively skewed, natural logarithmic transformation was used to normalize the distribution (natural logarithm of total homocysteine/folate concentrations, ln-total homocysteine/ln-folate). Descriptive statistics included mean values ± sd, geometric means, medians, full ranges and 10th to 90th percentile ranges for total homocysteine plasma levels, vitamin B₁₂ and folate levels. Separate comparisons of the total homocysteine plasma levels

<table>
<thead>
<tr>
<th>Table 1. Characteristics of 154 peritoneal dialysis patients</th>
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</thead>
<tbody>
<tr>
<td><strong>Sex female/male</strong></td>
</tr>
<tr>
<td><strong>Age years</strong></td>
</tr>
<tr>
<td><strong>Mean duration of peritoneal dialysis years</strong></td>
</tr>
<tr>
<td><strong>CAPD/APD patients</strong></td>
</tr>
<tr>
<td><strong>Mean weekly Kt/V</strong></td>
</tr>
<tr>
<td><strong>Mean total creatinine clearance liter/week</strong></td>
</tr>
<tr>
<td><strong>Residual renal clearance ml/min</strong></td>
</tr>
<tr>
<td><strong>Patients with vascular disease %</strong></td>
</tr>
<tr>
<td><strong>Diabetic patients %</strong></td>
</tr>
</tbody>
</table>

Data are given as means ± sd. Abbreviations are: CAPD continuous ambulatory peritoneal dialysis; APD automated peritoneal dialysis.

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**Table 2.** Genotypes and allelic frequency of the C677T MTHFR polymorphism in peritoneal dialysis patients and age- and sex-matched control subjects

<table>
<thead>
<tr>
<th>MTHFR (C677T)</th>
<th>Peritoneal dialysis patients (N = 154)</th>
<th>Control subjects (N = 154)</th>
<th>Diabetic patients (N = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T/T)</td>
<td>12 (7.8%)</td>
<td>21 (13.6%)</td>
<td>3 (6.4%)</td>
</tr>
<tr>
<td>(C/T)</td>
<td>65 (42.2%)</td>
<td>64 (41.6%)</td>
<td>21 (44.7%)</td>
</tr>
<tr>
<td>(C/C)</td>
<td>77 (50.0%)</td>
<td>69 (44.8%)</td>
<td>23 (48.9%)</td>
</tr>
</tbody>
</table>

Allelic frequency 0.29 0.34 0.29

There were no significant differences in the frequencies of different MTHFR genotypes or allelic frequencies between peritoneal dialysis patients, the subgroup of diabetic patients and control subjects.

between (C/C), (C/T) or (T/T) subjects were performed by analysis of covariance. The covariates were folate, vitamin B12, age, gender, albumin, serum creatinine, weekly Kt/V, weekly creatinine clearance, residual renal function, dialysis center, mode of peritoneal dialysis (CAPD or APD) and vitamin intake in peritoneal dialysis patients. Individual comparisons between the groups were conducted by post hoc analysis (Scheffe’s test).

For both the patient and the control group (factor: group) an analysis of covariance with the factors MTHFR [dummy variables: (C/C) versus (T/T) and (C/T) versus (T/T)] and group, the covariates age, gender, folate and B12 level, which were available for both groups (patients and controls), and the calculations for interaction terms group × MTHFR, group × age, group × gender, group × plasma folate levels and group × B12 levels on the total homocysteine plasma levels were performed.

A similar model was applied for the analysis of influence of MTHFR (dummy variables, see above) and group as factors, and the covariates age and gender and the interaction terms group × MTHFR, group × age, group × gender on plasma folate levels.

Unpaired comparisons of patient characteristics in subjects with and without cardiovascular disease and comparison of VDS 1 and VDS 2 in patients with total homocysteine levels above versus below the sample median or above versus below the cut off value of the normal range (≤ 15.0 μmol/liter) were performed by Student’s t-test (two tailed). All calculations were performed by the statistical software package SAS (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**MTHFR gene polymorphism**

In peritoneal dialysis patients the allelic frequency of the 677 C to T transition in the MTHFR gene was 0.29 and in age- and sex-matched healthy controls it was 0.34. Twelve of 154 patients (7.8%) showed a homozygous C677T mutation (T/T), 65 patients (42.2%) were heterozygous (C/T) and 77 patients (50.0%) did not carry this mutation (C/C). In the control group 21 of 154 healthy individuals (13.6%) were homozygous for the T allele, 64 (41.6%) were (C/T) and in 69 control subjects (44.8%) the mutation was not present. Comparison of the frequencies of the different MTHFR alleles in healthy subjects and peritoneal dialysis patients revealed no significant difference. The allelic frequency in the group of diabetic patients (0.29; N = 47; Table 2) was similar to the allelic frequency in non-diabetic patients.

**Total homocysteine plasma levels**

Total homocysteine plasma levels were transformed to natural logarithmic data to normalize the distribution. The mean total homocysteine level in peritoneal dialysis patients was 25.6 ± 19.8 μmol/liter versus 9.7 ± 3.3 μmol/liter in control subjects.

Forty-six of 154 peritoneal dialysis patients (29.9%) had total homocysteine levels ≤ 15.0 μmol/liter, in contrast to 145 of 154 healthy subjects (94.2%). Of these 46 patients 23 patients were (C/T) and 22 patients (C/C), respectively, only one was homozygous for the MTHFR gene variation, whereas in the control group 19 of 145 subjects with total homocysteine levels ≤ 15.0 μmol/liter were identified as homozygotes for the MTHFR mutation. The geometric mean total homocysteine levels in (T/T) patients were more than two times higher than total homocysteine levels of (C/T) patients and patients without the mutation (Table 3).

There was a significant influence of the MTHFR gene-polymorphism (P = 0.0001), vitamin B12 (P = 0.0001), folate (P = 0.0005), and vitamin C (P = 0.016), serum albumin levels (P = 0.0157) and dialysis center (P = 0.0173) on ln-total homocysteine plasma levels in peritoneal dialysis patients. The mean total homocysteine level was 61.7 ± 40.1 μmol/liter in (T/T) peritoneal dialysis patients [P < 0.05 vs. (C/T) and (C/C) patients] and 10.4 ± 5.7 μmol/liter in (T/T) controls. In (C/T) patients the mean total homocysteine concentration was 23.1 ± 15.8 μmol/liter versus 9.8 ± 2.9 μmol/liter in (C/T) controls. In (C/C) peritoneal dialysis patients the mean total homocysteine level was 22.2 ± 11.1 μmol/liter versus 9.4 ± 2.5 μmol/liter in (C/C) controls (Fig. 1). The (T/T) allele resulted in an estimated increase in total homocysteine levels of 127% (more than doubling) compared to the (C/T) and as much as 150% compared to the (C/C) patients. An increase of the vitamin B12 level of 100 pmol/liter resulted in an estimated decrease of total homocysteine levels of 8.9%, and an increase of folate levels of 10 mmol/liter resulted in a decrease of 4%. In contrast, an increase of serum albumin level of 1 g/liter resulted in an estimated increase of 2% and an increase of creatinine of 1 mg/dl resulted in an estimated increase of total homocysteine levels of 7%. The significant center effect in our analysis was due to relative many (T/T) patients with high total homocysteine plasma levels in one small center. The other covariates (weekly Kt/V, residual renal clearance, weekly creatinine clearance, age, gender, duration of dialysis, and vitamin intake) showed no significant influence on total homocysteine levels.

For the overall analysis of ln-total homocysteine levels of patients and controls, there was a significant influence of the factor group (P = 0.0001), of the covariates age (P = 0.0006) and gender (P = 0.0003), and significant interaction terms group × MTHFR [for (C/C) versus (T/T), P = 0.0001, for (C/T) versus (T/T), P = 0.0001], group × age (P = 0.0002), and group × B12 level (P = 0.0002). The total homocysteine levels were more than twofold higher in the patient group than in the control group. Furthermore, total homocysteine levels significantly varied among the MTHFR groups of the patient group and the control group. The most striking finding was that patients homozygous for the T allele had particularly high total homocysteine values (Table 3). The total homocysteine levels in the control group were increasing in older control people, whereas in the patient group the total homocysteine levels were nearly constant with advancing age. In the control group there was no influence of vitamin B12 levels on...
Vitamin B12 concentrations

The mean folate concentration was 22.9 ± 36.6 nmol/liter (normal range, 3.4 to 38 nmol/liter) in peritoneal dialysis patients versus 16.1 ± 8.2 nmol/liter in control subjects. For the analysis of folate levels the factor MTHFR (only the dummy (C/C) versus (T/T) was significant, \( P = 0.016 \) and group \( (P = 0.0146) \) were significant. This reflects that the behavior of the folate level is slightly different between control and patient group and between (C/C) and (T/T) mutated MTHFR alleles. The mean vitamin B12 concentration was 336.1 ± 242.1 pmol/liter in the patient group (normal range, 118 to 720 pmol/liter) versus 263.9 ± 184.7 pmol/liter in healthy subjects. Not one of the peritoneal dialysis patients or the healthy controls had a folate deficiency. Deficiency of vitamin B12 was identified in 9 of 154 patients (5.8%) and in 8 patients or the healthy controls had a folate deficiency. Deficiency of vitamin B12 was identified in 9 of 154 patients (5.8%) and in 8 of 154 control subjects (5.2%). The mean vitamin B12 level was 96.1 ± 17.7 pmol/liter in B12 deficient patients and 99.5 ± 12.0 pmol/liter in B12 deficient control subjects. The mean total homocysteine level was 35.0 ± 18.9 μmol/liter in this patient group and 11.6 ± 2.4 μmol/liter in the control individuals, including 5 patients and 3 controls with a (C/T) and 3 patients and 2 controls with a (T/T) genotype. Folate and vitamin B12 levels according to MTHFR subgroups are indicated in Table 3.

Cardiovascular disease and relation to total homocysteine levels

Sixty-seven of 154 patients had evidence of vascular disease (43.5%). In patients with cardiovascular disorders, the geometric mean total homocysteine level was 18.5 μmol/liter versus 22.8 μmol/liter in those without vascular disease (NS). The only significant differences between these two patient groups were the patients’ gender and age, creatinine and serum albumin levels and the number of diabetic patients (Table 4). Vascular disease scores were analyzed in all patients with cardiovascular disease and in vascular disease patients with and without diabetes mellitus. The mean VDS 1 and mean VDS 2 in patients with cardiovascular disease and in patients with and without diabetes mellitus with respect to total homocysteine concentrations are summarized in Table 5. Diabetic patients with total homocysteine levels above the sample median had a higher mean VDS 1 and VDS 2 compared to patients with total homocysteine levels below the sample median (Table 5).

**DISCUSSION**

The present study demonstrates that peritoneal dialysis patients who are homozygous for the C677T polymorphism in the
Table 4. Main characteristics of 154 peritoneal dialysis patients with and without cardiovascular disease

<table>
<thead>
<tr>
<th></th>
<th>With vascular disease</th>
<th>Without vascular disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients %</td>
<td>67 (43.5)</td>
<td>87 (56.5)</td>
</tr>
<tr>
<td>Gender female/male</td>
<td>23/44</td>
<td>46/44*</td>
</tr>
<tr>
<td>Age years</td>
<td>59.4 (60.6 ± 12.1)</td>
<td>46.5 (49.0 ± 15.2)*</td>
</tr>
<tr>
<td>Duration of peritoneal</td>
<td>0.9 (1.5 ± 1.4)</td>
<td>0.9 (1.4 ± 1.2)</td>
</tr>
<tr>
<td>Total homocysteine</td>
<td>18.5 (22.7 ± 18.9)</td>
<td>22.8 (27.8 ± 20.3)</td>
</tr>
<tr>
<td>MTHFR transition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with tHcy levels</td>
<td>24 (35.8)</td>
<td>22 (25.3)</td>
</tr>
<tr>
<td>&gt;15 μmol/liter %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate level μmol/liter</td>
<td>16.7 (22.3 ± 26.1)</td>
<td>17.4 (23.4 ± 43.1)</td>
</tr>
<tr>
<td>Vitamin B12 level μmol/</td>
<td>278.5 (330.3 ± 221.7)</td>
<td>276.8 (340.6 ± 257.9)</td>
</tr>
<tr>
<td>liter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin concentration</td>
<td>36.4 (37.0 ± 6.3)</td>
<td>39.6 (39.9 ± 4.8)*</td>
</tr>
<tr>
<td>gliter/μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine mg/dl</td>
<td>7.3 (7.5 ± 2.0)</td>
<td>8.1 (8.4 ± 2.0)*</td>
</tr>
<tr>
<td>Weekly Kt/V</td>
<td>2.1 (2.2 ± 0.4)</td>
<td>2.2 (2.3 ± 0.5)</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>71.7 (75.4 ± 25.6)</td>
<td>73.6 (78.3 ± 29.8)</td>
</tr>
<tr>
<td>liter/week</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as geometric means and means ± standard deviation in parentheses.
tHcy is total homocysteine.
*P < 0.05

The MTHFR gene have significantly higher total homocysteine plasma levels (61.7 ± 40.1 μmol/liter) than heterozygous patients (23.1 ± 15.8 μmol/liter) or non-carriers (22.2 ± 11.1 μmol/liter). Vitamin B12, folate, creatinine, albumin concentrations and dialysis center, but not age, gender, weekly Kt/V, weekly creatinine clearance, residual renal function, duration and mode of peritoneal dialysis and vitamin intake had a significant influence on plasma total homocysteine levels. The MTHFR polymorphism also significantly influenced plasma folate levels of peritoneal dialysis patients and healthy subjects. In diabetic patients with vascular disease the vascular disease morbidity was more pronounced if total homocysteine plasma levels above the sample median were present. The allelic frequency of the (C677T) transition in the MTHFR gene was not different between healthy subjects, all peritoneal dialysis patients and the subgroup of diabetic patients.

Elevated homocysteine levels were initially suspected in end-stage renal disease patients in 1972 by Robins and colleagues [18] and were later confirmed by others [19–22]. Subsequently, hyperhomocysteinemia has been shown to be associated with an increased frequency of atherosclerotic vascular disease and was established as an independent risk factor for cardiovascular complications in chronic renal failure patients [1–5, 23].

In the first study conducted in a small population of CAPD patients, Hultberg, Andersson and Sterner [11] compared total homocysteine levels in three different patient groups receiving folate and vitamin B12 substitution therapy. The median total homocysteine plasma level was 24.0 μmol/liter in 17 CAPD patients, 27.9 μmol/liter in 29 hemodialysis patients, and 23.5 μmol/liter in 30 chronic renal failure patients. In this report, the authors also studied the diurnal variations of total homocysteine levels in a subset of these patients and did not find a significant increase of total homocysteine levels following a meat-rich meal. Also, in another recent study by Janssen and associates no daytime variations of total homocysteine plasma concentrations in CAPD patients have been observed [13].

It is well established that folate supplementation therapy has a lowering effect on homocysteine concentrations [24, 25]. Total homocysteine plasma levels decreased from 50.2 ± 5.6 μmol/liter to 16.6 ± 1.9 μmol/liter during six weeks of folate therapy (5 mg/day) in 10 CAPD patients compared to a decrease of 55.7 ± 10.1 μmol/liter to 24.0 ± 2.0 μmol/liter in 10 hemodialysis patients [12]. After 12 and 26 weeks following cessation of folate substitution, total homocysteine levels gradually increased again but did not reach the pretreatment values [12]. Kim and coworkers [14] compared total homocysteine plasma levels in 12 CAPD patients, 5 hemodialysis patients and 10 healthy controls who did not receive vitamin substitution therapy. Total homocysteine plasma levels in these patients were 32.7 ± 18.5 μmol/liter, 25.2 ± 10.9 μmol/liter, and 14.0 ± 4.0 μmol/liter, respectively. The authors did not find a correlation with age, vitamin B6 levels, residual renal function, protein catabolic rate or Kt/V. However, a significant correlation with an arbitrary atherosclerosis score was observed.

Robinson and collaborators [3] investigated the risk of development of cardiovascular disease in 176 end-stage renal failure patients with hyperhomocysteinemia. They found an independent odds ratio of 2.9 (confidence interval 1.4 to 5.8, P < 0.007) for vascular complications in patients with a total homocysteine concentration in the upper two quintiles (> 27.8 μmol/liter). Hemodialysis patients (N = 130) in this study showed higher total homocysteine levels (29.5 ± 1.7 μmol/liter) than peritoneal dialysis patients (N = 46; 19.5 ± 1.7 μmol/liter, P < 0.01). Ninety-five percent of patients with vascular disease and 54% of patients without vascular disease were on hemodialysis treatment [3].

The pathogenesis leading to elevated homocysteine concentrations in renal failure patients is only partially understood. The kidney itself is supposed to play a substantial role in homocysteine metabolism, and hyperhomocysteinemia has been shown to progress in parallel with the decline of renal function [5, 11, 26–29] despite the negligible urinary excretion of homocysteine [30]. Bostom and colleagues demonstrated that the total homocysteine content in renal vein blood of rats is much lower compared to the total homocysteine concentration in the renal artery [30]. Based on this observation it was speculated that homocysteine might not reach the renal metabolic site in case of a decreased glomerular filtration rate. Furthermore, uremic toxins are supposed to contribute to hyperhomocysteinemia by inhibition of enzymes required for homocysteine metabolism. Perna and colleagues demonstrated that hyperhomocysteinemia can lead to intracellular accumulation of S-adenosyl-homocysteine, resulting in an impairment of remethylation pathways in uremia [31–33].

Besides age, gender and nutritional factors including vitamin status and albumin concentration [3, 4], a genetic defect in the MTHFR gene (C677T transition) was recently shown to result in elevated total homocysteine concentrations in homozygous hemodialysis patients [10]. The enzyme MTHFR represents an important cofactor involved in the homocysteine remethylation pathway. Decreased MTHFR activity results in inadequate production
of 5-methyltetrahydrofolate, which is required for the remethylation of homocysteine to methionine, with the consequence of elevated plasma total homocysteine concentrations [34, 35]. The frequency of the different MTHFR alleles in our patients was comparable to the frequency observed in age- and gender-matched healthy individuals.

Recently, the influence of the MTHFR polymorphism on total homocysteine plasma levels in subjects with normal renal function was shown to be limited to those having suboptimal folate levels [9]. Even though the homozygous (T/T) MTHFR polymorphism may lead to elevated total homocysteine levels in healthy subjects, the increase was not significant in this study.

In contrast, total homocysteine levels in (T/T) homozygous peritoneal dialysis patients were significantly higher (more than twofold) compared to (C/T) and (C/C) patients. This increase is much higher compared to the levels in hemodialysis patients [43% increase of total homocysteine levels in (T/T) versus (C/C) patients] [10]. This striking difference in total homocysteine concentrations of (T/T) hemodialysis and (T/T) peritoneal dialysis patients might be associated with, at least in part, the lower, but still normal folate and vitamin B12 levels observed in the group of (T/T) peritoneal dialysis patients.

In several studies hemodialysis patients had higher total homocysteine concentrations compared to peritoneal dialysis patients [3, 11, 14]. These data are in line with our own observation on hemodialysis patients showing a mean total homocysteine level of 28.7 ± 11 μmol/liter [10] compared to the mean total homocysteine level of 25.6 ± 19.8 μmol/liter for peritoneal dialysis patients of this study. Peritoneal dialysis patients presented with lower mean folate but higher mean vitamin B12 plasma levels compared to the hemodialysis patients from our previous study (folate levels, 22.9 nmol/liter vs. 26.6 nmol/liter; vitamin B12 levels, 336.1 pmol/liter vs. 276.9 pmol/liter, respectively). Therefore, separate analyses of the total homocysteine levels, of the influence of the (C677T) MTHFR polymorphism, and of cofactors involved in homocysteine metabolism, have to be performed in patients with different modes of renal replacement therapy. Furthermore, a separate investigation of cardiovascular risk factors, morbidity and mortality in hemodialysis and peritoneal dialysis patients appears to be mandatory for future studies.

There was a significant influence of vitamin B12, folate, creatinine, and albumin levels on total homocysteine plasma concentrations of peritoneal dialysis patients in the present study, which is in line with previous observations in renal failure patients [3, 4]. It can be speculated that total homocysteine concentrations may reflect the nutritional status because we observed a significant influence of albumin concentrations in our analyses. However, the distribution of patients with moderate hyperhomocysteinemia (30 to 100 μmol/liter) was comparable in patients with albumin levels between 30 and 40 g/liter and between 40 and 50 g/liter with respect to the different MTHFR alleles (Fig. 2). Low dose vitamin supplementation in some of the patients had no influence on total homocysteine concentrations in our study.

High dose folate therapy has been demonstrated to lower total homocysteine plasma concentrations in end-stage renal failure patients despite the commonly observed high folate levels of these patients, which is probably linked to intracellular folate deficiency [13, 16, 25]. A potential effect of low intracellular folate concentrations on total homocysteine plasma levels in our peritoneal dialysis patients cannot be ruled out. Nevertheless, there was a significant influence of folate plasma levels on total homocysteine concentrations in our analyses. The homozygous C677T MTHFR transition was recently shown to affect red cell folate and plasma folate concentrations in pregnant women and red cell folate levels in nonpregnant women [37]. Our study also demonstrates that the MTHFR polymorphism significantly influences plasma folate levels in peritoneal dialysis patients and healthy subjects (the median folate concentration was lower in (TT) subjects compared with (CT) and (CC) subjects; Table 3). Thus, low 5-methyltetrahydrofolate levels in individuals with the (T/T) MTHFR allele do not necessarily reflect a suboptimal folate intake, but may point to decreased formation of active folate resulting from the presence of a thermolabile MTHFR variant. This status leads to elevated total homocysteine levels due to decreased conversion of homocysteine to methionin, which might be overcome by increased folate intake [24, 25].

It can be hypothesized that the extent of hyperhomocysteinemia in dialysis patients might be influenced by geographical factors. Recently, a European-wide survey on total homocysteine levels in subjects without renal failure revealed significant variations in total homocysteine plasma levels in different cities and countries [36]. In this study genetic and nutritional influences on total homocysteine levels were not considered [36]. In the present study a center effect on total homocysteine levels was observed, which was due to the relatively greater proportion of (T/T) patients with high total homocysteine levels in a center with few patients. In healthy subjects age and sex influence total homocysteine plasma concentrations, whereas in patients with renal failure the MTHFR polymorphism, folate and vitamin B12 concentrations are much more important.

### Table 5. Vascular disease scores of all 67 patients with vascular disease, of 33 vascular disease patients with diabetes mellitus and of 34 non-diabetic vascular disease patients in relation to high and low total homocysteine plasma levels

<table>
<thead>
<tr>
<th>tHcy plasma levels</th>
<th>All patients with vascular disease (N = 67)</th>
<th>Diabetic patients (N = 33)</th>
<th>Non-diabetic patients (N = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number VDS 1 VDS 2</td>
<td>Number VDS 1 VDS 2</td>
<td>Number VDS 1 VDS 2</td>
</tr>
<tr>
<td>Sample median</td>
<td>34 1.53 2.44</td>
<td>17 1.47 2.35</td>
<td>17 1.59 2.53</td>
</tr>
<tr>
<td>&gt;Sample median</td>
<td>33 1.76 3.00</td>
<td>16 2.06 3.50</td>
<td>17 1.47 2.53</td>
</tr>
<tr>
<td>Normal (≤15 μmol/liter)</td>
<td>24 1.54 2.46</td>
<td>11 1.55 2.45</td>
<td>13 1.54 2.46</td>
</tr>
<tr>
<td>&gt;Normal</td>
<td>43 1.70 2.86</td>
<td>22 1.86 3.14</td>
<td>21 1.52 2.57</td>
</tr>
</tbody>
</table>

Abbreviations are: VDS 1, vascular disease score 1; VDS 2, vascular disease score 2; tHcy, total homocysteine. The median total homocysteine level was 18.4 μmol/liter for all patients with vascular disease, 18.4 μmol/liter for diabetic patients with vascular disease and 17.9 μmol/liter for non-diabetic patients with vascular disease. 

[a] P = 0.0211 vs. VDS 1, and [b] P = 0.020 vs. VDS 2 in diabetic patients with vascular disease and total homocysteine levels ≤ sample median.
In hemodialysis patients, plasma total homocysteine concentrations can be transiently lowered by dialysis treatment [11, 18]. In the present study, however, no significant influence of duration and mode of peritoneal dialysis treatment, weekly Kt/V, residual renal function and weekly creatinine clearance on total homocysteine levels was observed in peritoneal dialysis patients. These findings extend the results obtained in previous studies describing no influence of Kt/V on total homocysteine levels in a small patient population [14].

The cardiovascular morbidity and mortality of dialysis patients is increased manyfold compared to healthy subjects [37–39]. This finding was suggested to be associated with elevated total homocysteine plasma levels [1–4], although no separate analysis in hemodialysis and peritoneal dialysis patients was conducted in these studies. It was not the goal of the present study to demonstrate that total homocysteine levels represent an independent risk factor for cardiovascular disease. Therefore, lipid profiles, Lp(a), fibrinogen levels and other known cardiovascular risk factors were not considered in our analyses. Interestingly, a high proportion of our patients presented with total homocysteine concentrations within the normal range and comparison of patients with and without evidence of cardiovascular disease revealed no significant difference of total homocysteine levels in either patient groups. It has to be mentioned, however, that there were significantly more diabetic patients in the group with vascular disease. Furthermore, the mean age in patients with vascular disease was significantly higher and the mean albumin level was significantly lower in these patients. Interestingly, serum creatinine levels in patients with vascular disease were lower compared to those without vascular disease. Nevertheless, we found a significant increase of vascular morbidity in diabetic patients with total homocysteine levels above the sample median. This finding points to the multifactorial genesis of atherosclerosis in end-stage renal failure patients.

In conclusion, the present study provides evidence that homocysteine levels in peritoneal dialysis patients can be transiently lowered by dialysis treatment and that weekly Kt/V, residual renal function and weekly creatinine clearance are not significant factors influencing total homocysteine levels. These findings extend the results obtained in previous studies describing no influence of Kt/V on total homocysteine levels in a small patient population [14].

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