Patients with chronic kidney disease who are on dialysis or with a kidney transplant have higher total plasma homocysteine concentrations than individuals who are free from kidney disease. Several single-nucleotide polymorphisms of genes encoding enzymes that are involved in homocysteine metabolism have been studied in these patients. These polymorphisms are located in genes encoding of 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase reductase, methionine synthase, cystathionine β-synthase, glutamate carboxypeptidase II, reduced folate carrier 1, and transcobalamin II. Among the single-nucleotide polymorphisms studied, only MTHFR 677C>T was associated consistently with total plasma homocysteine levels, but there currently is no evidence of any association between MTHFR 677C>T genotype and long-term outcomes.

KEYWORDS chronic kidney disease, homocysteine, genetics, MTHFR, hyperhomocysteinemia

Among the determinants and correlates of total plasma homocysteine (tHcy) levels, the genetics of enzymes involved in the metabolism of homocysteine has yielded a large and fast-growing body of scientific literature. As a result, several single-nucleotide polymorphisms (SNPs) were identified and their associations with tHcy concentrations were tested. Rare autosomal-recessive disorders such as severe deficiencies of cystathionine β-synthase (CBS)1 or 5,10-methylenetetrahydrofolate reductase (MTHFR)2 often are associated with severe hyperhomocysteinemia (plasma tHcy concentrations > 100 μmol/L) and urinary excretion of excess homocysteine (homocystinuria). Patients suffering from severe CBS or MTHFR deficiency frequently experience vascular complications early in life, providing the basis for the homocysteine theory of atherosclerosis.3

By contrast, several SNPs of proteins involved in vitamin flux or of Hcy-converting enzymes are prevalent4 and can be associated with mild hyperhomocysteinemia (tHcy plasma concentrations, 10-15 μmol/L, the higher normal range) or moderately increased tHcy levels (tHcy plasma concentrations, 15-30 μmol/L). The most interesting SNPs including MTHFR 677C>T,5 MTHFR 1298A>C,6 MTHFR 1793G>A,7 MTRR 66A>G,8 MTRR 997C>G,9 MTR 2756A>G,10 CBS 844ins68,11 GCP2 1561C>T,12 RFC1 80G>A,13 and TCN2 776C>G14 are summarized in Table 1.

Genetic Polymorphisms of MTHFR and tHcy Plasma Concentrations in Chronic Kidney Disease Patients

MTHFR (EC 1.5.1.20) is a key enzyme of the folate cycle.15 It catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, thus generating the active form of folate that is required for remethylation of homocysteine to methionine.

In the MTHFR gene (OMIM 607093), several SNPs have been identified, such as at nucleotide position 677 (MTHFR 677C>T),5 position 1298 (MTHFR 1298A>C),6 MTHFR 1793G>A,7 MTRR 66A>G,8 MTRR 997C>G,9 MTR 2756A>G,10 CBS 844ins68,11 GCP2 1561C>T,12 RFC1 80G>A,13 and TCN2 776C>G14 are summarized in Table 1.
(R594Q). MTHFR 677C>T, MTHFR 1298A>C, and compound heterozygosity for 1289A>C and 677C>T are associated with a reduced enzyme activity of 45%, 68%, and 42%, respectively. The functional consequences of the 1793G>A mutation currently are unknown.

Bagley and Selhub showed that red blood cell folate is represented exclusively by 5-methyltetrahydrofolate in MTHFR 677CC individuals, whereas individuals with the 677TT genotype accumulated formylated folates, which pointed to a disruption of the folate cycle. Functional analysis of recombinant expressed human MTHFR showed that 677TT led to an enhanced propensity to dissociate into monomers and to losing its flavin adenine dinucleotide co-factor on dilution, resulting in a loss of activity. In this context it was shown that riboflavin deficiency aggravated hyperhomocysteinemia in MTHFR 677TT individuals and in end-stage renal disease patients. The 677TT genotype also was associated with alterations of genomic DNA methylation, which is essential for the regulation of gene expression and genomic integrity. Hypomethylation of DNA was correlated positively with formylated folates, hyperhomocysteinemia, and correlated inversely with methylated folate derivatives.

The effect of MTHFR 677C>T on plasma tHcy plasma concentrations has been investigated in several studies of chronic kidney disease patients. Hemodialysis, peritoneal dialysis patients, and kidney graft recipients who were homozygous for the MTHFR 677TT allele had higher tHcy plasma concentrations as compared with patients with wild-type alleles (Table 3). In the majority of studies, this effect even was sustained when patients received folic acid (≥10 mg/d). By contrast, neither the MTHFR 1298A>C polymorphism alone nor compound heterozygosity for MTHFR 677T and MTHFR 1298C had a major effect on tHcy plasma concentrations.

### Table 1 SNPs With Potential Relevance to Hcy Metabolism

<table>
<thead>
<tr>
<th>Gene Locus</th>
<th>Sequence Variation</th>
<th>Study</th>
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<tbody>
<tr>
<td>5,10-methylenetetrahydrofolate reductase</td>
<td>MTHFR</td>
<td>677C&gt;T</td>
</tr>
<tr>
<td>Methionine synthase reductase</td>
<td>MTRR</td>
<td>66A&gt;G</td>
</tr>
<tr>
<td>Methionine synthase</td>
<td>MTR</td>
<td>997C&gt;G</td>
</tr>
<tr>
<td>Cystathionine β-synthase</td>
<td>CBS</td>
<td>844ins68</td>
</tr>
<tr>
<td>Glutamate carboxypeptidase II</td>
<td>GCP2</td>
<td>1561C&gt;T</td>
</tr>
<tr>
<td>Reduced folate carrier</td>
<td>RFC1</td>
<td>80G&gt;A</td>
</tr>
<tr>
<td>Transcobalamin II</td>
<td>TCN2</td>
<td>776C&gt;G</td>
</tr>
</tbody>
</table>

### Table 2 Milestones of MTHFR 677C>T

<table>
<thead>
<tr>
<th>Year</th>
<th>Finding</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>Thermolabile MTHFR Associated with Coronary Artery Disease and tHcy</td>
<td>Kang et al</td>
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<tr>
<td>1994</td>
<td>cDNA cloning of MTHFR gene</td>
<td>Goyette et al</td>
</tr>
<tr>
<td>1995</td>
<td>Description of 677C&gt;T: association of TT genotype with reduced enzyme activity and elevated plasma tHcy</td>
<td>Frosst et al</td>
</tr>
<tr>
<td>1996</td>
<td>Interaction of 677TT with poor folate status is responsible for increase of tHcy</td>
<td>Jacques et al</td>
</tr>
<tr>
<td>1997</td>
<td>Association of 677TT with better tHcy response to folic acid therapy</td>
<td>Malinow et al</td>
</tr>
<tr>
<td>1997/1998</td>
<td>Association of 677TT with low cellular 5-CH3-tetrahydrofolate</td>
<td>Molloy et al; Bagley and Selhub</td>
</tr>
<tr>
<td>1999</td>
<td>Association of 677TT with enhanced vitamin B2 dissociation rate</td>
<td>Guenther et al</td>
</tr>
<tr>
<td>2002</td>
<td>Association of 677TT with impaired DNA methylation</td>
<td>Friso et al</td>
</tr>
<tr>
<td>2002</td>
<td>Meta-analysis of the association of 677TT with coronary artery disease risk</td>
<td>Klerk et al</td>
</tr>
<tr>
<td>2003</td>
<td>Meta-analysis of 677C&gt;T / 1298A&gt;C haplotypes</td>
<td>Ogino and Wilson</td>
</tr>
</tbody>
</table>

### Table 3 Mean tHcy Plasma Concentrations (μmol/L) According to MTHFR 677C>T Genotypes in Individuals From Austria

<table>
<thead>
<tr>
<th>Patient Population</th>
<th>MTHFR 677C&gt;T Genotype</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>25.4</td>
<td>28.7</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>22.2</td>
<td>23.1</td>
</tr>
<tr>
<td>Kidney transplant recipients</td>
<td>14.9</td>
<td>14.6</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>9.7</td>
<td>9.9</td>
</tr>
</tbody>
</table>
concentration of hemodialysis and peritoneal dialysis patients, or kidney graft recipients. The MTHFR 1298A>C mutation was associated weakly with low folate concentrations as shown in MTHFR 677T/1298C compound heterozygous kidney graft recipients (Table 4). In other studies, heterozygosity or homozygosity for MTHFR 1793G>A was not associated independently with plasma levels of tHcy or vitamin B12 in kidney graft recipients, but showed an association with higher folate concentrations. In these studies, Winkelmayer et al also found evidence for strong positive interactions between the MTHFR 1793G>A and 1298A>C mutations on vitamin B12 concentrations.

Pharmacogenetics of Homocysteine-Decreasing Therapies in Chronic Kidney Disease Patients

The response to homocysteine-decreasing therapy of renal failure patients appears to be influenced by genetic polymorphisms in MTHFR. Hemodialysis patients with the MTHFR 677TT genotype receiving high-dose folic acid orally attained normal total homocysteine levels more frequently than patients with the CC or CT genotype. A better response to therapy also was observed in hemodialysis patients with the MTHFR 677TT/1298CA and the MTHFR 677CT/1298AC genotype who were treated intravenously with 15 mg of folic acid and an equimolar amount of folic acid, and among MTHFR 677TT genotype patients receiving 15 mg of folic acid orally. The more pronounced response to folate therapy of MTHFR 677TT patients possibly is caused by the correction of the intracellular deficiency of 5-methyltetrahydrofolate in TT genotype patients.

MTHFR 677C>T and Outcomes of Chronic Kidney Disease Patients

In one cross-sectional study of dialysis patients, the MTHFR 677C>T mutation was associated with greater rates of cardiovascular disease. When following-up these patients for 2 years, no association between this mutation and cardiovascular disease outcomes was detected. However, the observation of an independent role of MTHFR 677C>T in cardiovascular disease risk received support by a recent meta-analysis of 11,162 cases with coronary artery disease and 12,758 control individuals without renal failure. In that study, it was shown clearly that patients with the MTHFR 677TT genotype had a significantly higher risk for coronary heart disease, particularly in the presence of low folate levels. The idea of an independent association of MTHFR 677C>T with vascular disease risk was supported further by the observation of an association between the MTHFR 677T allele with increased intima-media thickness of the common carotid arteries and an increased risk for vascular access thrombosis.

Based on these observations, the MTHFR 677C>T mutation in individuals with and without renal failure may be an independent cardiovascular disease risk factor, which sup-

<table>
<thead>
<tr>
<th>Types of Cases</th>
<th>N</th>
<th>tHcy</th>
<th>Plasma Folate</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR 677C&gt;T</td>
<td>HD</td>
<td>69</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MTHFR 677C&gt;T</td>
<td>PD</td>
<td>178</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MTHFR 677C&gt;T</td>
<td>KTR</td>
<td>636</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MTHFR 1298A&gt;C</td>
<td>KTR</td>
<td>733</td>
<td>No*</td>
<td>Yes</td>
</tr>
<tr>
<td>MTHFR 1298A&gt;C</td>
<td>HD, PD</td>
<td>415</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>MTHFR 1793G&gt;A</td>
<td>KTR</td>
<td>730</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>MTR 2756A&gt;G</td>
<td>KTR, HD, PD, CO</td>
<td>1716</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>MTRR 66A&gt;G</td>
<td>KTR</td>
<td>733</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: HD, hemodialysis patients; PD, peritoneal dialysis patients; KTR, kidney transplant recipients; CO, controls.

*Compound heterozygosity for MTHFR 677C>T/1298 A>C.
ports the concept that impaired folate metabolism is related causally to increased coronary heart disease and vascular disease risk.

It has been speculated that hyperhomocysteinemia in kidney transplant patients might contribute to an increased risk for graft loss.31 In this context, MTHFR 677C→T and MTHFR 1298A→C mutations were studied for a putative relation with kidney transplant survival. The studies available thus far have shown that (1) neither MTHFR 677C→T nor MTHFR 1298A→C were important determinants of renal transplant survival, (2) the MTHFR genotype of the donor kidney did not influence tHcy plasma levels,27 and (3) the MTHFR 677TT genotype might be associated with occurrence of acute rejection episodes within 90 days after kidney transplantation. In this small study the TT genotype had no effect on 1-year graft survival rate.44

Other Common Genetic Polymorphisms and Hyperhomocysteinemia in Chronic Kidney Disease Patients

Several other SNPs in candidate genes encoding enzymes that are involved in the metabolism of folate and homocysteine (Tables 4-6) have been investigated in renal failure patients.24 These include MTR 2756A→G of the gene encoding methionine synthase,45 MTRR 66G→A and 997C→G of the gene coding for methionine synthase reductase,46 RFC1 80G→A of the gene coding for the reduced folate carrier 1,47,48 GCP2 1561C→T encoding glutamate carboxypeptidase II,37,46 and TCN2 776C→G coding for transcobalamin II.49,50 Among these SNPs only GCP2 1561C→T showed a significant influence on folate status, whereas none of these SNPs showed a major association with tHcy plasma concentrations (Table 7).

Summary

In summary, tHcy plasma concentrations in patients with chronic kidney disease are increased compared with patients without kidney disease, and several genetic factors were tested for association with tHcy levels in these patients. Polymorphisms have also been identified in genes potentially modifying the metabolism of folate or vitamin B_{12}, and thus affecting tHcy plasma concentrations. The most consistent effect on tHcy plasma concentrations, however, only was observed for 677C→T of MTHFR, whereas all other polymorphisms investigated to date showed no major effect on tHcy concentrations.

References

nine synthase reductase deficiency in patients belonging to the cblE
complementation group of disorders in folate/cobalamin metabolism.
Hum Mol Genet 8:2009-2016, 1999

cDNA cloning and identification of mutations in patients of the cblG
complementation group of folate/cobalamin disorders. Hum Mol Genet
5:1867-1874, 1996

mocysteine: Association of two prevalent mutations, the 844ins68 of
cystathionine β-synthase and A2756G of methionine synthase, with
lowered plasma homocysteine levels. Atherosclerosis 149:131-137,
2000

12. Devlin AM, Ling-E-H, Peerson JM, et al: Glutamate carboxypeptidase II:
A polymorphism associated with lower levels of serum folate and hy-

(80G>A) in the reduced folate carrier gene and its associations with

259 polymorphism, vitamin A and C in Causians: Relation to
transcobalamin and homocysteine concentration in blood.
Blood 97:1092-1098, 2001

15. Födinger M, Hörf WH, Sunder-Plassmann G: Molecular biology of

5,10-methylenetetrahydrofolate reductase locus in human ovarian car-

mutation in the methylenetetrahydrofolate reductase gene: An addi-
tional risk factor for neural-tube defects? Am J Hum Genet 62:1044-
1051, 1998

variant, with
methylenetetrahydrofolate reductase gene aggravates hyperhomocys-
teinemia in peritoneal dialysis patients. Kidney Int 53:1775-
1782, 1998

polymorphisms and renal failure, in Ueland PM, Rozen R (eds): MTHFR Polymorphisms and
Disease. Georgetown, TX, Landes Bioscience (www.eurekah.com),
2004

polymorphisms with total homocysteine plasma levels in dialysis pa-

21. Hustad S, Ueland PM, Vollset SE, et al: Riboflavin as a determinant of
plasma total homocysteine: Effect modification by the methylenetetra-
hydrofolate reductase C677T variant, with
methylenetetrahydrofolate reductase gene affects genomic DNA meth-
lation to transcobalamin and homocysteine concentration in blood.
Proc Natl Acad Sci USA 99:5606-5611, 2002

22. Skoupy S, Födinger M, Veitl M, et al: Riboflavin is a determinant of total
homocysteine in hemodialysis patients. J Am Soc Nephrol 13:1314-1319,
2002

23. Födinger M, Sunder-Plassmann G, Huber A, et al: Patterns of co-
ocurrence of three single nucleotide polymorphisms of the 5,10-
methylenetetrahydrofolate reductase gene in kidney transplant recipi-
ents. Eur J Clin Invest 34:613-618, 2004

MTHFR 1793G>A and plasma total homocysteine, folate, and vitamin
11:1918-1925, 2000

co-occurrence of three single nucleotide polymorphisms of the 5,10-
methylenetetrahydrofolate reductase gene in kidney transplant recipi-
ents. Eur J Clin Invest 34:613-618, 2004

MTHFR 1793G>A and plasma total homocysteine, folate, and vitamin

dose folie acid therapy on hyperhomocysteinemia in hemodialysis pa-
tients: Results of the Vienna Multicenter Study. J Am Soc Nephrol
11:1106-1116, 2000

co-occurrence of three single nucleotide polymorphisms of the 5,10-
methylenetetrahydrofolate reductase gene in kidney transplant recipi-
ents. Eur J Clin Invest 34:613-618, 2004

c acid for the correction of hyperhomocysteinemia in hemodialysis pa-

30. Billon S, Tribout B, Cadet E, et al: Hyperhomocysteinaemia, folate and
vitamin B12 in unsupplemented haemodialysis patients: Effect of oral
therapy with folic acid and vitamin B12. Nephrol Dial Transplant 17:
455-461, 2002

hypermocysteine, and cardiovascular comorbidity in renal disease. Kidney
Int 60:1106-1113, 2001

acid for prevention of cardiovascular events in end-stage renal disease.

and risk of coronary heart disease. A meta-analysis. JAMA 288:2023-
2031, 2002

34. Lim PS, Hung WR, Wei YH: Polymorphism in methylenetetrahydrofo-
late reductase gene: Its impact on plasma homocysteine levels and
paracodiartherosclerosis in ESRD patients receiving hemodialysis.
Nephron 87:249-256, 2001

hydrofolate reductase C677T point mutation is a risk factor for vascular
access thrombosis in hemodialysis patients. Am J Kidney Dis 41:637-
642, 2003

holocysteine levels and mortality and allograft loss in kidney trans-
2005

and hyperhomocysteinemia on patient and graft survival in kidney trans-

38. Liangos O, Kreutz R, Beige J, et al: Methylenetetrahydrofolate-reduc-
tase gene C677T variant and kidney-transplant survival. Nephrol Dial

plantation in patients with inherited thrombophilia: Data of a prospec-

combined MTR and MTHFR genotypes among individuals with se-
verely elevated total homocysteine plasma levels. Am J Kidney Dis
38:956-964, 2001

41. Feix A, Winkelmayer WC, Eberle C, et al: Methionine synthase reduc-
tase MTRR 66A>G has no effect on total homocysteine, folate, and
vitamin B12 concentrations in renal transplant patients. Atherosclerosis
174:43-48, 2004

42. Födinger M, Dierkes J, Skoupy S, et al: Effect of glutamate carboxypep-
tidase II and reduced folate carrier polymorphisms on folate and total
homocysteine concentrations in dialysis patients. J Am Soc Nephrol
14:1313-1319, 2003