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Genetic Aspects of Hyperhomocysteinemia in Chronic Kidney Disease

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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 carboxy peptidase 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 prevalent[4] 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>G[14] 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),[6,16,17] position 1317 (*MTHFR* 1317T>C),[6] and position 1793 (*MTHFR* 1793G>A).[7] *MTHFR* 677C>T is located at the folate-binding site, changing an alanine into a valine residue (A222V). The most important milestones of this SNP are listed in Table 2. *MTHFR* 1298A>C is located in the presumptive regulatory domain, changing glutamic acid into an alanine residue (E429A), whereas *MTHFR* 1317T>C is a silent mutation. *MTHFR* 1793G>A results in an amino acid substitution

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Table 1 SNPs With Potential Relevance to Hcy Metabolism

	Gene Locus	Sequence Variation	Study
5,10-methylenetetrahydrofolate reductase	*MTHFR*	677C>T	Frosst et al[5]
		1298A>C	Weisberg et al[6]
		1793 G>A	Rady et al[7]
Methionine synthase reductase	*MTRR*	66A>G	Wilson et al[8]
		997C>G	Wilson et al[9]
Methionine synthase	*MTR*	2756A>G	Leclerc et al[10]
Cystathionine β-synthase	*CBS*	844ins68	Tsai et al[11]
Glutamate carboxy peptidase II	*GCP2*	1561C>T	Devlin et al[12]
Reduced folate carrier 1	*RFC1*	80G>A	Chango et al[13]
Transcobalamin II	*TCN2*	776C>G	Namour et al[14]

(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.[18] The functional consequences of the 1793G>A mutation currently are unknown.

Bagley and Selhub[19] 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 cofactor on dilution, resulting in a loss of activity.[20] In this context it was shown that riboflavin deficiency aggravated hyperhomocysteinemia in *MTHFR* 677TT individuals[21] and in end-stage renal disease patients.[22] 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.[23]

The effect of MTHFR 677C>T on plasma tHcy plasma concentrations has been investigated in several studies of chronic kidney disease patients.[24] Hemodialysis,[25] peritoneal dialysis patients,[26] and kidney graft recipients[27] who were homozygous for the *MTHFR* 677T 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 ($\geq$10 mg/d).[28] 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

Table 2 Milestones of *MTHFR* 677C>T

Year	Finding	Reference
1991	Thermolabile MTHFR Associated with Coronary Artery Disease and tHcy	Kang et al[51]
1994	cDNA cloning of *MTHFR* gene	Goyette et al[52]
1995	Description of 677C>T: association of TT genotype with reduced enzyme activity and elevated plasma tHcy	Frosst et al[5]
1996	Interaction of 677TT with poor folate status is responsible for increase of tHcy	Jacques et al[53]
1997	Association of 677TT with better tHcy response to folic acid therapy	Malinow et al[54]
1997/1998	Association of 677TT with low cellular 5-CH_3-tetrahydrofolate	Molloy et al[55]; Bagley and Selhub[19]
1999	Association of 677TT with enhanced vitamin B_2 dissociation rate	Guenther et al[56]
2002	Association of 677TT with impaired DNA methylation	Friso et al[23]
2002	Meta-analysis of the association of 677TT with coronary artery disease risk	Klerk et al[38]
2003	Meta-analysis of 677C>T / 1298A>C haplotypes	Ogino and Wilson[57]

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

	MTHFR 677C>T Genotype			
Patient Population	CC	CT	TT	Study
Hemodialysis	25.4	28.7	36.4	Födinger et al[25]
Peritoneal dialysis	22.2	23.1	61.7	Vychytil et al[26]
Kidney transplant recipients	14.9	14.6	18.6	Födinger et al[27]
Healthy controls	9.7	9.9	12.2	Födinger et al[25]

Table 4 Associations Between Common Genetic Polymorphisms of *MTHFR*, *MTR*, and *MTRR* on tHcy Concentrations in Chronic Kidney Disease Patients

	Types of Cases	N	tHcy	Plasma Folate	Study
MTHFR 677C>T	HD	69	Yes	Yes	Födinger et al[25]
MTHFR 677C>T	PD	178	Yes	Yes	Vychytil et al[26]
MTHFR 677C>T	KTR	636	Yes	Yes	Födinger et al[27]
MTHFR 677C>T	HD, PD	415	Yes	No	Födinger et al[29]
MTHFR 1298A>C	KTR	733	No	Yes*	Födinger et al[30]
MTHFR 1298A>C	HD, PD	415	No	No	Födinger et al[29]
MTHFR 1793G>A	KTR	730	No	Yes	Winkelmayer et al[32]
MTR 2756A>G	KTR, HD, PD, CO	1716	No	No	Feix et al[45]
MTRR 66A>G	KTR	733	No	No	Feix et al[46]

Abbreviations: HD, hemodialysis patients; PD, peritoneal dialysis patients; KTR, kidney transplant recipients; CO, controls.
*Compound heterozygosity for *MTHFR* 677C>T/1298 A>C.

concentration of hemodialysis and peritoneal dialysis patients,[29] or kidney graft recipients.[30] 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).[30]

In other studies,[31,32] heterozygosity or homozygosity for *MTHFR* 1793G>A was not associated independently with plasma levels of tHcy or vitamin B_{12} in kidney graft recipients, but showed an association with higher folate concentrations. In these studies, Winkelmayer et al[31,32] also found evidence for strong positive interactions between the *MTHFR* 1793G>A and 1298A>C mutations on vitamin B_{12} 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.[33] A better response to therapy also was observed in hemodialysis patients with the *MTHFR* 677TT/1298AA and the *MTHFR* 677CT/1298AC genotype who were treated intravenously with 15 mg of folic acid and an equimolar amount of folinic acid,[34] and among *MTHFR* 677TT genotype patients receiving 15 mg of folic acid orally.[35] 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.[19]

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.[36] When following-up these patients for 2 years, no association between this mutation and cardiovascular disease outcomes was detected.[37] 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.[38] 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[39] and an increased risk for vascular access thrombosis.[40]

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 5 Associations Between Common Genetic Polymorphisms of *GCP2* and *RFC1* on tHcy, Plasma Folate, and Red Blood Cell Folate Levels in Chronic Kidney Disease Patients

	Types of Cases	N	tHcy	Folate	Red Blood Cell Folate	Study
GCP2 1561C>T	HD, PD	120	No	No	Yes	Födinger et al[47]
GCP2 1561C>T	KTR	730	No	Yes	ND	Winkelmayer et al[48]
RFC1 80G>A	HD, PD	120	No	No	No	Födinger et al[47]
RFC1 80G>A	KTR	730	No	No	ND	Winkelmayer et al[48]

Abbreviations: HD, hemodialysis patients; PD, peritoneal dialysis patients; KTR, kidney transplant recipients; ND, not done.

Table 6 Associations Between a Common Genetic Polymorphism in *TCN2* on tHcy, Vitamin B_{12} and Holotranscobalamin II Levels in Chronic Kidney Disease Patients

	Types of Cases	N	tHcy	Vitamin B_{12}	Holotranscobalamin II	Study
TCN2 776C>G	HD, PD	120	No	No	No	Födinger et al[49]
TCN2 776C>G	KTR	732	No	No	ND	Winkelmayer et al[50]

Abbreviations: HD, hemodialysis patients; PD, peritoneal dialysis patients, KTR, kidney transplant recipients; ND, not done.

Table 7 Associations Between *GCP2* C>T on Plasma or Red Blood Cell Folate Concentrations.

		Mean Red Blood Cell or Plasma Folate (nmol/L)			
Types of Cases	N	CC	CT	TT	Study
Framingham offspring study	1,913	12.7* (n = 1,713)	13.5* (n = 190)	13.5* (n = 190)	Vargas-Martinez et al[58]
HD, PD	120	1,337† (n = 111)	2,055† (n = 9)	2,055† (n = 9)	Födinger et al[47]
KTR	730	14.7* (n = 654)	15.6* (n = 74)	24.7* (n = 2)	Winkelmayer et al[48]
CO	180	685† (n = 161)	863† (n = 19)	—	Melse-Boonstra et al[59]

Abbreviations: HD, hemodialysis patients; PD, peritoneal dialysis patients; KTR, kidney transplant recipients; CO, controls.
*Plasma folate.
†Red blood cell folate.

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.[41] 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[42] nor *MTHFR* 1298A>C[42,43] 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 carboxypetidase II,[47,48] 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

1. Mudd SH, Levy HL, Skovby F: Disorders of transsulfuration, in Scriver CR, Beaudet AL, Sly WS, et al (eds): The metabolic and molecular bases of inherited disease (7th ed). New York, McGraw-Hill, Inc., 1995, pp 1279-1327
2. Rosenblatt DS: Inherited disorders of folate transport and metabolism, in Scriver CR, Beaudet AL, Sly WS, et al (eds): The Metabolic and Molecular Bases of Inherited Disease (7th ed). New York, McGraw-Hill, Inc., 1995, pp 3111-3128
3. McCully KS, Wilson RB: Homocysteine theory of arteriosclerosis. Atherosclerosis 22:215-227, 1975
4. Födinger M, Buchmayer H, Sunder-Plassmann G: Molecular genetics of homocysteine metabolism. Miner Electrolyte Metab 25:269-278, 1999
5. Frosst P, Blom HJ, Milos R, et al: A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase [letter]. Nat Genet 10:111-113, 1995
6. Weisberg I, Tran P, Christensen B, et al: A second genetic polymorphism in methylenetetrahydrofolate reductase (*MTHFR*) associated with decreased enzyme activity. Mol Genet Metab 64:169-172, 1998
7. Rady PL, Szucs S, Grady J, et al: Genetic polymorphisms of methylenetetrahydrofolate reductase (*MTHFR*) and methionine synthase reductase (*MTRR*) in ethnic populations in Texas; a report of a novel *MTHFR* polymorphic site, G1793A. Am J Med Genet 107:162-168, 2002
8. Wilson A, Platt R, Wu Q, et al: A common variant in methionine synthase reductase combined with low cobalamin (vitamin B_{12}) increases risk for spina bifida. Mol Genet Metab 67:317-323, 1999

9. Wilson A, Leclerc D, Rosenblatt DS, et al: Molecular basis for methionine synthase reductase deficiency in patients belonging to the *cblE* complementation group of disorders in folate/cobalamin metabolism. Hum Mol Genet 8:2009-2016, 1999
10. Leclerc D, Campeau E, Goyette P, et al: Human methionine synthase: cDNA cloning and identification of mutations in patients of the *cblG* complementation group of folate/cobalamin disorders. Hum Mol Genet 5:1867-1874, 1996
11. Tsai MY, Bignell M, Yang F, et al: Polygenic influence on plasma homocysteine: 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 hyperhomocysteinemia. Hum Mol Genet 9:2837-2844, 2000
13. Chango A, Emery-Fillon N, Portier de Courcy G, et al: A polymorphism (80G>A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia. Mol Genet Metab 70:310-315, 2000
14. Namour F, Olivier JL, Abdelmouttaleb I, et al: Transcobalamin codon 259 polymorphism in HT-29 and Caco-2 cells and in Caucasians: Relation to transcobalamin and homocysteine concentration in blood. Blood 97:1092-1098, 2001
15. Födinger M, Hörl WH, Sunder-Plassmann G: Molecular biology of 5,10-methylenetetrahydrofolate reductase. J Nephrol 13:20-33, 2000
16. Viel A, Dall'Agnese L, Simone F, et al: Loss of heterozygosity at the 5,10-methylenetetrahydrofolate reductase locus in human ovarian carcinomas. Br J Cancer 75:1105-1110, 1997
17. van der Put NMJ, Gabreëls F, Stevens EMB, et al: A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural-tube defects? Am J Hum Genet 62:1044-1051, 1998
18. Weisberg IS, Jacques PF, Selhub J, et al: The 1298A>C polymorphism in methylenetetrahydrofolate reductase (MTHFR): In vitro expression and association with homocysteine. Atherosclerosis 156:409-415, 2001
19. Bagley PJ, Selhub J: A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci U S A 95:13217-13220, 1998
20. Yamada K, Chen Z, Rozen R, et al: Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci U S A 98:14853-14858, 2001
21. Hustad S, Ueland PM, Vollset SE, et al: Riboflavin as a determinant of plasma total homocysteine: Effect modification by the methylenetetrahydrofolate reductase C677T polymorphism. Clin Chem 46:1065-1071, 2000
22. Skoupy S, Födinger M, Veitl M, et al: Riboflavin is a determinant of total homocysteine plasma concentrations in end-stage renal disease patients. J Am Soc Nephrol 13:1331-1337, 2002
23. Friso S, Choi SW, Girelli D, et al: A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A 99:5606-5611, 2002
24. Födinger M, Wagner OF, Hörl WH, et al: Recent insights into the molecular genetics of the homocysteine metabolism. Kidney Int 59: S238-S242, 2001 (suppl 78)
25. Födinger M, Mannhalter C, Wölfl G, et al: Mutation (677 C to T) in the methylenetetrahydrofolate reductase gene aggravates hyperhomocysteinemia in hemodialysis patients. Kidney Int 52:517-523, 1997
26. Vychytil A, Födinger M, Wölfl G, et al: Major determinants of hyperhomocysteinemia in peritoneal dialysis patients. Kidney Int 53:1775-1782, 1998
27. Födinger M, Wölfl G, Fischer G, et al: Effect of *MTHFR* 677C>T on plasma total homocysteine levels in renal graft recipients. Kidney Int 55:1072-1080, 1999
28. Födinger M, Sunder-Plassmann G: *MTHFR* polymorphisms and renal failure, in Ueland PM, Rozen R (eds): *MTHFR* Polymorphisms and Disease. Georgetown, TX, Landes Bioscience (www.eurekah.com), 2004
29. Födinger M, Buchmayer H, Heinz G, et al: Association of two *MTHFR* polymorphisms with total homocysteine plasma levels in dialysis patients. Am J Kidney Dis 38:77-84, 2001
30. Födinger M, Buchmayer H, Heinz G, et al: Effect of *MTHFR* 1298A>C and *MTHFR* 677C>T genotypes on total homocysteine, folate, and vitamin B_{12} plasma concentrations in kidney graft recipients. J Am Soc Nephrol 11:1918-1925, 2000
31. Winkelmayer WC, Sunder-Plassmann G, Huber A, et al: Patterns of co-occurrence of three single nucleotide polymorphisms of the 5,10-methylenetetrahydrofolate reductase gene in kidney transplant recipients. Eur J Clin Invest 34:613-618, 2004
32. Winkelmayer WC, Huber A, Wagner OF, et al: Associations between MTHFR 1793G>A and plasma total homocysteine, folate, and vitamin B in kidney transplant recipients. Kidney Int 67:1980-1985, 2005
33. Sunder-Plassmann G, Födinger M, Buchmayer H, et al: Effect of high dose folic acid therapy on hyperhomocysteinemia in hemodialysis patients: Results of the Vienna Multicenter Study. J Am Soc Nephrol 11:1106-1116, 2000
34. Hauser AC, Hagen W, Rehak PH, et al: Efficacy of folinic versus folic acid for the correction of hyperhomocysteinemia in hemodialysis patients. Am J Kidney Dis 37:758-765, 2001
35. Billion 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
36. Wrone EM, Zehnder JL, Hornberger JM, et al: An *MTHFR* variant, homocysteine, and cardiovascular comorbidity in renal disease. Kidney Int 60:1106-1113, 2001
37. Wrone EM, Hornberger JM, Zehnder JL, et al: Randomized trial of folic acid for prevention of cardiovascular events in end-stage renal disease. J Am Soc Nephrol 15:420-426, 2004
38. Klerk M, Verhoef P, Clarke R, et al: *MTHFR* 677C>T polymorphism and risk of coronary heart disease. A meta-analysis. JAMA 288:2023-2031, 2002
39. Lim PS, Hung WR, Wei YH: Polymorphism in methylenetetrahydrofolate reductase gene: Its impact on plasma homocysteine levels and carotid atherosclerosis in ESRD patients receiving hemodialysis. Nephron 87:249-256, 2001
40. Fukasawa M, Matsushita K, Kamiyama M, et al: The methylentetrahydrofolate reductase C677T point mutation is a risk factor for vascular access thrombosis in hemodialysis patients. Am J Kidney Dis 41:637-642, 2003
41. Winkelmayer WC, Kramar R, Curhan GC, et al: Fasting plasma total homocysteine levels and mortality and allograft loss in kidney transplant recipients: A prospective study. J Am Soc Nephrol 16:255-260, 2005
42. Hagen W, Födinger M, Heinz G, et al: Effect of *MTHFR* genotypes and hyperhomocysteinemia on patient and graft survival in kidney transplant recipients. Kidney Int 59:S253-S257, 2001 (suppl 78)
43. Liangos O, Kreutz R, Beige J, et al: Methylenetetrahydrofolate-reductase gene C677T variant and kidney-transplant survival. Nephrol Dial Transplant 13:2351-2354, 1998
44. Heidenreich S, Junker R, Wolters H, et al: Outcome of kidney transplantation in patients with inherited thrombophilia: Data of a prospective study. J Am Soc Nephrol 14:234-239, 2003
45. Feix A, Fritsche-Polanz R, Kletzmayr J, et al: Increased prevalence of combined *MTR* and *MTHFR* genotypes among individuals with severely elevated total homocysteine plasma levels. Am J Kidney Dis 38:956-964, 2001
46. Feix A, Winkelmayer WC, Eberle C, et al: Methionine synthase reductase MTRR 66A>G has no effect on total homocysteine, folate, and vitamin B_{12} concentrations in renal transplant patients. Atherosclerosis 174:43-48, 2004
47. Födinger M, Dierkes J, Skoupy S, et al: Effect of glutamate carboxypeptidase II and reduced folate carrier polymorphisms on folate and total homocysteine concentrations in dialysis patients. J Am Soc Nephrol 14:1314-1319, 2003

48. Winkelmayer WC, Eberle C, Sunder-Plassmann G, et al: Effects of the glutamate carboxypeptidase II (*GCP2* 1561C>T) and reduced folate carrier (*RFC1* 80G>A) allelic variants on folate and total homocysteine levels in kidney transplant patients. Kidney Int 63:2280-2285, 2003
49. Födinger M, Veitl M, Skoupy S, et al: Effect of *TCN2* 776C>G on vitamin B_{12} availability in end-stage renal disease patients. Kidney Int 64:1095-1100, 2003
50. Winkelmayer WC, Skoupy S, Eberle C, et al: Effects of *TCN2* 776C>G on vitamin B_{12}, folate and total homocysteine levels in kidney transplant patients. Kidney Int 65:1877-1881, 2004
51. Kang SS, Wong PW, Susmano A, et al: Thermolabile methylenetetrahydrofolate reductase: An inherited risk factor for coronary artery disease. Am J Hum Genet 48:536-545, 1991
52. Goyette P, Sumner JS, Milos R, et al: Human methylenetetrahydrofolate reductase: Isolation of cDNA, mapping and mutation identification. Nat Genet 7:195-200, 1994
53. Jacques PF, Bostom AG, Williams RR, et al: Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 93:7-9, 1996
54. Malinow MR, Nieto FJ, Kruger WD, et al: The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. Arterioscler Thromb Vasc Biol 17:1157-1162, 1997
55. Molloy AM, Daly S, Mills JL, et al: Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: Implications for folate intake recommendations. Lancet 349:1591-1593, 1997
56. Guenther BD, Sheppard CA, Tran P, et al: The structure and properties of methylenetetrahydrofolate reductase from Escherichia coli suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol 6:359-365, 1999
57. Ogino S, Wilson RB: Genotype and haplotype distributions of MTHFR677C>T and 1298A>C single nucleotide polymorphisms: A meta-analysis. J Hum Genet 48:1-7, 2003
58. Vargas-Martinez C, Ordovas JM, Wilson PW, et al: The glutamate carboxypeptidase gene II (C>T) polymorphism does not affect folate status in the Framingham Offspring Cohort. J Nutr 132:1176-1179, 2002
59. Melse-Boonstra A, Lievers KJ, Blom HJ, et al: Bioavailability of polyglutamyl folic acid relative to that of monoglutamyl folic acid in subjects with different genotypes of the glutamate carboxypeptidase II gene. Am J Clin Nutr 80:700-704, 2004