

Case Control Study

Relationship of *MTHFR* gene polymorphisms with renal and cardiac disease

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Abstract

AIM: To investigate the effects of different methylenetetrahydrofolate reductase (*MTHFR*) 677C>T gene polymorphism and hyperhomocysteinemia for the development of renal failure and cardiovascular events, which are controversial.

METHODS: We challenged the relationship, if any, of *MTHFR* 677C>T and *MTHFR* 1298A>C polymorphisms with renal and heart function. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common *MTHFR* polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure requiring hemodialysis. *MTHFR* polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure. A similar finding was reported in fatty liver disease in which it is suggested that *MTHFR* polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism. We studied a total of 630 Italian Caucasian subject aged 54.60 ± 16.35 years, addressing to the increased hazard of hemodialysis, if any, according to the studied *MTHFR* genetic polymorphisms.

RESULTS: A favorable association with normal renal function of *MTHFR* polymorphisms, and notably of *MTHFR* C677T is present independently of the negative effects of left ventricular hypertrophy, increased Intra-Renal arterial Resistance and hyperparathyroidism.

CONCLUSION: *MTHFR* gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among our dialysis patients in end-stage renal failure; differently, the association with left ventricular hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism.

Key words: Homocysteine; Glomerular filtration rate; Renal function; Mediterranean diet; Genetic; Methylenetetrahydrofolate reductase polymorphism; Insulin

resistance; Obesity; Left ventricular hypertrophy; Echocardiography

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Core tip: We investigated the effects of different methylenetetrahydrofolate reductase (MTHFR) 677C>T gene polymorphism and hyperhomocysteinemia for the development of renal failure and cardiovascular events, which are controversial, and challenged the relationship, if any, of MTHFR 677C>T and MTHFR 1298A>C polymorphisms with renal and heart function. *MTHFR* gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among our dialysis patients in end-stage renal failure; differently, the association with left ventricular hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism.

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INTRODUCTION

Hyperhomocysteinemia is a frequent condition among patients both in end-stage renal disease and on dialysis and may represent an additional risk factor for increased cardiovascular disease^[1]. It is recognized that supplementation with folic acid may often reduce, but not always and permanently correct hyperhomocysteinemia. More important, this approach does not reduce cardiovascular events in patients with kidney disease so that folic acid based regimens are not recommended as a generalized approach in the prevention of cardiovascular events in chronic kidney disease^[2]. Some polymorphism of the human methylenetetrahydrofolate reductase (*MTHFR*) gene have been associated with increased homocysteine levels: this was suspected to increase risks of cardio-vascular disease^[3] (CVD) especially in the natural story of chronic kidney disease^[4]. The more common *MTHFR* polymorphism (nucleotide 677 C>T) results in a thermolabile enzyme, lower folate levels and an inefficient homocysteine metabolism^[5]. In recent years evidence has accumulated that the total homocysteine plasma level of patients under different forms of renal replacement therapy is influenced by a common polymorphism at nucleotide position 677 of the gene coding for 5,10-methylenetetrahydrofolate reductase (*MTHFR* 677C-->T). Furthermore, compound heterozygosity for the 677T allele and a novel A-->C polymorphism at nucleotide position 1298 of *MTHFR* was suggested to correlate with a decrease of folate

plasma concentrations^[6]. Hyperhomocysteinemia appears independent from other risk factors and subsequent reports increased concerns around the related common genetic polymorphism^[7] despite earlier studies already challenged this concept^[8] since this polymorphism prevalence in the elderly is not lower than in the young^[9]. A very relevant question for the putative detrimental role of the allele 677T of the *MTHFR* gene is related to the evidence that this polymorphism is the best explaining protective factor against cervical carcinogenesis^[10], and for colonic cancer^[11,12], seemingly associated with longer and healthier survival^[13]. Nonetheless, according to other studies, *MTHFR* 677TT homozygous and systolic blood pressure independently influence intima-media thickness^[14] as other non-genetic markers^[15] and nutritional conditions do^[16]. Also mild-moderate renal impairment is associated with mortality, increased left ventricular (LV) myocardial mass^[17], lower Ejection Fraction and increased E/A ratio at echocardiography^[18]. Insulin resistance accounts significantly for LV mass increase in normotensive individuals^[19]. A linear relationship between LV myocardial mass/m² (LVMMi) vs cardiovascular events, a J-shape relationship between LVMMi vs all-cause death^[20] and NT-proBNP increase in patients with LV hypertrophy (LVH) suggest a common pathway, through the increase of measured myocardial mass, toward cardiac insufficiency^[21]. Relevance of hyper-homocysteinemia stems from many considerations. Among them, in general population with no history of cardiovascular disease, concentrations of homocysteine alone could accurately identify those at high risk of cardiovascular mortality, whereas classic risk factors included in the Framingham risk score do not^[22], suggesting the need of intervention^[23]. *MTHFR* polymorphisms^[24,25] seemingly intervene, not only inducing hyperhomocysteinemia, within a cluster of different and even interrelated conditions, diseases and indexes. Dietary profiles are the background of any adequate nutrients intake and particularly of a normal B vitamin intake and availability: they can be modified by conditions impairing renal function^[26]. *MTHFR* gene-mediterranean diet interaction on homocysteine metabolism was reported: this dietary profile may reduce homocysteine concentrations and consequently influence coronary risk in genetically high-risk individuals by quality and proportion of nutrients^[27]. The accompanying body size increase is not invariably detrimental since, actually, patients with established chronic disease benefit of large body size^[28]. This finding, defined the obesity paradox, is shared over a variety of cardiovascular, pulmonary, and renal diseases: it challenges the concept about differences for optimal body size in health and disease^[29]. The cornerstone is how several metabolic factors affect renal circulation and, as a consequence, renal function. The increase of intra-renal artery resistance, measured by renal artery resistive index (RRI), affects the natural history of atherosclerosis and arterial hypertension, which was found to correlate with LVH and carotid intimal thickening^[29], with cardiovascular

risk score and impaired renal outcome and death^[30]. Also endocrine factors are very relevant: among them, Parathyroid Hormone intervenes in several mechanisms of disease progression, including LVH^[31], impairment of renal function^[32] and increase of intrarenal arterial resistance^[33,34]. We reported that renal insufficiency in non-diabetic subjects is explained by interactions of MTHFR C677T polymorphism mutation with LVH, high-sensitivity C-reactive protein (hsCRP), intact parathyroid hormone (iPTH), and RRI. Sign of these predictive effects is opposite: subjects with MTHFR 677C>T polymorphism have lower likelihood of renal insufficiency; differently, wild-type MTHFR genotype subjects have lower glomerular filtration rate (GFR) and greater hsCRP, iPTH, RRI, and LVH^[35]. Even with the limitations of an observational study, the concept that MTHFR polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure, appears reasonable and deserving further and more systematic research. A similar finding was reported in fatty liver disease in which it is suggested that MTHFR polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism^[36]. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure. Patients considered were on hemodialysis or on maintenance therapy, and GFR, RRI and LVMM and systolic/diastolic function, dietary profile, hsCRP, iPTH, insulin resistance were assessed.

MATERIALS AND METHODS

We studied a total of 630 Italian Caucasian subject aged 54.60 ± 16.35 years. Body mass index (BMI) 27.70 ± 5.76 kg/m² consecutively admitted according to the request of their primary care doctors for nutritional assessment and work-up. One hundred and sixty of all subjects were with advanced renal insufficiency, treated by hemodialysis (HD); the other 470 were patients without or with slight-moderate renal impairment, quantified by serum creatinine. These patients were briefly defined No-HD. All patients were managed within a protocol which included medical history, physical examination, nutritional and physical activity assessment, electrocardiogram, Chest X-ray, echocardiography and clinical abdomen and thyroid ultrasound. According to MTHFR genotype, 94 of them were MTHFR C677CC (Wild genotype), 118 heterozygous MTHFR C677CT and 104 homozygous MTHFR C677TT (thermolabile polymorphism) subjects. Of the A1298C subjects 80 were homozygous A1298CC, 76 were heterozygous A1298AC; 158 subjects were with a compound MTHFR heterozygous polymorphism, *i.e.*, both MTHFR A1298AC/C677CT. These data are summarized in Table 1. Routine

laboratory tests included virus hepatitis [hepatitis A virus (HAV), HBV, and HCV] and cancer biomarkers (AFP, CEA, Ca125, Ca15-3), thyroid hormones, thyroid stimulating hormone, aspartate Aminotransferase, Alanine Aminotransferase, γ -Glutamyl transpeptidase, ferritin, total protein, and albumin. Human insulin and Folic acid were assayed using immulite 2000 Analyzer, by a solid-phase 2-site chemiluminescent immunometric assay. hsCRP concentrations were assayed by a standard detection limit of 0.175 mg/L (CardioPhase high-sensitivity hsCRP method-Siemens Medical System, Milan, Italy). Homocysteine and B12 Vitamin assay in the blood were performed by ADVIA Centaur® XP Immunoassay (Siemens Medical System, Milan, Italy)^[37]. iPTH and NT-proBNP (IMMULITE® 2000 Siemens Medical System, Milan, Italy) were assessed by a solid phase two-site chemiluminescent immunometric assay. PTH values considered normal were < 70 pg/mL for subjects without severe renal insufficiency^[38]. Body weight (BW) was measured in light clothing, without shoes, in kilograms, and height (H) was measured in meters, using a scale-integrated stadiometer. BMI was calculated as BW/H² and patients were categorized as normal weight (< 25.0 kg/m²), overweight (≥ 25.0 and ≤ 29.9 kg/m²), and obese (≥ 30.0 kg/m²). Insulin resistance was assessed by the homoeostasis model-insulin resistance index (HOMA), according to the following formulas: fasting insulin value x fasting blood sugar level/405. The HOMA threshold for insulin resistance is conventionally considered as > 1.7 , according to the likelihood ratios for 11-year cardiovascular disease prediction^[39]. The Waist-to-Hip (W/H) ratio was assessed in all patients. Ultrasound (US) examinations were performed by echographers unaware of laboratory details at the time of the procedure. An echo-color-doppler machine (Siemens Acuson S2000™, Siemens AG, Muenchen Germany), high resolution, with real-time sectional scan transducers was used. Renal color Doppler echography is performed assessing intra-parenchymal *renal arterial* resistive index, RRI (peak systolic velocity-end diastolic velocity/peak systolic velocity)^[40]. First measurement is the size of the left and right kidney. For orientation purposes, perfusion in the whole of the left and right kidneys is then checked using color Doppler ultrasonography and the main trunk of the renal artery is displayed. Three measurements for each kidney are taken by pulsed Doppler within 5 min in the vicinity of the interlobar artery. RRI is calculated as the average value of all measurements taken. RRI threshold to define higher RRI measurements is defined by the 75th percentile derived by measurements of all eligible patients^[40]. Echocardiographic studies were performed with two-dimensional guided M-mode echocardiography according to methods established by the American Society of Echocardiography (ASE)^[41-44] with transducer frequencies appropriate for body size. Siemens Acuson S2000™, Siemens AG, Muenchen Germany or a GE echo-color-doppler device [GE Logiq7 Expert US, manufactured by GE Medical Systems-Milwaukee-Wisconsin (United States)], high resolution,

Table 1 Differences between methylenetetrahydrofolate reductase groups in all patients

	Wild genotype (n = 94)	Heterozygous MTHFR C677T (n = 118)	Heterozygous MTHFR 1298 AC (n = 76)	Compound heterozygous C677T and A1298C (n = 154)	Homozygous MTHFR 1298 CC (n = 80)	Homozygous MTHFR 677TT (n = 104)	P
Age, yr	53.30 ± 11.89	51.59 ± 17.39	56.74 ± 16.55	57.81 ± 17.04	57.85 ± 14.70	50.12 ± 17.09	< 0.0001
BMI, kg/m ²	27.26 ± 5.04	27.28 ± 5.95	28.04 ± 5.84	27.90 ± 6.13	27.01 ± 3.92	28.72 ± 6.61	0.316
GFR	48.84 ± 32.90	66.05 ± 36.70	64.16 ± 36.32	63.31 ± 38.35	61.57 ± 34.91	68.85 ± 27.33	0.002
Triglycerides, mg/dL	109.96 ± 75.73	113.21 ± 57.91	107.63 ± 42.67	128.72 ± 85.29	95.46 ± 37.07	103.94 ± 48.93	0.003
Total cholesterol, mg/dL	191.67 ± 41.71	206.29 ± 52.53	203.84 ± 38.73	198.28 ± 41.37	196.06 ± 54.99	201.54 ± 34.49	0.206
HDL cholesterol, mg/dL	58.11 ± 20.91	55.17 ± 15.75	55.28 ± 18.46	52.52 ± 18.49	52.51 ± 18.50	56.27 ± 16.35	0.175
LDL cholesterol, mg/dL	111.57 ± 34.89	128.47 ± 48.01	127.04 ± 31.67	120.81 ± 35.73	124.46 ± 48.10	124.89 ± 35.30	0.039
AST, U/L	19.50 ± 6.30	23.32 ± 14.23	27.93 ± 17.69	20.49 ± 6.91	21.76 ± 12.26	19.72 ± 5.99	< 0.0001
ALT, U/L	15.82 ± 4.59	16.46 ± 5.38	18.51 ± 5.75	16.46 ± 5.93	16.59 ± 5.74	15.92 ± 5.90	0.031
γGT, U/L	24.63 ± 12.20	33.82 ± 25.96	37.45 ± 38.03	42.71 ± 48.64	28.94 ± 15.17	25.37 ± 16.29	< 0.0001
HOMA	2.00 ± 1.13	3.18 ± 3.49	3.04 ± 2.27	4.04 ± 4.87	2.28 ± 1.07	2.76 ± 2.77	< 0.0001
PTH, pg/mL	84.94 ± 100.37	84.49 ± 170.97	78.37 ± 65.95	86.30 ± 76.75	86.53 ± 95.97	84.83 ± 81.83	0.997
hsCRP, mg/dL	2.58 ± 4.41	2.15 ± 2.79	6.30 ± 13.55	4.30 ± 8.66	3.99 ± 6.42	3.51 ± 4.95	< 0.001
RRI	0.60 ± 0.05	0.59 ± 0.05	0.59 ± 0.04	0.58 ± 0.05	0.59 ± 0.07	0.59 ± 0.06	0.392
EF, %	67.05 ± 8.18	66.94 ± 9.19	65.99 ± 9.15	63.52 ± 12.04	67.15 ± 11.63	66.51 ± 7.26	0.035
E/A	1.15 ± 0.36	1.20 ± 0.26	1.23 ± 0.34	1.09 ± 0.40	1.01 ± 0.26	1.18 ± 0.31	< 0.0001
LVMM/m ²	100.48 ± 54.70	105.44 ± 33.79	107.69 ± 48.47	109.21 ± 41.02	110.38 ± 46.63	97.11 ± 28.69	0.179
AMDS	34.94 ± 2.52	34.97 ± 3.03	33.42 ± 3.88	34.23 ± 3.02	34.93 ± 2.68	34.46 ± 3.18	0.005
Homocysteine μmol/L	17.41 ± 3.00	25.53 ± 8.12	28.58 ± 9.23	18.68 ± 9.01	21.26 ± 9.17	18.83 ± 6.25	< 0.0001

BMI: Body mass index; GFR: Glomerular filtration rate; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: γ-Glutamyl Transpeptidase; hsCRP: High-sensitivity C-reactive protein; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; AMDS: Adherence mediterranean diet score; MTHFR: Methylene tetrahydrofolate reductase; PTH: Parathyroid hormone; HOMA: Homoeostasis model-insulin resistance index.

with real-time sectional scan transducers were used. An average of two echocardiographic measurements was taken and the cardiologist reading them was blinded to the clinical information of the patient. Measurements were obtained for LV end-diastolic and end-systolic dimension, septal wall thickness and posterior wall thickness in diastole. LVM was calculated with the method of Devereux *et al*^[44] and indexed by dividing by body surface area/m². All the exams were stored on digital media for subsequent analysis. LV diameters and wall thickness were measured according to the ASE guidelines and LV ejection fraction (LVEF) accordingly^[41]. LVEF was considered abnormal if < 50%. GFR is assessed as estimated GFR by the modification of diet in renal disease formula in mL/min per 1.73 m², according to the Clinical Practice Guidelines for Chronic Kidney Disease KDOQI^[38]. Genotypes of the MTHFR C677T and A1298C polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). DNA was extracted from peripheral blood by a commercially available DNA isolation method (QIAamp DNA Blood Mini Kit QIAGEN, Milan, Italy). Restriction enzyme analysis of amplified product (PCR-RFLP) analysis was carried out for direct genotypes detection of single nucleotide polymorphisms, C667T (rs1801133) and A1298C (rs1801131). PCR products were obtained using specific primers (NCBI Reference Sequence: NG_013351.1): C667T (5'-GTCCTGTGCTCTTCATCC-3'/R5'-GGTGGCCAAGCAAGCTGTG-3'); A1298C (5'-CTTCTACCTGAGAGCAAGTC-3'/R5'-CACATGTCACAGCATGGAC-3'). Both amplicons were successively digested by HinfI and MboII restriction enzymes for C667T and A1298C respectively, and DNA fragment visualized in a 4% agarose gel stained with Synergy Brand Inc safe (Life Technologies Italia, Monza, Italy); electrophoresis pattern was used to determined MTHFR genotypes^[45]. Informed consent was obtained from each patient, relatively also to the use of genetic information, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Statistical analysis

The fit to the Hardy-Weinberg equilibrium was analyzed. Student's *t* test was used to assess the difference between subject with advanced renal insufficiency, treated by

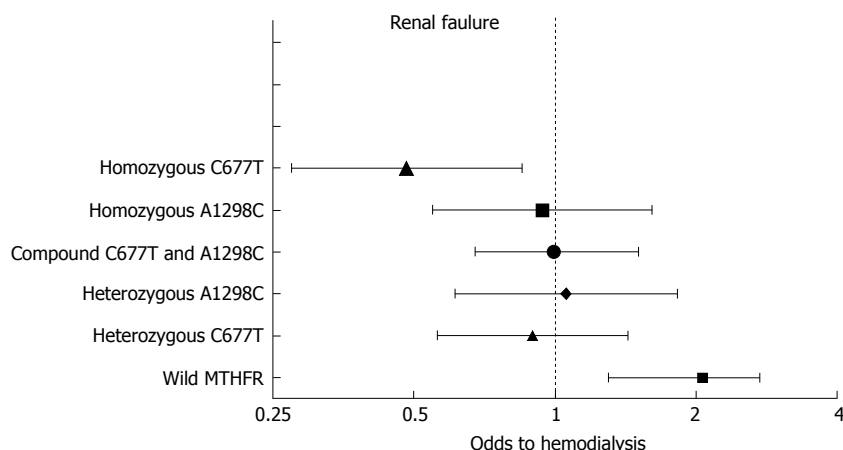


Figure 1 Odds to renal failure-hemodialysis. Comparison of the wild MTHFR genotype in dialysis patients (36/160; 22.5%) vs the No-HD group (58/470; 12.3%): $P < 0.003$; OR = 2.062 (95%CI: 1.3-3.273), *i.e.*, the wild MTHFR genotype bears a double risk of renal failure in comparison with all MTHFR polymorphisms and a four-fold risk vs the Homozygous C677T MTHFR polymorphism. The individual odds of renal failure, according to the specific MTHFR polymorphism status (hemodialysis patients 160 vs No-HD group 470) are not significant, with the exception of the Homozygous C677T MTHFR polymorphism which exhibits a protective behavior. Heterozygous C677T (28/160 vs 90/470); $P = \text{NS}$. OR = 0.896 (95%CI: 0.561-1.43); Heterozygous A1298C (20/160 vs 56/470); $P = \text{NS}$. OR = 1.056 (95%CI: 0.612-1.822); Compound Heterozygous C677T and A1298C (40/160 vs 118/470); $P = \text{NS}$. OR = 0.994 (95%CI: 0.657-1.505); Homozygous A1298C (20/160 vs 62/470); $P = \text{NS}$. OR = 0.94 (95%CI: 0.548-1.612); Homozygous C677T (16/160 vs 88/470); $P = 0.015$. OR = 0.482 (95%CI: 0.274-0.85). MTHFR: Methylene tetrahydrofolate reductase; OR: Odds ratio; NS: Not significant.

hemodialysis and No-HD group. ANOVA was used to assess the difference in averages between subjects with MTHFR heterozygous, compound and homozygous polymorphism. Descriptive results of continuous variables are expressed as averages (\pm SD). Two-sided $P < 0.05$ was considered statistically significant. The distributions of MTHFR alleles and genotypes in studied group, *i.e.*, normal-impaired renal function vs hemodialysis patients were checked by χ^2 test or Fisher's exact test. Higher quartiles of age, homocysteine, iPTH, RRI, hsCRP and of other continue measures were defined; thereafter, the associations of older age, higher hsCRP, iPTH, RRI, Left Ventricular Hypertrophy (LVMMi ≥ 135 g/m² in men, ≥ 110 g/m² in women^[46]) and MTHFR polymorphisms were assessed as odds ratios (ORs) to severe chronic renal failure in hemodialysis with 95% CIs. Statistical analyses were performed using SPSS 18.0 for Windows (SPSS, Chicago, IL), Likelihood Ratio was assessed and sensitivity, specificity and predictivity were calculated by the CEBM Statistics Calculator, by Courtesy of CEBM, and graphs by Prism-Graphpad. Venn Diagram Plotter was used by courtesy of Pacific Northwest National Laboratory.

RESULTS

The differences of averages of measures between patients with MTHFR 677C>T heterozygous and homozygous polymorphism, of heterozygous and homozygous MTHFR 1298A>C polymorphism and of compound heterozygous MTHFR 677C>T/MTHFR 1298A>C polymorphism vs wild genotype subjects are shown in Table 1. Glomerular filtration rate is significantly higher in all the polymorphism groups vs wild genotype subjects, with figures greater of about 30%-35% more. Difference of age, even significant, are actually minor and, in

any case, subjects with polymorphisms are older; homocysteine and low-density lipoprotein cholesterol are slightly higher in the MTHFR 677C>T polymorphism group vs wild genotype subjects. There are internal relationships between most measures: a significant linear correlation of GFR vs LVMMi ($r = -0.37$; $P < 0.0001$) is observed. Significant inverse correlation of age vs GFR ($r = -0.56$; $P < 0.0001$) and direct correlations of age vs RRI ($r = 0.41$; $P < 0.0001$), and vs LVMMi ($r = 0.29$; $P < 0.001$) are observed. iPTH shows significant inverse correlation vs GFR ($r = -0.34$; $P < 0.0001$), whereas a direct trend of iPTH is observed vs RRI ($r = 0.32$; $P < 0.001$) and vs LVMMi ($r = 0.14$; $P < 0.05$). No significant correlation is observed both for hsCRP and insulin resistance (HOMA) vs GFR, LVMMi and RRI.

Characteristic of study population and differences between HD patients (HD) and No-HD are reported in Table 2.

A significant difference is observed, overall, for the prevalence of wild MTHFR genotype in dialysis patients (36/160; 22.5%) vs the No-HD group (58/470; 12.3%): $P < 0.003$; OR = 2.062 (95%CI: 1.3-3.273), *i.e.*, the wild MTHFR genotype bears a double risk of renal failure in comparison with MTHFR polymorphisms and a four-fold risk vs the Homozygous C677T MTHFR polymorphism. The individual odds of renal failure, according to the specific MTHFR polymorphism status (hemodialysis patients 160 vs No-HD group 470) are not significant, with the exception of the Homozygous C677T MTHFR polymorphism which exhibits a protective behavior (Figure 1).

Likelihood ratio was assessed and sensitivity, specificity and predictivity, which were all very weak and substantially non-contributory: Homozygous C677T MTHFR polymorphism displays a Sensitivity of 0.154

Table 2 Characteristic of study population and differences between hemodialysis patients and no-hemodialysis *n* (%)

	Total (<i>n</i> = 630)	Dialysis patients (<i>n</i> = 160)	Patients with maintained Renal function (<i>n</i> = 470)	<i>P</i>
Women	336 (53.3)	72	264	0.014 ¹
Obese patients	196 (31.1)	24	172	< 0.000 ¹
Patients with GFR < 90	514 (81.6)	160	354	< 0.000 ¹
NAFLD patients	256 (40.6)	28	228	< 0.000 ¹
MTHFR group				
Wild genotype	94 (14.9)	36	58	0.016 ¹
MTHFR C677T	118 (18.7)	28	90	
MTHFR 1298 AC	76 (12.1)	20	56	
Compound heterozygous C677T and A1298C	158 (25.1)	40	118	
MTHFR 1298 CC	80 (12.7)	20	60	
MTHFR 677TT	104 (16.5)	16	88	
Age, yr	54.60 ± 16.35	67.48 ± 14.57	50.22 ± 14.51	< 0.0001
BMI, kg/m ²	27.70 ± 5.76	25.29 ± 3.97	28.52 ± 6.04	< 0.0001
Blood glucose, mg/dL	96.42 ± 26.42	95.33 ± 34.80	96.79 ± 22.91	0.545
Blood urea, mg/dL	52.47 ± 35.74	100.45 ± 41.07	36.13 ± 9.40	< 0.0001
Creatinin, mg/dL	2.36 ± 2.98	6.75 ± 2.99	0.86 ± 0.21	< 0.0001
GFR	62.46 ± 35.32	9.28 ± 3.60	80.56 ± 19.38	< 0.0001
Triglycerides, mg/dL	112.16 ± 64.71	131.90 ± 87.21	105.44 ± 53.48	< 0.0001
Total cholesterol, mg/dL	199.72 ± 44.43	175.80 ± 42.67	207.86 ± 42.05	< 0.0001
HDL cholesterol, mg/dL	54.81 ± 18.10	48.20 ± 15.63	57.07 ± 18.34	< 0.0001
LDL cholesterol, mg/dL	122.75 ± 39.63	101.22 ± 33.04	130.09 ± 39.05	< 0.0001
AST, U/L	21.81 ± 11.16	14.38 ± 4.07	24.34 ± 11.66	< 0.0001
ALT, U/L	16.54 ± 5.63	12.75 ± 4.15	17.83 ± 5.49	< 0.0001
γGT, U/L	33.10 ± 32.11	31.78 ± 19.18	33.55 ± 35.46	0.546
Insulin	11.84 ± 9.73	11.44 ± 10.77	11.98 ± 9.36	0.547
HOMA	3.02 ± 3.30	3.08 ± 3.94	3.00 ± 3.05	0.797
PTH, pg/mL	84.58 ± 105.79	162.38 ± 178.81	57.99 ± 36.85	< 0.0001
hsCRP, mg/dL	3.52 ± 7.01	2.62 ± 2.45	3.82 ± 7.98	0.107
Albumin, g/dL	4.60 ± 0.37	4.64 ± 0.35	4.58 ± 0.37	0.119
Albumin, %	62.39 ± 3.60	62.60 ± 3.03	62.31 ± 3.77	0.388
RRI	0.62 ± 0.06	0.68 ± 0.03	0.60 ± 0.06	< 0.0001
EF, %	65.93 ± 9.99	61.03 ± 12.62	67.87 ± 7.95	< 0.0001
E/A	1.14 ± 0.33	1.03 ± 0.39	1.18 ± 0.30	< 0.0001
LVMM/m ²	104.95 ± 42.10	135.37 ± 55.56	93.84 ± 28.91	< 0.0001
AMDS	34.51 ± 3.09	35.93 ± 1.69	34.02 ± 3.31	< 0.0001
Homocysteine, μmol/L	2.1 ± 5.4	36.8 ± 8.5	21.2 ± 7.7	< 0.0001

¹Pearson χ^2 . GFR: Glomerular filtration rate; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: γ-Glutamyl transpeptidase; hsCRP: High-sensitivity C-reactive protein; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; AMDS: Adherence mediterranean diet score; MTHFR: Methylene-tetrahydrofolate reductase; PTH: Parathyroid hormone; HOMA: Homoeostasis model-insulin resistance index; NAFLD: Non-alcoholic fatty liver disease.

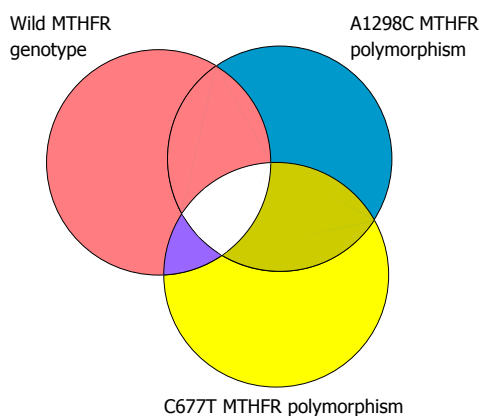


Figure 2 Venn diagram showing proportionally the overlap of methylene-tetrahydrofolate reductase genetic polymorphisms A1298C and C677T with the wild one. The three groups have very relevant overlaps in the studied population. MTHFR: Methylene-tetrahydrofolate reductase.

(0.097-0.235), with a Specificity of 0.726 (0.687-0.763); the positive predictive value is 0.1 (0.062-0.156) and the negative predictive value is 0.813 (0.775-0.845); the positive likelihood ratio is LR+ 0.562 (0.351-0.901), the negative likelihood ratio is LR- 1.165 (1.057-1.284). Similarly, for the wild MTHFR genotype, Sensitivity is 0.383 (0.291-0.484); Specificity is 0.769 (0.731-0.802); PPV is 0.225 (0.167-0.296); NPV is 0.877 (0.844-0.903); LR+ is 1.655 (1.227-2.233) and LR- is 0.803 (0.68-0.948).

In Figure 2 the polymorphism overlap is displayed by Venn diagram showing proportionally the overlap of MTHFR genetic polymorphisms A1298C and C677T with the wild one. The three groups have very relevant overlaps in the studied population.

Odds to LVH (assessed as increased Left Ventricular Myocardial Mass by Echocardiography), by the comparison of the prevalence of LVH within the wild

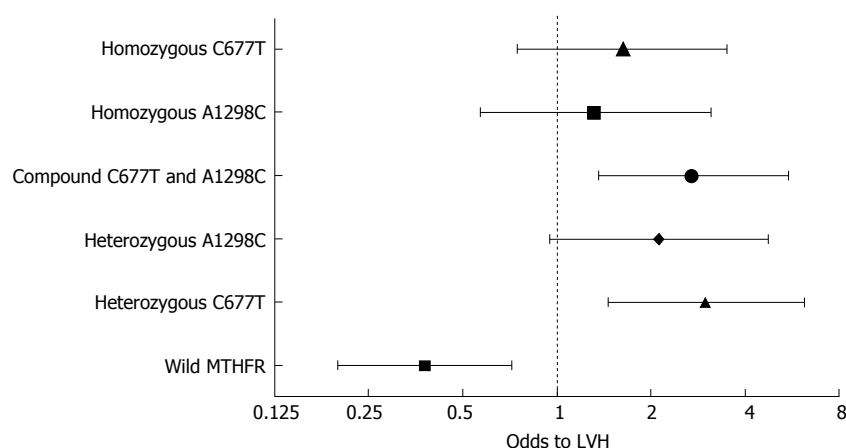


Figure 3 Odds to left ventricular hypertrophy (increased left ventricular myocardial mass assessed by echocardiography). Comparison of prevalence of LVH within the wild MTHFR genotype (12/94; 12.7%) vs the polymorphism MTHFR group (131/470; 27.9%); OR = 0.3787; 95%CI: 0.2000 to 0.7171; Z statistic 2.981; $P = 0.0029$, *i.e.*, the wild MTHFR genotype bears a significantly lower risk of LVH in comparison with all MTHFR polymorphisms. The individual odds of LVH, according to the specific MTHFR polymorphism status are as follows: Heterozygous C677T (12/94; 12.7% vs 36/118); OR = 3.0000, 95%CI: 1.4581 to 6.1725, Z statistic 2.985, $P = 0.0028$; Heterozygous A1298C (12/94; 12.7% vs 18/76); OR = 2.1207, 95%CI: 0.9490 to 4.7393, Z statistic 1.832, $P = 0.0669$; Compound Heterozygous C677T and A1298C (12/94; 12.7% vs 44/154); OR = 2.7333, 95%CI: 1.3581 to 5.5012, Z statistic 2.818, $P = 0.0048$; Homozygous A1298C (12/94; 12.7% vs 13/80); OR = 1.3259, 95%CI: 0.5676 to 3.0972, Z statistic 0.652, $P = 0.5146$; Homozygous C677T (12/94; 12.7% vs 20/104); OR = 1.6270, 95%CI: 0.7475 to 3.5410, Z statistic 1.227, $P = 0.2199$. LVH: Left ventricular hypertrophy; MTHFR: Methylene tetrahydrofolate reductase; OR: Odds ratio.

Table 3 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation ($E/A > 1$), and left ventricular hypertrophy (all patients)

	Wild MTHFR (n = 94)	Heterozygous C677T (n = 118)	Heterozygous A1298C (n = 76)	Compound heterozygous C677T and A1298C (n = 154)	Homozygous A1298C (n = 80)	Homozygous C677T (n = 104)	χ^2	P
high RRI	24	30	16	38	28	22	5.746	0.332
EF < 50%	4	4	4	14	4	0	11.188	0.048
E/A > 1	68	100	66	74	42	70	53.497	< 0.0001
LVH (high LVMM)	12	36	18	44	13	20	14.923	0.011

Pearson χ^2 . MTHFR: Methylene tetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; LVH: Left ventricular hypertrophy.

MTHFR genotype (12/94; 12.7%) vs the polymorphism MTHFR group (131/470; 27.9 %) displays an OR = 0.3787; 95%CI: 0.2000-0.7171; Z statistic 2.981; $P = 0.0029$, *i.e.*, the wild MTHFR genotype bears a significantly lower risk of LVH in comparison with all MTHFR polymorphisms (Figure 3). The individual odds of LVH, according to the specific MTHFR polymorphism status, confirm substantially this result, *i.e.*, that MTHFR polymorphisms are associated with LVH. Differences are not significant assessing Ejection fraction and Renal Resistive index. The E/A ratio, *i.e.*, the measurement of left ventricular transmitral filling, and index of overall left ventricular distensibility, is higher, *i.e.*, better, in subjects with the wild MTHFR genotype (Tables 3-5).

DISCUSSION

According to our study, the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show an association with chronic renal failure patients requiring hemodialysis which suggests some protective

role in comparison with the wild MTHFR genotype. Despite the apparent disagreement with the available studies with renal disease patients, this result is less surprising of what can appear at the first glance. Even with the limitations of an observational study, based on the reappraisal of the information available in our data base investigating within a greater population that includes a subgroup of dialysis patients, we find that the concept that MTHFR polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure, appears reasonable and deserving further and more systematic research. A similar finding was reported in fatty liver disease in which it is suggested that MTHFR polymorphisms could have maintained and maintain their persistence by an heterozygosity advantage mechanism^[35].

Homocysteine is settled as a putative risk factor for cardiovascular disease^[47] and mechanisms for glomerular injury and progression of renal insufficiency are envisaged^[48]. Although high-dose folic acid would slow the progression of atherosclerosis and reduce

Table 4 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation (E/A > 1), and left ventricular hypertrophy (chronic renal failure patients-hemodialysis)

	Wild MTHFR (n = 36)	Heterozygous C677T (n = 28)	Heterozygous A1298C (n = 20)	Compound heterozygous C677T and A1298C (n = 40)	Homozygous A1298C (n = 20)	Homozygous C677T (n = 16)	χ^2	P
High RRI	20	20	8	28	16	12	10.535	0.061
EF < 50%	4	4	4	12	4	0	9.114	0.105
E/A > 1	24	24	16	8	0	0	72.305	< 0.0001
High LVMM	8	16	4	32	5	4	38.428	< 0.0001

Pearson χ^2 . MTHFR: Methylene tetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial.

Table 5 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation (E/A > 1), and left ventricular hypertrophy (normal renal function patients)

	Wild MTHFR (n = 58)	Heterozygous C677T (n = 90)	Heterozygous A1298C (n = 56)	Compound heterozygous C677T and A1298C (n = 118)	Homozygous A1298C (n = 62)	Homozygous C677T (n = 88)	χ^2	P
high RRI	4	10	8	10	12	10	6.833	0.233
EF < 50%	0	0	0	2	0	0	5.798	0.326
E/A > 1	44	76	50	66	42	70	19.848	0.001
High LVMM	4	20	14	12	8	16	13.355	0.02

Pearson χ^2 . MTHFR: Methylene tetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial.

cardiovascular events in patients with chronic renal failure, counteracting effects of hyperhomocysteinemia, is still debated and not demonstrated^[49]. Differently, there is a good consistency of data that establishes renal involvement and LV hypertrophy as novel risk factors for morbidity and mortality in diabetes mellitus^[50]. Cardiac remodeling, also with increase of LVMM, is a premise toward the development of heart insufficiency^[51], which could be redefined also encompassing serological biomarkers^[52]. The favorable relevance of adherence to healthier nutritional profile and lifestyle changes is well established and warranted in cardiac disease^[53,54] and also, by more recent contributions, in renal disease^[55]. In earlier studies^[56,57] relationship of MTHFR C677T mutation with renal and cardiac involvement was associated with precocious target organ damage. Actually, in younger subjects^[58] and in other reports^[59] homozygosity for the C677T mutation is not unequivocally associated with increased risk for cardiovascular disease, irrespective of folate intake. This is confirmed by a recent extensive epidemiological study, in which despite lower serum folate and higher homocysteine, MTHFR 677TT genotype, used as a proxy for lifelong high blood homocysteine concentrations, is associated with a significantly lower risk of CVD mortality^[60]. Hyperhomocysteinemia is common in patients with severe heart failure, and plasma homocysteine levels are uniformly elevated regardless of the etiology of heart failure. Elevated plasma homocysteine levels are likely a consequence of heart failure-related renal insufficiency^[61]. Moreover, high homocysteine levels in patients with end-stage renal disease were not associated with incidence of vascular access thrombosis^[62]. In our study, MTHFR

C677T mutation occurs in a population which has still a relatively low prevalence of cardiovascular^[5] and renal disease^[55]. It is possible that this polymorphism, even associated with greater LVMMi, could have maintained its persistence in human populations by an heterozygosis-mutant advantage mechanism exerted over more critical conditions, including the occurrence of renal insufficiency. All-cause and coronary heart disease death rates are low in cohorts with greater adherence to Mediterranean Diet.

In conclusion, MTHFR 677C>T and A1298A>C gene polymorphisms could have a protective role on renal function as suggested by the lower frequency of both polymorphisms among our dialysis patients in end-stage renal failure; differently, the association with LV hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism related to MTHFR polymorphisms.

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COMMENTS

Background

Hyperhomocysteinemia is a frequent condition among patients both in end-stage renal disease and on dialysis and may represent an additional risk factor for increased cardiovascular disease. It is recognized that supplementation with folic acid may often reduce, but not always and permanently correct hyperhomocysteinemia. More important, this approach does not reduce cardiovascular events in patients with kidney disease so that Folic acid based regimens are not recommended as a generalized approach in the prevention of

cardiovascular events in chronic kidney disease.

Research frontiers

A similar finding was reported in fatty liver disease in which it is suggested that methylenetetrahydrofolate reductase (MTHFR) polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure.

Innovations and breakthroughs

The authors reported that renal insufficiency in non-diabetic subjects is explained by interactions of MTHFR C677T polymorphism mutation with left ventricular (LV) hypertrophy (LVH), high-sensitivity C-reactive protein (hsCRP), intact parathyroid hormone (iPTH), and renal artery resistive index (RRI). Sign of these predictive effects is opposite: subjects with MTHFR 677C>T polymorphism have lower likelihood of renal insufficiency; differently, wild-type MTHFR genotype subjects have lower glomerular filtration rate and greater hsCRP, iPTH, RRI, and LVH.

Applications

MTHFR gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among the authors dialysis patients in end-stage renal failure; differently, the association with LVH and reduced LV relaxation suggest some type of indirect, or concurrent mechanism.

Peer-review

This is a well written manuscript analysing the effect of MTHFR gene polymorphisms on renal and cardiac function.

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