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

Trovato FM *et al*. Heart and kidney disease MTHFR-associated

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

**Francesca M Trovato, Daniela Catalano, Clara Pirri, Concetta Di Nora, Guglielmo M Trovato,** Internal Medicine Department, University of Catania, 95100 Catania, Italy

**Angela Ragusa, Maria Antonietta Buccheri,** AOU Prenatal Diagnosis and Medical Genetics, University of Catania, 95100 Catania, Italy

**G Fabio Martines,** Internal and Emergency Medicine Department, University of Catania, 95100 Catania, Italy

**Author contributions:** All the authors solely contributed to this paper.

**Correspondence to: Guglielmo M Trovato, MD,** Department of Internal Medicine, University of Catania, Via Sant’Orsola 30, 95100 Catania, Italy. trovato.eu@gmail.com

**Telephone:** +39-95-3781533 **Fax:** +39-95-3781549

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**Abstract**

**AIM:** To investigate the effects of different of 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 MTHFR677C>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 polimorphisms.

**RESULTS:** A favorable association with normal renal function of MTHFR polymorphisms, and notably of MTHFR C677T is present independently by 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.

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**Key words:** Homocysteine; Glomerular filtration rate; Renal function; Mediterranean diet; Genetic; Methylenetetrahydrofolate reductase polymorphism; Insulin resistance; Obesity; Left ventricular hypertrophy; Echocardiography

**Core tip:** Weinvestigated the effects of different of 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 MTHFR677C>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]. Hypermocysteinemia 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 I 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 left myocardial ventricular mass/m2 (LVMMi) *vs* cardiovascular events, a J-shape relationship between LVMMi vs. all-cause death[20] and NT-proBNP increase in patients with left ventricular 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 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 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 glomerular filtration rate (GFR), renal artery resistive index (RRI) and with left ventricular myocardial mass (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. 160 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, EKG, 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 (termolabile 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, HBV, and HCV) and cancer biomarkers (AFP, CEA, Ca125, Ca15-3), thyroid hormones, TSH, 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 (HCY) and B12 Vitamin assay in the blood were performed by ADVIA Centaur® XP Immunoassay (Siemens Medical System, Milan, Italy)[37]. iPTH (intact Parathyroid Hormone) 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 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] 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 (USA)], high resolution, 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*[45] and indexed by dividing by body surface area (BSA)/m2. All the exams were stored on digital media for subsequent analysis. LV diameters and wall thickness were measured according the ASE guidelines and LV ejection fraction (LVEF) accordingly[41]. LVEF was considered abnormal if < 50%. GFR is assessed as estimated glomerular filtration rate (eGFR) by the modification of diet in renal disease (MDRD) 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 (RFLP-PCR) analysis were carried out for direct genotypes detection of SNPs, C667T (rs1801133) and A1298C (rs1801131). PCR products were obtained using specific primers (NCBI Reference Sequence: NG\_013351.1): C667T (F5’-GTCCCTGTGGTCTCTTCATCC-3’/R5’-GGTGGCCAAGCAACGCTGTG-3’); A1298C (F5’-CTTCTACCTGAAGAGCAAGTC-3’/R5’-CACATGTCCACAGCATGGAC-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 SYBR 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 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 (± SD). Two-sided *P* value < 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 MTHFR677C>T heterozygous and homozygous polymorphism, of heterozygous and homozygous MTHFR 1298A>C polymorphism and of compound heterozygous MTHFR677C>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 LDL cholesterol are slightly higher in the MTHFR677C>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 hemo-dialysis 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 to 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 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).

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 [0.097 to 0.235], with a Specificity of 0.726 [0.687 to 0.763]; the positive predictive value is PPV 0.1 [0.062 to 0.156] and the negative predictive value is NPV 0.813 [0.775 to 0.845]; the positive likelihood ratio is LR+ 0.562 [0.351 to 0.901], the negative likelihood ratio is LR- 1.165 [1.057 to 1.284]. Similarly, for the wild MTHFR genotype, Sensitivity is 0.383 [0.291 to 0.484]; Specificity is 0.769 [0.731 to 0.802]; PPV is 0.225 [0.167 to 0.296]; NPV is 0.877 [0.844 to 0.903]; LR+ is 1.655 [1.227 to 2.233] and LR- is 0.803 [0.68 to 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 MTHFR genotype (12/94; 12.7%) *vs* the polymorphism MTHFR group (131/470; 27.9 %) displays an Odds ratio 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, 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, is 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 suggest 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.

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[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, MTHFR677TT 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 left ventricular 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 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 GFR 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 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.

***Peer review***

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

**REFERENCES**

1 **Saifan 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 [PMID: 23898227 DOI: 10.2147/VHRM.S47255]

2 **Jardine MJ**, Kang A, Zoungas S, Navaneethan SD, Ninomiya T, Nigwekar 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**, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996; **93**: 7-9 [PMID: 8616944 DOI: 10.1161/01.CIR.93.1.7]

4 **Fung MM**, Salem RM, Lipkowitz MS, Bhatnagar V, Pandey B, Schork NJ, O'Connor DT; AASK Study Investigators. Methylenetetrahydrofolate reductase (MTHFR) polymorphism A1298C (Glu429Ala) predicts decline in renal function over time in the African-American Study of Kidney Disease and Hypertension (AASK) Trial and Veterans Affairs Hypertension Cohort (VAHC). *Nephrol Dial Transplant* 2012; **27**: 197-205 [PMID: 21613384 DOI: 10.1093/ndt/gfr257]

5 **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.88.4.1463]

6 **Födinger 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: 11169018]

7 **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]

8 **Gülec S**, Aras O, Akar E, Tutar E, Omürlü K, Avci 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]

9 **Brattström L**, Zhang Y, Hurtig M, Refsum H, Ostensson S, Fransson L, Jonés 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]

10 **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]

11 **Martínez ME**, Thompson P, Jacobs ET, Giovannucci E, Jiang R, Klimecki 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]

12 **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* 2012; **23**: 537-545 [PMID: 22367721 DOI: 10.1007/s10552-012-9911-3]

13 **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(99)00417-7]

14 **Ravera M**, Viazzi F, Berruti V, Leoncini G, Zagami P, Bezante GP, Rosatto N, Ravazzolo R, Pontremoli R, Deferrari G. 5,10-Methylenetetrahydrofolate reductase polymorphism and early organ damage in primary hypertension. *Am J Hypertens* 2001; **14**: 371-376 [PMID: 11336184 DOI: 10.1016/S0895-7061(00)01296-6]

15 **Dedoussis GV**, Panagiotakos DB, Pitsavos C, Chrysohoou C, Skoumas J, Choumerianou D, Stefanadis C; ATTICA Study Group. An association between the methylenetetrahydrofolate reductase (MTHFR) C677T mutation and inflammation markers related to cardiovascular disease. *Int J Cardiol* 2005; **100**: 409-414 [PMID: 15837084 DOI: 10.3109/0886022X.2010.516853]

16 **Greaves K**, Chen R, Ge L, Wei M, Tong B, Cai N, Senior R, Hemingway H. Mild to moderate renal impairment is associated with increased left ventricular mass. *Int J Cardiol* 2008; **124**: 384-386 [PMID: 17399818 DOI: 10.1016/j.ijcard.2006.12.054]

17 **Hsieh MC**, Su HM, Wang SY, Tsai DH, Lin SD, Chen SC, Chen HC. Significant correlation between left ventricular systolic and diastolic dysfunction and decreased glomerular filtration rate. *Ren Fail* 2011; **33**: 977-982 [PMID: 22013930 DOI: 10.3109/0886022X.2011.618792]

18 **Rodrigues SL**, Angelo LC, Pereira AC, Krieger JE, Mill JG. Determinants of left ventricular mass and presence of metabolic risk factors in normotensive individuals. *Int J Cardiol* 2009; **135**: 323-330 [PMID: 18929416 DOI: 10.1016/j.ijcard.2008.03.066]

19 **Lai CL**, Chien KL, Hsu HC, Su TC, Chen MF, Lee YT. Left ventricular mass and risk of cardiovascular events and all-cause death among ethnic Chinese--the Chin-Shan Community Cardiovascular Cohort study. *Int J Cardiol* 2011; **149**: 347-352 [PMID: 20202708 DOI: 10.1016/j.ijcard.2010.02.015]

20 **Rivera Otero JM**, Taléns-Visconti R, Salvador A, Bertomeu V, Miró V, Jordán A, Sogorb F, Cortés R, Payá R, Diago JL, Grau G; Grupo de Disfunción VI, Comunidad Valencian. Ventricular hypertrophy increases NT-proBNP in subjects with and without hypertension. *Int J Cardiol* 2004; **96**: 265-271 [PMID: 15262044 DOI: 10.1016/j.ijcard.2003.07.019]

21 **de Ruijter W**, Westendorp RG, Assendelft WJ, den Elzen WP, de Craen AJ, le Cessie S, Gussekloo J. Use of Framingham risk score and new biomarkers to predict cardiovascular mortality in older people: population based observational cohort study. *BMJ* 2009; **338**: a3083 [PMID: 19131384 DOI: 10.1136/bmj.a3083]

22 **Wald DS**, Law M, Morris JK. The dose-response relation between serum homocysteine and cardiovascular disease: implications for treatment and screening. *Eur J Cardiovasc Prev Rehabil* 2004; **11**: 250-253 [PMID: 15179109 DOI: 10.1097/01.hjr.0000129742.15346.ab]

23 **Brattström L**, Wilcken DE. Homocysteine and cardiovascular disease: cause or effect? *Am J Clin Nutr* 2000; **72**: 315-323 [PMID: 10919920]

24 **Ueland PM**, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. *Am J Clin Nutr* 2000; **72**: 324-332 [PMID: 10919921]

25 **Kopple JD**. The phenomenon of altered risk factor patterns or reverse epidemiology in persons with advanced chronic kidney failure. *Am J Clin Nutr* 2005; **81**: 1257-1266 [PMID: 15941874]

26 **Dedoussis GV**, Panagiotakos DB, Chrysohoou C, Pitsavos C, Zampelas A, Choumerianou D, Stefanadis C. Effect of interaction between adherence to a Mediterranean diet and the methylenetetrahydrofolate reductase 677C--& gt; T mutation on homocysteine concentrations in healthy adults: the ATTICA Study. *Am J Clin Nutr* 2004; **80**: 849-854 [PMID: 15447889]

27 **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]

28 **Tublin ME**, Bude RO, Platt JF. Review. The resistive index in renal Doppler sonography: where do we stand? *AJR Am J Roentgenol* 2003; **180**: 885-892 [PMID: 12646425]

29 **Heine GH**, Reichart B, Ulrich C, Köhler H, Girndt M. Do ultrasound renal resistance indices reflect systemic rather than renal vascular damage in chronic kidney disease? *Nephrol Dial Transplant* 2007; **22**: 163-170 [PMID: 16936334 DOI: 10.1093/ndt/gfl484]

30 **Saleh FN**, Schirmer H, Sundsfjord J, Jorde R. Parathyroid hormone and left ventricular hypertrophy. *Eur Heart J* 2003; **24**: 2054-2060 [PMID: 14613742 DOI: 10.1016/j.ehj.2003.09.010]

31 **Anderson JL**, Vanwoerkom RC, Horne BD, Bair TL, May HT, Lappé DL, Muhlestein JB. Parathyroid hormone, vitamin D, renal dysfunction, and cardiovascular disease: dependent or independent risk factors? *Am Heart J* 2011; **162**: 331-339.e2 [PMID: 21835295 DOI: 10.1016/j.ahj.2011.05.005]

32 **Trovato GM**, Martines GF, Trovato FM, Pirri C, Pace P, Catalano D. Renal resistive index and parathyroid hormone relationship with renal function in nondiabetic patients. *Endocr Res* 2012; **37**: 47-58 [PMID: 22007967 DOI: 10.3109/07435800.2011.625513]

33 **Bell DS**. Insulin resistance. An often unrecognized problem accompanying chronic medical disorders. *Postgrad Med* 1993; **93**: 99-103, 106-7 [PMID: 8098525]

34 **Trovato GM**, Catalano D, Ragusa A, Martines GF, Tonzuso A, Pirri C, Buccheri MA, Di Nora C, Trovato FM. Renal insufficiency in non-diabetic subjects: relationship of MTHFR C677t gene polymorphism and left ventricular hypertrophy. *Ren Fail* 2013; **35**: 615-623 [PMID: 23534584 DOI: 10.3109/0886022X.2013.779895]

35 **Catalano D**, Trovato GM, Ragusa A, Martines GF, Tonzuso A, Pirri C, Buccheri MA, Trovato FM. Non-alcoholic fatty liver disease (NAFLD) and MTHFR 1298A & gt; C gene polymorphism. *Eur Rev Med Pharmacol Sci* 2014; **18**: 151-159 [PMID: 24488901]

36 **La'ulu SL**, Rawlins ML, Pfeiffer CM, Zhang M, Roberts WL. Performance characteristics of six homocysteine assays. *Am J Clin Pathol* 2008; **130**: 969-975 [PMID: 19019776 DOI: 10.1309/AJCP64BJIPNPSQDJ]

37 **National Kidney Foundation.** K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 2003; **42**: S1-201 [PMID: 14520607]

38 **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]

39 **Trovato GM**, 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]

40 **Trovato GM**, Pirri C, Martines GF, Tonzuso A, Trovato F, Catalano D. Lifestyle interventions, insulin resistance, and renal artery stiffness in essential hypertension. *Clin Exp Hypertens* 2010; **32**: 262-269 [PMID: 20662726 DOI: 10.3109/10641960903265204]

41 **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 adult transthoracic echocardiography: a report from the American Society of Echocardiography's Nomenclature and Standards Committee and Task Force for a Standardized Echocardiography Report. *J Am Soc Echocardiogr* 2002; **15**: 275-290 [PMID: 11875394 DOI: 10.1067/mje.2002.121536]

42 **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: 709763 DOI: 10.1161/01.CIR.58.6.1072]

43 **Woythaler JN**, Singer SL, Kwan OL, Meltzer RS, Reubner B, Bommer W, DeMaria A. Accuracy of echocardiography versus electrocardiography in detecting left ventricular hypertrophy: comparison with postmortem mass measurements. *J Am Coll Cardiol* 1983; **2**: 305-311 [PMID: 6223063 DOI: 10.1016/S0735-1097(83)80167-3]

44 **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]

45 **Guéant-Rodriguez RM**, Guéant JL, Debard R, Thirion S, Hong LX, Bronowicki JP, Namour F, Chabi NW, Sanni A, Anello G, Bosco P, Romano C, Amouzou E, Arrieta HR, Sánchez BE, Romano A, Herbeth B, Guilland JC, Mutchinick OM. Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study in Mexican, West African, and European populations. *Am J Clin Nutr* 2006; **83**: 701-707 [PMID: 16522920]

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

47 **Maurer M**, Burri S, de Marchi S, Hullin R, Martinelli M, Mohacsi 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]

48 **Yi F**, Li PL. Mechanisms of homocysteine-induced glomerular injury and sclerosis. *Am J Nephrol* 2008; **28**: 254-264 [PMID: 17989498]

49 **Zoungas 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]

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

51 **Florea VG**, Mareyev VY, 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/S0167-5273(98)00362-3]

52 **Thomas MD**, Fox KF, Coats AJ. Redefining heart failure. *Int J Cardiol* 2006; **112**: 139-141 [PMID: 16581144 DOI: 10.1016/j.ijcard.2005.11.071]

53 **de Lorgeril M**, Salen P. Mediterranean diet in secondary prevention of CHD. *Public Health Nutr* 2011; **14**: 2333-2337 [PMID: 22166192 DOI: 10.1017/S136898001100259X]

54 **Coats AJ**. Advances in the non-drug, non-surgical, non-device management of chronic heart failure. *Int J Cardiol* 2005; **100**: 1-4 [PMID: 15820278 DOI: 10.1016/j.ijcard.2005.01.005]

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](http://dx.doi.org/10.1053/j.jrn.2009.08.006" \t "_blank)]

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]

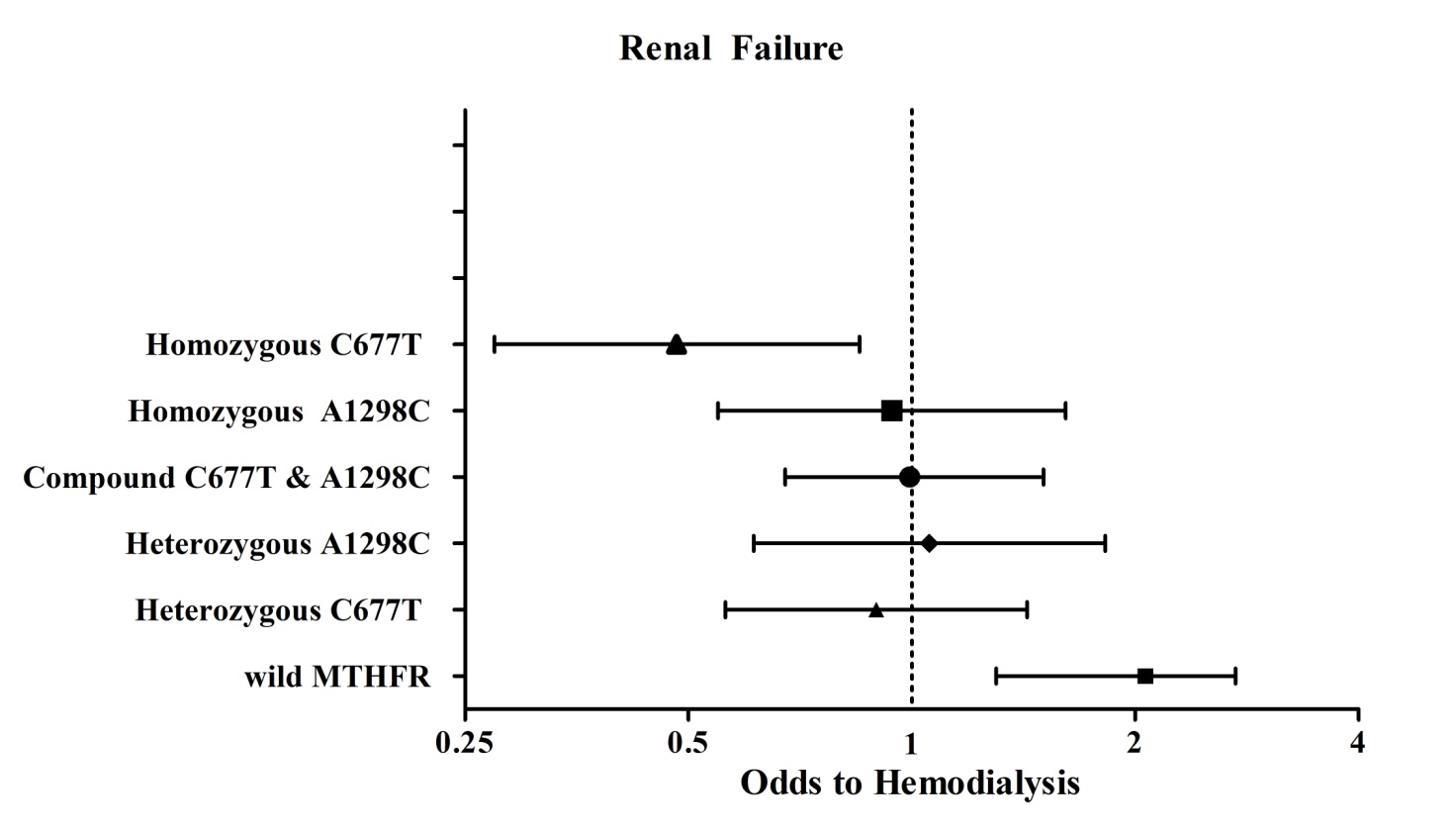
59 **Schmitz C**, Lindpaintner K, Verhoef P, Gaziano JM, Buring J. Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction. A case-control study. *Circulation* 1996; **94**: 1812-1814 [PMID: 8873653 DOI: 10.1161/01.CIR.94.8.1812]

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]

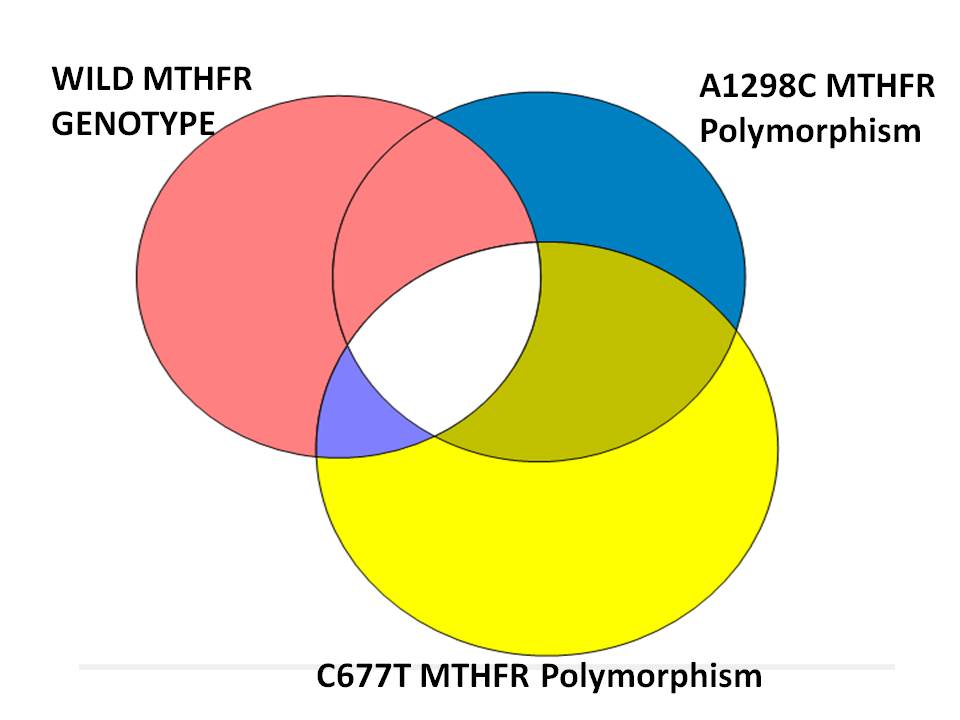
61 **Schofield RS**, Wessel TR, Walker TC, Cleeton TS, Hill JA, Aranda JM. Hyperhomocysteinemia in patients with heart failure referred for cardiac transplantation: preliminary observations. *Clin Cardiol* 2003; **26**: 407-410 [PMID: 14524595 DOI: 10.1002/clc.4960260904]

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]

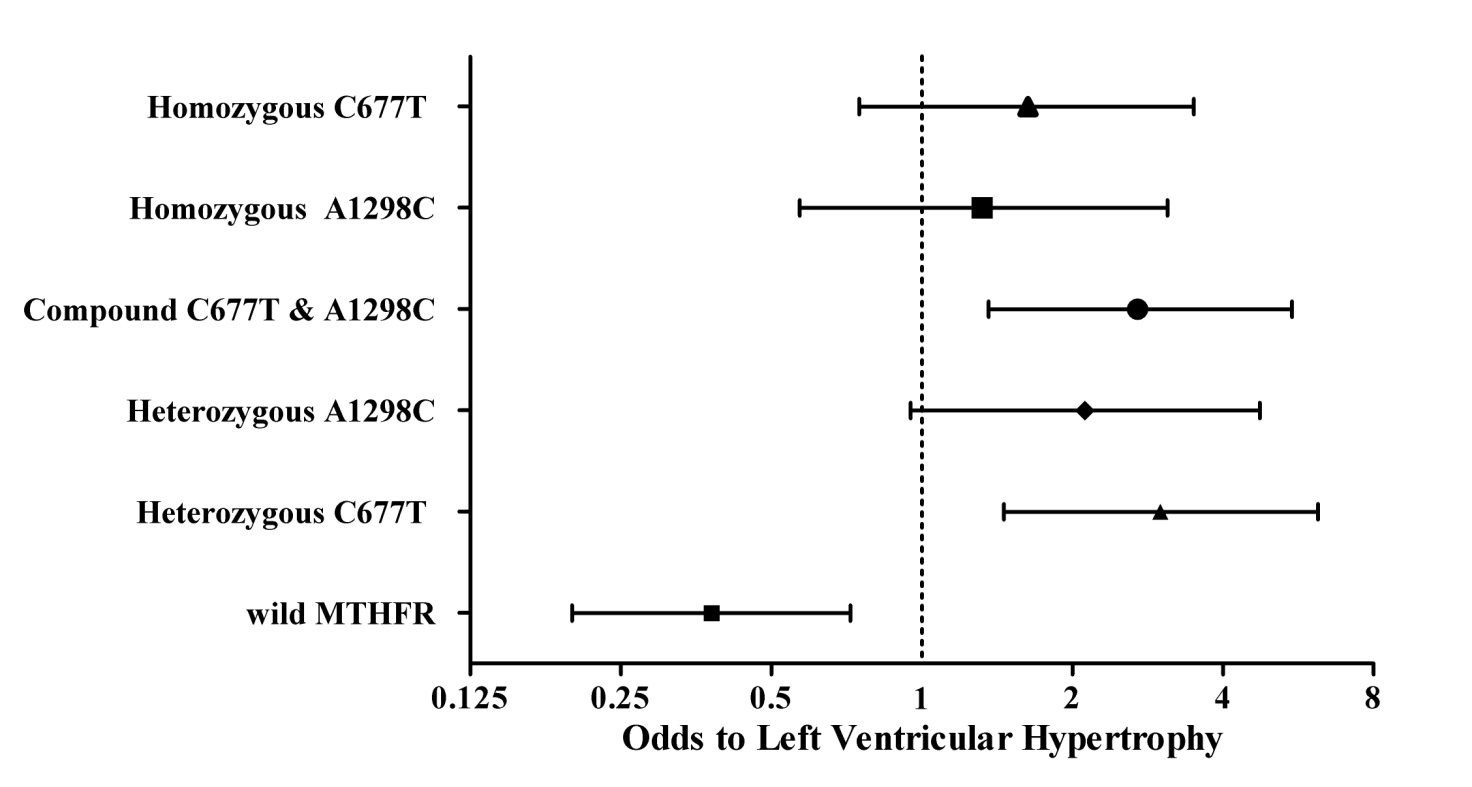
**P-Reviewer:** Kettering K, Trimarchi H, Yorioka N **S-Editor:** Song XX **L-Editor:** **E-Editor:**



**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 to 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 to 1.43]; Heterozygous A1298C (20/160 *vs* 56/470); *P* = NS. OR 1.056 [95%CI: 0.612 to 1.822]; Compound Heterozygous C677T and A1298C (40/160 *vs* 118/470); *P*: ns. OR 0.994 [95%CI: 0.657 to 1.505]; Homozygous A1298C (20/160 *vs* 62/470); *P* = ns. OR 0.94 [95%CI: 0.548 to 1.612]; Homozygous C677T (16/160 *vs* 88/470); *P* = 0.015. OR 0.482 [95%CI: 0.274 to 0.85]. MTHFR: Methylenetetrahydrofolate reductase; OR: Odds ratio; NS: Not significant.



**Figure 2 Venn diagram showing proportionally the overlap of Methylenetetrahydrofolate reductase genetic polymorphisms A1298C and C677T with the wild one.** The three groups have very relevant overlaps in the studied population.



**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: Methylenetetrahydrofolate reductase; OR: Odds ratio.

**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 | 57.91 ± 17.04 | 57.85 ± 14.70 | 50.12 ± 17.09 | <0.0001 |
| BMI, kg/m2 | 27.26 ± 5.04 | 27.28 ± 5.95 | 28.04 ± 5.84 | 27.80 ± 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/m2 | 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; 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; BLS: Bright liver score; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; AMDS: Adherence Mediterranean Diet Score.

**Table 2 Characteristic of study population and differences between hemo-dialysis patients and No- hemo-dialysis *n* (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Total  (*n* = 630) | Dialysis patients  (*n* = 160) | Patients with maintained  Renal function  (*n* = 470) | *P* |
| Women | 336(53.3) | 72 | 264 | 0.0141 |
| Obese patients | 196(31.1) | 24 | 172 | <0.0001 |
| Patients with GFR<90 | 514(81.6) | 160 | 354 | <0.0001 |
| NAFLD patients | 256(40.6) | 28 | 228 | <0.0001 |
| MTHFRGroup | |  |  |  |
| Wild genotype | 94(14.9) | 36 | 58 | 0.0161 |
| 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/m2 | 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/m2 | 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. 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; BLS: Bright liver score; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; AMDS: Adherence Mediterranean Diet Score.

**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* |
| highRRI | 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. LVH: Left ventricular hypertrophy.

**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 (CRF 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. LVH: Left ventricular hypertrophy.

**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* |
| highRRI | 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 |
| HIGHLVMM | 4 | 20 | 14 | 12 | 8 | 16 | 13,355 | 0.020 |

Pearson *χ*2. LVH: Left ventricular hypertrophy.