Relationship of MTHFR gene polymorphisms with renal and cardiac disease

Francesca M Trovato, Daniela Catalano, Angela Ragusa, G Fabio Martines, Clara Pirri, Maria Antonietta Buccheri, Concetta Di Nora, Guglielmo M Trovato

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
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.

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. 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. 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 (CVD) especially in the natural story of chronic kidney disease. The more common MTHFR polymorphism (nucleotide 677 C>T) results in a thermolabile enzyme, lower folate levels and an inefficient homocysteine metabolism. 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. Hyperhomocysteinemia appears independent from other risk factors and subsequent reports increased concerns around the related common genetic polymorphism despite earlier studies already challenged this concept. Since this polymorphism prevalence in the elderly is not lower than in the young, 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, and for colonic cancer, seemingly associated with longer and healthier survival. Nonetheless, according to other studies, MTHFR 677TT homozygous and systolic blood pressure independently influence intima-media thickness as other non-genetic markers and nutritional conditions do. Also mild-moderate renal impairment is associated with mortality, increased left ventricular (LV) myocardial mass, lower Ejection Fraction and increased E/A ratio at echocardiography. Insulin resistance accounts significantly for LV mass increase in normotensive individuals. A linear relationship between LV myocardial mass/m² (LVMMI) vs cardiovascular events, a J-shape relationship between LVMMI vs all-cause death and NT-proBNP increase in patients with LV hypertrophy (LVH) suggest a common pathway, through the increase of measured myocardial mass, toward cardiac insufficiency. 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, suggesting the need of intervention. MTHFR polymorphisms 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. 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. The accompanying body size increase is not invariably detrimental since, actually, patients with established chronic disease benefit of large body size. 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. 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, 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 LVM 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 with 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 echographists 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] (A-Phi)® with transducer frequencies appropriate for body size. Siemens Acuson S2000™, Siemens AG, Muenchen Germany or a GE echo-color-doppler device (GE Logic7 Expert US, manufactured by GE Medical Systems-Milwaukee-Wisconsin (United States)), high resolution,
Table 1 Differences between methylenetetrahydrofolate reductase genotypes in all patients

| Genotype | LV end-diastolic dimension (cm) | LV ejection fraction (%) | Total cholesterol (mg/dL) | HDL cholesterol (mg/dL) | LDL cholesterol (mg/dL) | Triglycerides (mg/dL) | hsCRP (mg/dL) | P value |
|----------|--------------------------------|--------------------------|--------------------------|-------------------------|------------------------|----------------------|---------------|---------|
| Wild      | 3.99 ± 6.42                    | 53.30 ± 11.89            | 2.28 ± 1.07              | < 0.0001                | 127.04 ± 31.67         | 1.09 ± 0.40         | 0.392         | < 0.0001 |
| Heterozygous MTHFR C677T | 201.54 ± 34.49 | 55.17 ± 15.75 | 1.23 ± 0.34 | 3.04 ± 2.27 | 52.51 ± 18.50 | 3.18 ± 3.49 | 18.51 ± 5.75 | 0.60 ± 0.05 | 0.0039 |
| Compound heterozygous C677T and A1298C | 84.94 ± 100.37 | 0.039 | 18.68 ± 9.01 | 34.46 ± 3.18 | 25.37 ± 16.29 | 0.031 | 110.38 ± 46.63 | 66.94 ± 9.19 | 27.28 ± 5.95 | 57.85 ± 14.70 | 107.69 ± 48.47 | 34.97 ± 3.03 | 33.82 ± 25.96 | 15.82 ± 4.59 | 206.29 ± 52.53 | 21.26 ± 9.17 | 20.49 ± 6.91 | 0.0001 |

BMI: Body mass index; GFR: Glomerular filtration rate; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GT: -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; PTH: Parathyroid hormone; HOMA: Homoeostasis model-insulin resistance index.
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 are as follows (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); $P =$ NS. OR = 0.896 (95% CI: 0.561-1.43); Heterozygous A1298C (20/160 vs 56/470); $P =$ NS. OR = 1.056 (95% CI: 0.612-1.822); Compound Heterozygous C677T and A1298C (40/160 vs 118/470); $P =$ NS. OR = 0.994 (95% CI: 0.657-1.505); Homozygous A1298C (20/160 vs 62/470); $P =$ 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.

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 are as follows (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); $P =$ NS. OR = 0.896 (95% CI: 0.561-1.43); Heterozygous A1298C (20/160 vs 56/470); $P =$ NS. OR = 1.056 (95% CI: 0.612-1.822); Compound Heterozygous C677T and A1298C (40/160 vs 118/470); $P =$ NS. OR = 0.994 (95% CI: 0.657-1.505); Homozygous A1298C (20/160 vs 62/470); $P =$ 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.

Licorice was used by courtesy of Pacific Northwest Laboratory.

RESULTS

The differences of averages of measures between patients with MTHFR 677C>T heterozygous and homozgyous 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. IPT shows significant inverse correlation vs GFR ($r = -0.34; P < 0.0001$). whereas a direct trend of IPT 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.

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 are as follows (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).

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.
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.

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

| Characteristic                        | Total (n = 630) | Dialysis patients (n = 160) | Patients with maintained Renal function (n = 470) |
|---------------------------------------|----------------|-----------------------------|---------------------------------------------------|
| Women                                 | 336 (53.3)     | 72                          | 264                                                |
| Obese patients                        | 196 (31.1)     | 24                          | 172                                                |
| Patients with GFR < 90                | 514 (81.6)     | 160                         | 354                                                |
| NAFLD patients                        | 256 (40.6)     | 28                          | 228                                                |
| MTHFR group                           |                |                             |                                                   |
| Wild genotype                         | 94 (14.9)      | 36                          | 58                                                 |
| 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                                      |
| BMI, kg/m²                             | 27.70 ± 5.76   | 25.29 ± 3.97                | 28.52 ± 6.04                                      |
| Blood glucose, mg/dL                  | 96.42 ± 26.42  | 95.33 ± 34.80               | 96.79 ± 22.91                                      |
| Blood urea, mg/dL                     | 52.47 ± 35.74  | 100.45 ± 41.07              | 36.13 ± 9.40                                       |
| Creatinin, mg/dL                      | 2.36 ± 2.98    | 6.75 ± 2.99                 | 0.86 ± 0.21                                       |
| GFR                                    | 62.46 ± 35.32  | 9.28 ± 3.60                 | 80.56 ± 19.38                                     |
| Triglycerides, mg/dL                  | 112.16 ± 64.71 | 131.90 ± 67.21              | 105.44 ± 53.48                                     |
| Total cholesterol, mg/dL              | 199.72 ± 44.43 | 175.80 ± 42.05              | 207.86 ± 42.05                                     |
| HDL cholesterol, mg/dL                | 54.81 ± 18.10  | 48.20 ± 15.63               | 57.07 ± 18.34                                     |
| LDL cholesterol, mg/dL                | 122.75 ± 39.63 | 101.22 ± 33.04              | 130.09 ± 39.05                                     |
| AST, U/L                               | 21.81 ± 11.16  | 14.38 ± 4.07                | 24.34 ± 11.66                                     |
| ALT, U/L                               | 16.54 ± 5.63   | 12.73 ± 4.15                | 17.83 ± 5.49                                      |
| γ-GT, U/L                              | 33.10 ± 32.11  | 31.78 ± 19.18               | 33.55 ± 35.46                                     |
| Insulin                                | 11.84 ± 9.75   | 11.44 ± 10.77               | 11.98 ± 9.36                                      |
| HOMA                                    | 3.02 ± 3.39    | 3.08 ± 3.94                 | 3.00 ± 3.05                                       |
| PTH, pg/mL                             | 84.58 ± 150.79 | 162.38 ± 178.81             | 57.99 ± 36.85                                     |
| hsCRP, mg/dL                           | 3.52 ± 7.01    | 2.62 ± 2.45                 | 3.82 ± 7.98                                       |
| Albumin, g/dL                          | 4.60 ± 0.37    | 4.64 ± 0.35                 | 4.58 ± 0.37                                       |
| Albumin, %                             | 62.39 ± 3.60   | 62.60 ± 3.03                | 62.31 ± 3.77                                      |
| RRI                                    | 62.02 ± 0.06   | 68.0 ± 0.63                 | 63.0 ± 0.66                                       |
| EF, %                                  | 65.93 ± 9.99   | 61.03 ± 12.62               | 67.87 ± 7.95                                      |
| E/A                                    | 1.14 ± 0.33    | 1.03 ± 0.39                 | 1.18 ± 0.30                                       |
| AMDS                                   | 104.95 ± 42.10 | 135.37 ± 55.56              | 93.84 ± 28.91                                     |
| LVMM/m²                                | 34.51 ± 3.09   | 35.93 ± 1.69                | 34.02 ± 4.31                                     |
| Homocysteine, μmol/L                   | 2.1 ± 5.4      | 36.8 ± 8.5                  | 21.2 ± 7.7                                        |

1Pearson χ², GFR: Glomerular filtration rate; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GT: 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: Homeostasis model-insulin resistance index; NAFLD: Non-alcoholic fatty liver disease.
**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 studied 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 heterozygosis advantage mechanism[48].

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
cardiovascular events in patients with chronic renal failure, counteracting effects of hyperhomocysteinemia, is still debated and not demonstrated\(^ \text{[55]} \). 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\(^ \text{[56]} \). Cardiac remodeling, also with increase of LVMM, is a premise toward the development of heart insufficiency\(^ \text{[57]} \), which could be redefined also encompassing serological biomarkers\(^ \text{[58]} \). The favorable relevance of adherence to healthier nutritional profile and lifestyle changes is well established and warranted in cardiac disease\(^ \text{[59]} \). The relationship of MTHFR C677T mutation with cardiac disease is still debated and not demonstrated\(^ \text{[60]} \). It is possible that this polymorphism, even associated with greater LVMM, 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 C677T>C and A1298A>C gene polymorphisms could have a protective role on renal function as suggested by the lower frequency of both polymorphisms in our dialysis patients and on dialysis patients with kidney disease. Conversely, the association with LV hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism related to MTHFR polymorphisms.

**ACKNOWLEDGMENTS**

These results were presented in part at the American Society for Nutrition 76th Annual Meeting held in San Diego, CA, April 21-25, 2012, and published as abstract on FASEB J (2012) 26: lb328.

**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

---

**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) |
|----------------|---------------------|-----------------------------|----------------------------|---------------------------------|--------------------------|--------------------------|
| High RRI       | 20                  | 20                          | 8                          | 28                              | 16                       | 12                       |
| EF < 50%       | 4                   | 4                           | 4                           | 4                               | 4                        | 0                        |
| E/A > 1        | 24                  | 24                          | 16                          | 8                               | 0                        | 0                        |
| High LVMM      | 8                   | 16                          | 4                           | 32                              | 5                        | 4                        |

Pearson $\chi^2$. 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) |
|----------------|---------------------|-----------------------------|----------------------------|---------------------------------|--------------------------|--------------------------|
| High RRI       | 4                   | 10                          | 8                          | 10                              | 12                       | 10                       |
| EF < 50%       | 0                   | 0                           | 0                           | 2                               | 0                        | 0                        |
| E/A > 1        | 44                  | 76                          | 50                          | 66                              | 42                       | 70                       |
| High LVMM      | 4                   | 20                          | 14                          | 12                              | 8                        | 16                       |

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

C677T mutation occurs in a population which has still a relatively low prevalence of cardiovascular disease\(^ \text{[55]} \) and renal disease\(^ \text{[56]} \). It is possible that this polymorphism, even associated with greater LVMM, 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 C677T>C and A1298A>C gene polymorphisms could have a protective role on renal function as suggested by the lower frequency of both polymorphisms in our dialysis patients and on dialysis patients with kidney disease. Conversely, the association with LV hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism related to MTHFR polymorphisms.
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 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.

**REFERENCES**

1. Siafan C, El-Charabaty E, El-Sayegh S. Hyperhomocysteinemia and vascular access thrombosis in hemodialysis patients: a retrospective study. *Vasc Health Risk Manag* 2013; 9: 361-364

2. Jardine MJ, Kang A, Zoumas S, Navaneethan SD, Ninomiya T, Nwigwekar SU, Gallagher MP, Cass A, Strippoli G, Perkovic V. The effect of folic acid based homocysteine lowering on cardiovascular events in people with kidney disease: systematic review and meta-analysis. *BMJ* 2012; 344: e3533 [PMID: 22695899 DOI: 10.1136/bmj.e3533]

3. Jacques PF, Boston AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg JH, Selhub J, Rozen R. Relation between folate status, methylenetetrahydrofolate reductase (MTHFR) polymorphism and colorectal cancer risk in three large nested case-control studies. *Cancer Causes Control* 2012; 23: 537-545 [PMID: 22677271 DOI: 10.1007/s10552-012-9911-3]

4. Rea IM, McMaster D, Woodside JV, Young IS, Archbold GP, Linton T, Lennox S, McNulty H, Harmon DL, Whitehead AS. Community-living nonagenarians in northern Ireland have lower plasma homocysteine but similar methylenetetrahydrofolate reductase thermolabile genotype prevalence compared to 70-89-year-old subjects. *Atherosclerosis* 2000; 149: 207-214 [PMID: 10704633 DOI: 10.1016/S0021-9150(00)00154-3]

5. Lee JE, Wei EK, Fuchs CS, Hunter DJ, Lee IM, Selhub J, Stampfer MJ, Willett WC, Ma J, Giovannucci E. Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. *Cancer Causes Control* 2013; 24: 1389-1400 [PMID: 23741611 DOI: 10.1007/s10552-013-9804-3]

6. Rea IM, Su HM, Wang SY, Tsai DH, Lin SD, Chen SC. Determinants of left ventricular mass and presence of metabolic syndrome in a large population, and inflammatory markers. *Atherosclerosis* 2006; 188: 307-312 [PMID: 16960239 DOI: 10.1016/j.atherosclerosis.2006.02.054]

7. Kang SS, Passen EL, Ruggie N, Wong PW, Sora H. Thermolabile defect of methylenetetrahydrofolate reductase in coronary artery disease. *Circulation* 1993; 88: 1463-1469 [PMID: 8403293 DOI: 10.1161/01.CIR.93.7.89]

8. Fudinger M, Wagner OF, Hörl WH, Sunder-Plassmann G. Recent insights into the molecular genetics of the homocysteine metabolism. *Kidney Int Suppl* 2001; 78: S238-S242 [PMID: 11690018]

9. Deloughery TG, Evans A, Sadeghi A, McWilliams J, Henner WD, Taylor LM, Press RD. Common mutation in methylenetetrahydrofolate reductase. Correlation with homocysteine metabolism and late-onset vascular disease. *Circulation* 1996; 94: 3074-3078 [PMID: 8989110 DOI: 10.1161/01.CIR.94.12.3074]

10. Güles S, Aras O, Akar E, Tutar E, Oznurk L, Avei F, Dinçer I, Akar N, Oral D. Methylenetetrahydrofolate reductase gene polymorphism and risk of premature myocardial infarction. *Clin Cardiol* 2001; 24: 281-284 [PMID: 11303694 DOI: 10.1002/clc.4960240405]

11. Brattström L, Zhang Y, Hurtig M, Refsum H, Ostenson C, Fransson L, Jonéus K, Landgren F, Brudin L, Ueland PM. A common methylenetetrahydrofolate reductase gene mutation and longevity. *Atherosclerosis* 1998; 141: 315-319 [PMID: 9862180 DOI: 10.1016/S0021-9150(98)00154-3]

12. Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC, Hunter DJ. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* 1996; 56: 4862-4864 [PMID: 8895734]

13. Martinez ME, Thompson P, Jacobs ET, Giovannucci E, Jiang R, Klimiwick W, Alberts DS. Dietary factors and biomarkers involved in the methylenetetrahydrofolate reductase genotype-colorectal adenoma pathway. *Gastroenterology* 2006; 131: 1706-1716 [PMID: 17087956 DOI: 10.1053/j.gastro.2006.09.010]

14. Lee JE, Wei EK, Fuchs CS, Hunter DJ, Lee IM, Selhub J, Stampfer MJ, Willett WC, Ma J, Giovannucci E. Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. *Cancer Causes Control* 2013; 24: 1389-1400 [PMID: 23741611 DOI: 10.1007/s10552-013-9804-3]
The phenomenon of altered risk factor patterns or reverse epidemiology in persons with advanced chronic kidney failure. Am J Clin Nutr 2003; 88: 849-854 [PMID: 15458899].

Doehner W, Clark A, Anker SD. The obesity paradox: weighing the benefit. Eur Heart J 2010; 31: 146-148 [PMID: 19734553 DOI: 10.1093/eurheartj/ehp339].

Tuñin ME, Bude RO, Platt JF. Review. The resistive index of renal artery and blood pressure in postmenopausal women. Am J Obstet Gynecol 2000; 183: 1296-1302 [PMID: 11062449].

Gardin JM, Adams DB, Douglas PS, Feigenbaum H, Forst DH, Fraser AG, Grayburn PA, Katz AS, Keller AM, Kerber RE, Khandheria BK, Klein AL, Lang RM, Pierard LA, Quinones MA, Schnittger I. Recommendations for a standardized report for left ventricular hypertrophy: comparison with postmortem mass measurements. J Am Coll Cardiol 1983; 2: 305-311 [PMID: 6223063 DOI: 10.1016/0735-1075(83)90167-3].

Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986; 57: 450-458 [PMID: 2936235 DOI: 10.1016/0002-9149(86)90771-X].

Guéant-Rodriguez RM, Guéant JL, Debard R, Thirion S, Hong LX, Browneicki JP, Nouria F, Chabi NW, Sanni A, Anello G, Bosco P, Romano C, Amouzou E, Arrieta HR, Sánchez BE, Romano A, Herbeth B, Guillard JC, Mutchinick OM. Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles in a European cohort: comparison with postmortem mass measurements. J Am Coll Cardiol 2005; 46: 1072-1083 [PMID: 7097763 DOI: 10.1161/01.CIR.58.6.1072].

Foppa M, Dunser BB, Rohde LE. Echocardiography-based left ventricular mass estimation. How should we define hypertrophy? Cardiovasc Ultrasound 2005; 3: 17 [PMID: 15963236].

Maurer M, Burri S, de Marchi S, Hullin R, Martinelli M, Mohacci P, Hess OM. Plasma homocysteine and cardiovascular risk in heart failure with and without cardiorenal syndrome. Int J Cardiol 2010; 141: 32-38 [PMID: 19181408 DOI: 10.1016/j.ijcard.2008.11.131].

Yi F, Li PL. Mechanisms of homocysteine-induced glomerular injury and sclerosis. Am J Nephrol 2008; 28: 254-264 [PMID: 17908498].

Zounas S, McGrath BP, Branley P, Kerr PG, Muske C, Wolfe R, Atkins RC, Nicholls K, Fraenkel M, Hutchison BG, Walker R, McNeil JJ. Cardiovascular morbidity and mortality in the Atherosclerosis and Folic Acid Supplementation Trial (ASFAST) in chronic renal failure: a multicenter, randomized, controlled trial. J Am Coll Cardiol 2006; 47: 1108-1116 [PMID: 16545638 DOI: 10.1016/j.jacc.2005.10.064].

Boner G. Renal involvement and left ventricular hypertrophy are novel risk factors for morbidity and mortality in diabetes mellitus. Diabetes Metab Rev 2011; 27: 425-429 [PMID: 21432982 DOI: 10.1002/dmr.1199].

Florea VG, Mareyev VV, Samko AN, Orlova IA, Coats AJ, Belenkov YN. Left ventricular remodelling: common process in patients with different primary myocardial disorders. Int J Cardiol 1999; 68: 281-287 [PMID: 10213279 DOI: 10.1016/S0167-5279(99)00062-3].

Rutter MK, Wilson PW, Sullivan LM, Fox CS, D’Agostino RB, Meigs JB. Use of alternative thresholds defining insulin resistance to predict incident type 2 diabetes mellitus and cardiovascular disease. Circulation 2008; 117: 1003-1009 [PMID: 18250267 DOI: 10.1161/CIRCULATIONAHA.107.727727].

Trovato FM, Catalano D, Sciacchitano G, Zuccalà G, Iannetti E. Resistive index of renal artery and blood pressure in postmenopausal women. Maturitas 2002; 41: 223-230 [PMID: 11886768 DOI: 10.1016/S0378-5122(01)00290-0].

Trovato GM, Pirri C, Martinis GF, Tonuzza A, Trovato F, Catalano D. Lifestyle interventions, insulin resistance, and arterial stiffness in essential hypertension. Clin Exp Hypertens 2010; 32: 262-269 [PMID: 20662726 DOI: 10.3109/10691961003262504].

Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978; 58: 1072-1083 [PMID: 7097763 DOI: 10.1161/01.CIR.58.5.1072].

Woythaler JN, Singer SL, Kwan OL, Meltzer RS, Reubner B, Bonner M, DeMaria A. Accuracy of echocardiography versus electrophysiology in detecting left ventricular hypertrophy: comparison with postmortem mass measurements. J Am Coll Cardiol 2004; 43: 151-159 [PMID: 15181414 DOI: 10.1016/j.jacc.2003.05.084].

Trovato GM, Catalano D, Ragusa A, Martinis GF, Tonuzza A, Pirri C, Buccheri MA, Di Nora C, Trovato FM. Renal insufficiency and left ventricular hypertrophy: relationship with renal function in non-diabetic patients. J Am Soc Nephrol 2005; 16: 331-339.e2 [PMID: 21852925 DOI: 10.1067/j.asn.2011.05.005].

Catalano D, Vanwoerkom RC, Horne BD, Bair TL, May HT, La’ulu SL, Bude RO. The resistive index in renal Doppler sonography: where do we stand? AJR Am J Roentgenol 2003; 180: 885-892 [PMID: 12664245].

Sahin BM, Bude RO, Platt JF. Review. The resistive index of renal artery and blood pressure in postmenopausal women. Am J Obstet Gynecol 2000; 183: 1296-1302 [PMID: 11062449].
55 Chrysohoou C, Panagiotakos DB, Pitsavos C, Skoumas J, Zeimbekis A, Kastorini CM, Stefanadis C. Adherence to the Mediterranean diet is associated with renal function among healthy adults: the ATTICA study. *J Ren Nutr* 2010; 20: 176-184 [PMID: 19819726 DOI: 10.1053/j.jrn.2009.08.006]

56 Pereira AC, Miyakawa AA, Lopes NH, Soares PR, de Oliveira SA, Cesar LA, Ramires JF, Hueb W, Krieger JE. Dynamic regulation of MTHFR mRNA expression and C677T genotype modulate mortality in coronary artery disease patients after revascularization. *Thromb Res* 2007; 121: 25-32 [PMID: 17604826 DOI: 10.1016/j.thromres.2007.03.004]

57 Kalina A, Czeizel AE. The methylenetetrahydrofolate reductase gene polymorphism (C677T) is associated with increased cardiovascular mortality in Hungary. *Int J Cardiol* 2004; 97: 333-334 [PMID: 15458711 DOI: 10.1016/j.ijcard.2003.08.021]

58 Collings A, Raitakari OT, Juonala M, Rontu R, Kähönen M, Hutri-Kähönen N, Rönnemaa T, Marniemi J, Viikari JS, Lehtimäki T. Associations of methylenetetrahydrofolate reductase C677T polymorphism with markers of subclinical atherosclerosis: the Cardiovascular Risk in Young Finns Study. *Scand J Clin Lab Invest* 2008; 68: 22-30 [PMID: 17934972 DOI: 10.1080/00365510701487735]

59 Schmitz C, Lindpaintner K, Verhoef P, Gaziano JM, Buring J. Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction. A case-control study. *Circulation* 1996; 94: 1812-1814 [PMID: 8873653 DOI: 10.1161/01.CIR.94.8.1812]

60 Yang Q, Bailey L, Clarke R, Flanders WD, Liu T, Yesupriya A, Khoury MJ, Friedman JM. Prospective study of methylenetetrahydrofolate reductase (MTHFR) variant C677T and risk of all-cause and cardiovascular disease mortality among 6000 US adults. *Am J Clin Nutr* 2012; 95: 1245-1253 [PMID: 22492374 DOI: 10.3945/ajcn.111.022384]

61 Schofield RS, Wessel TR, Walker TC, Cleeton TS, Hill JA, Aranda JM. Hyperhomocysteinemia in patients with heart failure referred for cardiac transplantation: preliminary observations. *Clin Cardiol* 2003; 26: 407-410 [PMID: 14524595 DOI: 10.1002/clc.4960260904]

62 Bowden RG, Wyatt FB, Wilson R, Wilborn C, Gentile M. Homocysteine and vascular access thrombosis in a cohort of end-stage renal disease patients. *Ren Fail* 2004; 26: 709-714 [PMID: 15600264 DOI: 10.1081/JDI-200037117]

P- Reviewer: Kettering K, Trimarchi H, Yorioka N
S- Editor: Song XX
L- Editor: A
E- Editor: Liu SQ
