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CLINICAL STUDY

Renal Insufficiency in Non-Diabetic Subjects: Relationship of MTHFR C677t Gene Polymorphism and Left Ventricular Hypertrophy

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Abstract

Background: Association of methylenetetrahydrofolate reductase (MTHFR) 677C>T gene polymorphism with hyperhomocysteinemia, renal failure, and cardiovascular events is controversial. We investigated the relationship of MTHFR 677C>T polymorphisms with left ventricular hypertrophy (LVH) and renal insufficiency. *Methods*: Glomerular filtration rate (GFR) and left myocardial ventricular mass/m2 were assessed in 138 non-diabetic subjects (age, 50.93 ± 14.85 years; body mass index, 27.95 ± 5.98 kg/m^2), 38 no-mutation wild MTHFR C677CC, 52 heterozygous MTHFR C677CT, and 48 homozygous MTHFR C677TT, all with adequate adherence to current international healthy dietary guidelines. Serum homocysteine, insulin resistance, high-sensitivity C-reactive-protein (hsCRP), parathyroid hormone, and renal artery resistive index (RRI) were challenged by odds ratio analysis and multiple linear regression models. *Results*: MTHFR 677C>T polymorphism showed higher GFR (73.8 ± 27.99 vs. 58.64 ± 29.95; $p = 0.001$) and lower renal failure odds (OR, 0.443; 95% confidence interval, 0.141–1.387) in comparison with wild MTHFR genotype. A favorable effect on GFR of MTHFR polymorphism is presented independently by the negative effects of LVH, increased intra-renal arterial resistance, and hyperparathyroidism; GFR is the significant predictive factor to LVH. *Conclusions*: Renal insufficiency in non-diabetic subjects is explained by interactions of MTHFR C677T polymorphism mutation with LVH, 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.

Keywords: homocysteine, GFR, renal function, Mediterranean diet, genetic, MTHFR polymorphism, insulin resistance, obesity, left ventricular hypertrophy, echocardiography

INTRODUCTION

Mutations of the human methylenetetrahydrofolate reductase (MTHFR) gene have been associated with increased homocysteine (HCY) levels: this was suspected to increase risks of cardiovascular disease (CVD) in various populations, and particularly, in patients with renal disease, especially when in hemodialysis.[1] Epidemiological studies have identified hyperhomocysteinemia as an independent risk factor for coronary artery disease, at least in some ethnic group[2] in which one of the more common MTHFR mutations (nucleotide 677C>T) results in a thermolabile enzyme,[3] lower folate levels, and an inefficient HCY metabolism.[4] Hyperhomocysteinemia appears independent from other risk factors,[5] and subsequent reports increased concerns around the related common genetic polymorphism.[6] Nonetheless, earlier studies already challenged this concept[7] and outlined that this mutation is not associated with premature death, since its prevalence in the elderly is not lower than in the young.[8] In other contexts, actually, the presence of the allele 677T of the MTHFR gene was the best explaining protective factor against cervical carcinogenesis[9] and for colonic cancer,[10–12] 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 myocardial mass (LVMM),[17] lower ejection fraction, and increased

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E/A ratio at echocardiography.[18] Insulin resistance accounts significantly for left ventricular (LV) mass increase in normotensive individuals.[19] A linear relationship between left myocardial ventricular mass/m2 (LVMMi) versus cardiovascular events, a J-shape relationship between LVMMi versus 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 hyperhomocysteinemia stems from many considerations. Among them, in general population with no history of CVD, concentrations of HCY 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 nutrient intakes and particularly of a normal B vitamin intake and availability: they can be modified by conditions impairing renal function.[26] MTHFR gene–Mediterranean Diet interactions on HCY metabolism was reported: this dietary profile may reduce HCY concentrations and consequently influence coronary risk in genetically high-risk individuals, by quality and proportion of nutrients.[27] The accompanying body size increase is not invariably detrimental since, actually, patients with established chronic disease benefit of large body size.[28] This finding, defined the obesity paradox, is shared over a variety of cardiovascular, pulmonary, and renal diseases: it challenges the concept about differences for optimal body size in health and disease.[29] The cornerstone is how several metabolic factors affect renal circulation and, as a consequence, renal function. The increase of intra-renal artery resistance, measured by renal artery resistive index (RRI), affects the natural history of atherosclerosis and arterial hypertension, which was found to correlate with LVH and carotid intimal thickening,[29] with cardiovascular risk score and impaired renal outcome and death.[30] Also endocrine factors are very relevant: among them, parathyroid hormone (PTH) intervenes in several mechanisms of disease progression, including LVH,[31] impairment of renal function,[32] and increase of intra-renal arterial resistance.[32–34] The aim of this study was to investigate relationship of MTHFR 677C>T polymorphisms with glomerular filtration rate (GFR) and with LVMM, dietary profile, high-sensitivity C-reactive-protein (hsCRP), intact parathyroid hormone (iPTH), insulin resistance, and RRI in non-diabetic subjects.

PATIENTS AND METHODS

We studied 138 Italian Caucasian subjects, aged 50.93 $\pm$ 14.85 years [body mass index (BMI) 27.95 $\pm$ 5.98 kg/m^2]: 38 MTHFR C677CC (wild genotype), 52 heterozygous MTHFR C677CT, and 48 homozygous MTHFR C677TT (tremolabile polymorphism) subjects. They were referred to the Internal Medicine Day hospital for clinical assessment and lifestyle–nutritional counseling. Preliminary exclusion criteria were as follows: (1) all patients with clinical/echographic signs of congestive heart failure, malignancies, severe chronic liver disease, apart from the lone finding of bright liver; (2) patients with any history of diabetes mellitus, established by a fasting glucose level $\geq$ 126 mg/dL or HbA1c $\geq$ 6.5%, or those under treatment for other diseases, apart from well-controlled arterial hypertension; and (3) individuals who were extremely obese (class III: BMI $\geq$ 40) and underweight subjects (BMI < 18.5). Subsequent exclusion criteria were the following: all patients with acute or chronic infectious disease, a history of alcohol abuse (exceeding 20 g/d), severe renal insufficiency (GFR < 30 mL/min/1.73 m^2 or detection of proteinuria), thyroid disease, polycystic ovary syndrome, and steroid use. Arterial hypertension, defined as >140 mmHg of systolic and >90 mmHg of diastolic blood pressure, was not an exclusion criterion, provided that an adequately stable normal blood pressure was achieved and maintained. Throughout the study, no patient was eligible if recently treated with statins, metformin, or other drugs with known effects on insulin resistance. Patients were managed by Mediterranean Diet and lifestyle counseling, including physical exercise prescription. Mediterranean Diet Adherence Profile was assessed as Adherence to Mediterranean Diet Score (AMDS) on the basis of a 1 week recall computerized questionnaire; this is a premise to personalized Mediterranean Diet prescriptions, with daily recommendations also derived from the specific software used (Dietosystem, Milan, Italy).[32] Physical activity increase and smoking withdrawal active counseling were also provided.[35] The criteria used for delivering the Mediterranean Diet Score included few modifications [35–37] in comparison with the original report.[38–40] Patients' assessment was performed at the end of the first month of observation after the initial enrollment, which included the prescription and planning of dietary and lifestyle changes. Suggestions and advice on individual "healthy" food purchase, storage, and cooking were provided; reliable feedback and evidence of patients' adherence were obtained by scheduled dietician's interviews. Physical activity was also encouraged in the form of walking using the "10,000 steps a day" suggestion, maintaining, if present, the current leisure or sport habits. A portable electronic pedometer (step counter) was also given as a monitoring and as a motivation tool[41] to enhance and maintain daily physical activity increase. Routine laboratory tests included virus hepatitis (hepatitis A virus, HBV, and HCV) and cancer biomarkers (AFP, CEA, Ca125, and 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). HCY and B12 vitamin

assay in the blood were performed by ADVIA Centaur® XP Immunoassay (Siemens Medical System, Milan, Italy).[42] iPTH and NT-proBNP (IMMULITE® 2000, Siemens Medical System, Milan, Italy) were assessed by a solid-phase two-site chemiluminescent immunometric assay. PTH values considered normal were <70 pg/mL for subjects without severe renal insufficiency.[43] 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/m^2), overweight (≥25.0 and ≤29.9 kg/m^2), and obese (≥30.0 kg/m^2). Insulin resistance was assessed by the homeostasis model-insulin resistance index (HOMA) according to the following formulas: fasting insulin value × fasting blood sugar level/405. The HOMA threshold for insulin resistance is conventionally considered >1.7, according to the likelihood ratios for 11-year CVD prediction.[44] 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 RRI (peak systolic velocity—end-diastolic velocity/peak systolic velocity).[28,45] 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.[46] Echocardiographic studies were performed with two-dimensional guided M-mode echocardiography according to methods established by the American Society of Echocardiography (ASE)[47–49] with transducer frequencies appropriate for body size. Siemens Acuson S2000™, Siemens AG, Munich, Germany, or a GE echo-color-doppler device (GE Logiq7 Expert US, manufactured by GE Medical Systems, Milwaukee, Wisconsin), high resolution, with real-time sectional scan transducers was 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.[50] and indexed by dividing by body surface area/m^2. All the exams were stored on digital media for subsequent analysis. LV diameters and wall thickness were measured according the ASE guidelines,[47] and LV ejection fraction (LVEF) was computed using the modified Simpson's formula. LVEF was considered abnormal if <50%. GFR is assessed as estimated GFR by the modification of diet in renal disease (MDRD) formula in mL/min per 1.73 m^2, according to the Clinical Practice Guidelines for Chronic Kidney Disease KDOQI.[42] Genotypes of the MTHFR C677T and A1298C polymorphisms were detected by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). DNA was extracted from peripheral blood by a commercially available DNA isolation method (QIAamp DNA Blood Mini Kit QIAGEN, Milan, Italy). Restriction enzyme analysis of amplified product (PCR-RFLP) analysis was carried out for direct genotypes detection of 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′-CACATG TCCACAGCATGGAC-3′). Both amplicons were successively digested by HinfI and MboII restriction enzymes for C667T and A1298C, respectively, and DNA fragment was visualized in a 4% agarose gel stained with SYBR safe (Life Technologies Italia, Monza, Italy); electrophoresis pattern was used to determined MTHFR genotypes.[51] Within all patients referred for clinical assessment and included in the MTHFR genetic diagnostic panel, A1298C homozygous, heterozygous, and compound MTHFR 677CT heterozygous polymorphism subjects ($n = 137$) were excluded from the analysis of data. 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. The distributions of MTHFR alleles and genotypes in studied group were checked by χ^2 test or Fisher's exact test. Descriptive results of continuous variables are expressed as averages (±SD). Student's *t*-test was used to assess the difference in averages between subjects with MTHFR heterozygous and homozygous polymorphism versus wild genotype subjects. Two-sided *p*-value <0.05 was considered statistically significant. Higher quartiles of age, homocysteine, iPTH, RRI, hsCRP, and other continue measures were defined; thereafter, the associations of older age, higher hsCRP, iPTH, RRI, LVH (LVMMi ≥150 g/m^2 in men, ≥120 g/m^2 in women[52]), and MTHFR C677T polymorphisms were assessed as odds ratios (ORs) to renal insufficiency (GFR ≤ 90 mL/min/m^2) with 95% confidence intervals (CI). A multiple linear regression (MLR) model, age-balanced, challenges MTHFR C677T polymorphism toward GFR and includes AMDS, RRI, HOMA, HCY, hsCRP, iPTH, B12 vitamin, folic acid, and LVMMi as predictive variants. An

Table 1. Characteristic of study population and differences between MTHFR polymorphism and control group.

	Total (*n.*138)	MTHFR Polymorphism (*n.* 100)	Wild MTHFR AA (*n.* 38)	*p*
Age, y	50.15 ± 15.44	49.47 ± 16.62	51.95 ± 11.81	0.402
BMI, Kg/m^2	28.00 ± 6.17	28.12 ± 6.44	>27.70 ± 5.47	0.726
hsCRP, mg/dL	2.77 ± 4.28	2.78 ± 4.09	2.74 ± 4.79	0.959
Blood glucose, mg/dL	93.74 ± 24.07	95.60 ± 27.31	88.84 ± 10.79	0.141
GFR	69.43 ± 29.12	73.68 ± 27.89	58.27 ± 29.69	**0.005**
Triglycerides, mg/dL	106.80 ± 60.56	103.46 ± 51.82	115.61 ± 79.27	0.294
Total cholesterol, mg/dL	203.73 ± 44.61	206.86 ± 44.93	195.49 ± 43.25	0.182
HDL cholesterol, mg/dL	57.32 ± 17.88	56.53 ± 16.09	59.39 ± 22.02	0.402
LDL cholesterol, mg/dL	125.20 ± 41.49	129.85 ± 42.25	112.97 ± 37.24	**0.032**
HOMA	2.72 ± 2.77	2.95 ± 3.14	2.12 ± 1.20	0.117
PTH, pg/mL	73.82 ± 100.78	72.90 ± 107.51	76.24 ± 81.69	0.863
Vit. B12, mcg/dL	488.17 ± 280.23	471.77 ± 237.69	531.34 ± 370.07	0.266
Folic acid, ng/mL	9.91 ± 19.33	10.13 ± 22.55	9.33 ± 4.72	0.830
AMDS	34.64 ± 3.04	34.58 ± 3.22	34.78 ± 2.53	0.739
NT-proBNP, pg/mL	65.77 ± 41.55	65.21 ± 44.23	67.24 ± 33.99	0.799
Homocysteine, μmol/L	20.75 ± 4.89	21.27 ± 5.02	19.37 ± 4.27	**0.040**
Albumin, g/dL	4.92 ± 3.81	5.03 ± 4.47	4.64 ± 0.29	0.596
RRI	0.61 ± 0.06	0.60 ± 0.07	0.62 ± 0.06	0.093
EF %	67.25 ± 8.07	67.13 ± 8.02	67.54 ± 8.30	0.803
E/A	1.19 ± 0.29	1.20 ± 0.28	1.14 ± 0.31	0.303
LVMM/m^2	97.50 ± 35.09	98.54 ± 30.36	94.76 ± 45.64	0.574
Women, *n*	75	56	19	0.659*

Notes: BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RRI, renal resistive index; EF, ejection fraction; LVMM, left ventricular mass myocardial. Bold fonts indicate significant results. *Pearson chi-square.

analogous MLR model, age-balanced, challenges MTHFR C677T polymorphism toward LVMM/m2 (LVMMi) and includes AMDS, RRI, HOMA, HCY, hsCRP, iPTH, B12 vitamin, folic acid, and GFR as predictive variants. All analyses were performed using SPSS 18.0 for Windows (SPSS, Chicago, IL, USA), Power analysis by G*Power 3.1 and graphs by GraphPad-Prism.

Table 2. Multiple linear regression predictive model for LVMM/m^2 and GFR.

			LVMM/m^2			
Predictors	*R*	R^2	*F*	*Significance*	*β*	*p*
	0.424	0.180	3.120	**0.002**		
HOMA					0.028	0.749
MTHFR polymorphism					−0.099	0.243
RRI					0.070	0.490
GFR					−0.338	**0.001**
iPTH, pg/mL					0.031	0.731
Homocysteine, μmol/L					0.129	0.127
Vit. B12, mcg/dL					0.051	0.556
Folic acid, ng/mL					−0.141	0.110
AMDS					−0.009	0.923
			GFR			
Predictors	*R*	R^2	*F*	*Significance*	*β*	*p*
	0.672	0.452	11.728	**<0.0001**		
HOMA					0.008	0.908
MTHFR polymorphism					0.154	**0.026**
RRI					−0.438	**<0.0001**
LVMM/m^2					−0.226	**<0.001**
iPTH, pg/mL					−0.193	**0.007**
Homocysteine, μmol/L					−0.050	0.475
Vit. B12, mcg/dL					−0.022	0.761
Folic acid, ng/mL					0.066	0.362
AMDS					−0.091	0.203

Notes: BMI, body mass index; RRI, renal resistive index; LVMM, left ventricular mass myocardial; AMDS, adherence Mediterranean diet score. Weighted by age least squares multiple regression. Bold fonts indicate significant predictive results.

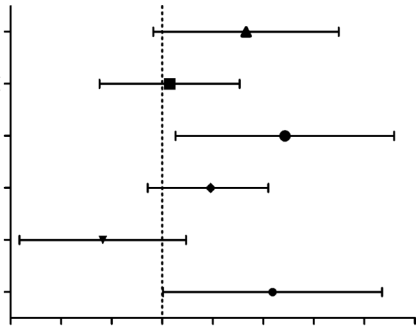

Figure 1. Higher concentration of parathyroid hormone (iPTH) (OR, 5.377; 95% CI, 1.202–24.051), greater levels of high-sensitivity C-reactive protein (hsCRP) (OR, 3.156; 95% CI, 0.885–11.255), older age (OR, 4.543; 95% CI, 1.012–20.397), and left ventricular hypertrophy (LVH) (OR, 1.942; 95% CI, 0.819–4.293) are associated significantly with renal insufficiency; MTHFR 677C>T polymorphism is associated significantly with lower odds of renal insufficiency (OR, 0.443; 95% CI, 0.141–1.387).

RESULTS

The differences in averages between patients with MTHFR 677C>T heterozygous and homozygous polymorphism versus wild genotype subjects are shown in Table 1. GFR is significantly higher in the MTHFR 677C>T polymorphism group versus wild genotype subjects (73.68 ± 27.89 vs. 58.27 ± 29.69; p: 0.005); HCY (21.27 ± 5.02 vs. 19.37 ± 4.27; p: 0.040), and LDL cholesterol (129.85 ± 42.25 vs. 112.97 ± 37.24; p: 0.032) are slightly higher in the MTHFR 677C>T polymorphism group versus wild genotype subjects. A significant linear correlation of GFR versus LVMMi ($r = -0.388$; $p < 0.0001$) is observed. Significant inverse correlation of age versus GFR ($r = -0.501$; $p < 0.0001$) and direct correlations of age versus RRI ($r = 0.491$; $p < 0.0001$) and versus LVMMi ($r = 0.275$; $p < 0.001$) are observed. iPTH shows significant inverse correlation versus GFR ($r = -0.366$; $p < 0.0001$), whereas a direct trend of iPTH is observed versus RRI ($r = 0.292$; $p < 0.001$) and versus LVMMi ($r = 0.162$; $p < 0.05$). No significant correlation is observed for both hsCRP and insulin resistance (HOMA) versus GFR, LVMMi, and RRI.

By MLR, age-balanced, GFR is the only factor that explains significantly 18.0% of the variance to LVH, assessed as LVMMi, in the MLR model (Table 2, top). A predictive effect of MTHFR 677C>T polymorphism versus lower GFR is significantly displayed, along with the opposite unfavorable effects of higher PTH, LVMMi, and RRI; these last are all conditions for lower GFR and, together, explain 45.2% of the variance toward GFR (Table 2, bottom).

By OR (Figure 1), higher iPTH (OR, 5.377; 95% CI, 1.202–24.051), greater hsCRP (OR, 3.156; 95% CI, 0.885–11.255), older age (OR, 4.543; 95% CI, 1.012–20.397), and LVH (OR, 1.942; 95% CI, 0.819–4.293) are associated significantly with renal insufficiency; MTHFR 677C>T polymorphism is associated significantly with lower odds of renal insufficiency (OR, 0.443; 95% CI, 0.141–1.387).

DISCUSSION

According to our results, renal insufficiency in adult nondiabetic subjects is explained by the interaction of MTHFR C677T polymorphism with other independent factors, that is, iPTH, LVMMi, and RRI. Myocardial LVH (assessed by LVMMi) has a single significant predictor, that is, lower GFR, while the other factors considered by our investigation, including MTHFR mutation, do not show this effect: our finding, age-independent, confirms the close relationship of GFR and LVMM and their parallel progression.[19,53] Mild–moderate renal insufficiency, assessed by GFR, is considered a comprehensive expression of the effects of multiple factors on renal function outcome,[53] while LVH, assessed as LVMM/m2, is used as a broad measure of the lasting effects of different mechanisms on myocardial mass. Mild–moderate renal insufficiency is associated with increased LVMMi.[17] LVH is a broad measure of the lasting effects of different mechanisms on myocardial anatomy and function.[19] We do not confirm the independent increased risk to LVH reported in association with greater CRP,[54] increased intra-renal arterial resistance, as assessed by RRI,[32] and increased serum iPTH.[55] Nonetheless, lower GFR is well recognized as a factor related with greater CRP,v[56,57] increased intra-renal arterial resistance, as assessed by RRI,[58,59] and increased serum iPTH.[32] MTHFR C677T polymorphism has predictive effects on GFR: subjects with MTHFR C677T polymorphism have a lower likelihood of renal insufficiency in comparison with subjects with the wild MTHFR genotype. This result is not surprising since this mutation is associated with a protective effect versus very prevalent cancer diseases[9–12] and is not disadvantageous for longevity.[13] HCY is settled as a putative risk factor for CVD,[60] and mechanisms for glomerular injury and progression of renal insufficiency are envisaged.[61] Nonetheless, related genetic background, such as MTHFR mutation, cannot be necessarily detrimental. Insulin resistance and obesity are recognized as LV mass determinants independent of blood pressure[19]; we failed to confirm this relationship, and probably the exclusion of diabetic patients is the reason of this result. Relationships of diabetes, insulin resistance, and subclinical hyperinsulinemia/hyperglycemia with cardiac structure and function are recognized: both were consistently implicated in concentric LV remodeling[62,63] and in development of chronic kidney disease with rapid decline in renal function.[64] 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.[65] Differently, there is a good consistency of data that establish renal involvement and LVH as novel risk factors for morbidity and mortality in diabetes mellitus.[66] Cardiac remodeling, also with increase of LVMM, is a premise toward the development of heart insufficiency,[67] which could be redefined as also encompassing serological biomarkers.[68] The favorable relevance of adherence to healthier nutritional profile and lifestyle changes is well established and warranted in cardiac disease[69,70] and also, by more recent contributions, in renal disease.[71] In earlier studies,[72,73] relationship of MTHFR C677T mutation with renal and cardiac involvement was associated with precocious target organ damage. Actually, in younger subjects[74] and in other reports,[75] homozygosity for the C677T mutation is not unequivocally associated with increased risk for CVD, irrespective of folate intake. This is confirmed by a recent extensive epidemiological study, in which despite lower serum folate and higher homocysteine, MTHFR 677TT genotype, used as a proxy for lifelong high blood HCY concentrations, is associated with a significantly lower risk of CVD mortality.[76] 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.[77] Moreover, high HCY levels in patients with end-stage renal disease were not associated with incidence of vascular access thrombosis.[78] In our study, MTHFR C677T mutation occurs in a population that has still a relatively low prevalence of cardiovascular[15] and renal disease.[71] It is possible that this polymorphism, even associated with greater LVMMi, could have maintained its persistence in human populations by a 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.[15]

CONCLUSION

MTHFR 677C>T gene polymorphism could have a protective role on renal function in non-diabetic patients without hyperhomocysteinemia and adequate alimentary regimen.

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APPENDIX

The traditional Mediterranean diet prescribed is characterized by a high intake of vegetables, legumes, fruits and nuts, and cereals, a high intake of olive oil, a low or no intake of saturated lipids, a moderately high intake of fish, a low-to-moderate intake of dairy products (mostly in the form of cheese or yogurt), and a low intake of meat and poultry. The subjects reported their daily or weekly average intake of several food items that they consumed during the last year. Then, the frequency of consumption was quantified approximately in terms of the number of times a month this food was consumed. Thus, daily consumption was multiplied by 30 and weekly consumption was multiplied by 4: a value of 0 was assigned to food items rarely or never consumed;[1] daily consumption of nonrefined cereals and products (e.g., whole-grain bread, pasta, brown rice, etc.), fruits (4–6 servings/day), vegetables (2–3 servings/day), olive oil (as the main added lipid), and non-fat or low-fat dairy products (1–2 servings/day); [2] weekly consumption of fish, poultry, potatoes, olives, pulses, and nuts (4–6 servings/week) and more rarely eggs and sweets (1–3 servings/week) and monthly consumption of red meat and meat products (4–5 servings/month). According to the previous dietary pattern and the reported monthly frequency consumption of these food groups, we calculated each participant's diet score, which assessed adherence to the Mediterranean diet (range 0–55).

Adherence to Mediterranean Diet Score criteria can be summarized as follows:

Mediterranean food (I Pasta and rice; II whole-grain bread, brown rice, legumes; III Fruit; IV Green vegetables; V Fish, poultry, No-fat or low-fat dairy products; VI olive oil) had assigned, each group of food, the following scores: 0 = no consumption; a score of 1 = 1–4 times/month; 2 = 5–8 times/month; 3 = 9–11 times/month; 4 = 12–14 times/month; and 5 = more than 14 times/month. "Westernized food": (VII Red meat; VIII Dairy products-butter; IX Potatoes and eggs; X Cakes) opposite scores were assigned, each group of food, the following scores: 5 = 0–4 monthly consumption; score 4 = 5–8 monthly consumption; 3 = 9–12 monthly consumption; 2 = 13–16 monthly consumption; 1 = 17–20 monthly

consumption; 0 = more than 20 monthly consumptions). XI Wine and alcoholics (on average daily base): (0–10 g of alcoholics from Red Wine for women score 5; 0–20 g of alcoholics from Red Wine for men score 5); each increment of 10 g, from the maximal allowed baseline, determines negative scores (20–30 = −1; 30–40 = −2; 40–50 = −3; 50–60 = −4; >60 = −5 for men; 10 less for women and for all non-wine alcoholics: 10–20 = −1; 20–30 = −2; 30–40 = −3; 40–50 = −4; >50 = −5). Overall Adherence to Mediterranean Diet Score (AMDS) has a range of 0–55 and currently we consider adequate a score with a cut-off above 30/55.