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Non-alcoholic fatty liver disease (NAFLD) and MTHFR 1298A > C gene polymorphism

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Abstract. – INTRODUCTION: Non-Alcoholic Fatty Liver Disease (NAFLD) is related to unhealthy habits, mainly to unfavorable dietary profiles. MTHFR gene encodes MethyleneTetraHydroFolate Reductase, a regulatory enzyme whose polymorphisms are associated with hyperhomocysteinemia. Among polymorphisms, C677T, a thermolabile form, but not A1298C, thermostable, was associated with fatty liver and insulin resistance.

AIM: to investigate if NAFLD, in subjects referred for nutritional assessment and counselling, has any difference of prevalence and severity when associated with isolated MTHFR A1298C polymorphism and hyperhomocysteinemia.

PATIENTS AND METHODS: 94 subjects, age 55.65±15.43 years, BMI 27.88±5.17 kg/m², 26 with MTHFR Wild type genotype (1298AA) and 68 with MTHFRA1298C single polymorphism were studied: of them, 35 were homozygous (MTHFR1298CC), 33 were heterozygous (MHTFR 1298AC). Insulin resistance was assessed by HOMA-IR, NAFLD by UltraSound Brigh-Liver-Score (BLS).

RESULTS: MTHFR subgroups (wild and A1298C single polymorphism) were not different for age, gender, dietary profile and BMI. In NAFLD, MTHFR 1298AC (heterozygous) vs. homozygous wild genotype (MTHFR 1298AA) patients had more severe NAFLD (BLS: 1.12±1.14 vs. 0.54±0.76, $p < 0.029$), greater insulin resistance (HOMA 3.20±2.35 vs. 2.12±1.12; $p < 0.036$), higher AST and gammaGT.

CONCLUSIONS: MTHFR1298AC gene heterozygous polymorphisms can be weakly predictive for NAFLD severity. This mutation occurs frequently in populations with low prevalence of overall mortality and of atherosclerosis-associated disease: it could have maintained and maintain its persistence by an heterozygosis advantage mechanism, within significant adherence to healthy nutritional profiles. Interactions of nutrition, genetics and health are a part of the aging process throughout the life span and a greater consideration to the genetic characteristics of populations and individuals is warranted.

Key Words:
Homocysteine, Fatty liver, Mediterranean diet, Genetic MTHFR polymorphism, Insulin resistance, Obesity.

Introduction

The most common factors of liver degeneration into hepato-steatosis are alcohol abuse, with consequent alcoholic liver disease (ALD) and dietary excess, with obesity; these results into Non-Alcoholic Fatty Liver Disease (NAFLD)[1]. Subtle interpatient genetic variations and environment may interact to produce disease phenotype determining its progression[1,2]. Few studies suggest a relationship of Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms with liver disease. This gene shows DNA sequence variants (genetic polymorphisms): C677T (rs1801133) and A1298C (rs1801131) are the two single nucleotide polymorphisms (SNP) more studied, more common and with evidence of association with disease[3]. An increase of the risk to develop hepatocarcinoma (HCC) in patients who consume a high alcohol diet was reported in transplanted patients with C677T MTHFR polymorphism[4]. Moreover, hyperhomocysteinemia and the MTHFR C677T polymorphism promote steatosis and fibrosis in chronic hepatitis C patients[5,6]. The protein encoded by the gene MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. Overall C677T mutation probably favors progression from NAFLD to NASH[7,8]. In methylenetetrahydrofolate reductase knockout mice additive adverse effect of Ang II-dependent hypertension and hyperhomocystinemia on endothelial function were reported[9]. Genetic variation of MTHFR increases susceptibility to occlusive vascular disease, mainly cerebral[10], to trombophilia[11] and to portal vein thrombosis in cirrhosis[12], but the same mutation consistently decreases susceptibility to colon cancer[13] and to acute lym-

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phoblastic leukemia[14]. The 677T allele, i.e. T (thymine) instead of C (cytosine) at position 677 (leading to a valine substitution at amino acid 222) encodes a thermolabile enzyme with reduced activity that can induce, in folic acid malnutrition, a recognizable hyperhomocysteinemia[15]. Differently, the 1298A wild allele (leading to a Glu at amino acid 429) can be substituted by the 1298C allele (leading to an Ala substitution at amino acid 429). The effect is a MTHFR enzyme not thermolabile and as effective as the other thermostable form: no effect is shown on homocysteine levels[16,17]. The most frequent cause of B vitamin deficits is malnutrition, which potentiates the effects of genetic predisposition[18,19]. MTHFR polymorphisms encodes biochemical changes that are not necessarily detrimental, as observed for C677T MTHR in cancer[13,14] and C-hepatitis patients[14]. The aim of this study is to investigate if NAFLD, in subjects consecutively referred for nutritional assessment and counselling, shows any difference of prevalence and severity when associated with isolated MTHFR A1298C polymorphism and hyperhomocysteinemia.

Patients and Methods

The study was carried out with 263 consecutive adult non-diabetic patients (121 M, 142 F), age 53.19±16.33 years, BMI 28.08±5.92 kg/m^2. They were tested for both MTHFR C677T and A1298C polymorphisms, with the purpose of excluding C677T polymorphisms from data analysis, and identifying MTHFR Wild genotype subjects. Mediterranean Diet Adherence Profile [appendix 1] was assessed on the basis of a 1 week recall computerized validated questionnaire as a premise to personalized Mediterranean Diet prescriptions, with daily recommendations derived also from the specific software used (Dietosystem, Milan, Italy®)[20]. Physical activity increase and smoking withdrawal active counselling were also provided. Overall Adherence to Mediterranean Diet Score[21] (AMDS) has a range of 0-55[20,22] and is considered sufficiently adequate above 30/55. Physical activity was encouraged also in the form of walking using the "10,000 steps a day" suggestion[23]. Human insulin and Folic acid were assayed using Immulite 2000 Analyzer (Siemens Medical System, Milan, Italy), by a solid-phase 2-site chemiluminescent immunometric assay. CRP concentrations were assayed by a standard detection limit of 0.175 mg/L (CardioPhase high-sensitivity CRP 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). BMI was calculated as BW/H^2 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). Patients who had class III (≥ 40.0 kg/m^2) obesity were not eligible for this study. Insulin resistance was assessed by the homoeostasis model-insulin resistance index (HOMA-IR), according to the following formula: fasting insulin value x fasting blood sugar level/22.5[24]. The HOMA threshold for insulin resistance is conventionally considered as > 1.7 according to the likelihood ratios for 11-year cardiovascular disease prediction[25]. The waist-to-hip (W/H) ratio was assessed in all patients. Ultrasound (US) examination processing and score assignment were performed by the senior specialist (D.C.), unaware of laboratory details at the time of the procedure. An echocolor-doppler machine (Siemens Acuson S2000™ , Siemens AG, Muenchen Germany), high resolution, with real-time sectional scan transducers was used. The liver was assessed for size, contour, echogenicity, structure, penetration of the US beam i.e. posterior beam attenuation and portal vessel wall distinction; these last criteria were assessed in the report of the degree of steatosis. The rating of bright liver considered for statistical evaluation was classified combining 3 subscale-grades focused on echogenicity, beam penetration, and portal vessel wall distinction, a method previously validated by FNAB US-guided liver biopsy[20,26]. Grade 0: normal or slightly reduced in comparison with right kidney echogenicity; grade 1: slightly increased in comparison with right kidney echogenicity; grade 2: clearly increased, with a decrease in echo amplitude (i.e., posterior beam attenuation owing to the high reflectivity of the fat liver parenchyma); and grade 3: markedly increased with a decrease in echo amplitude and impaired portal vessel wall distinction, that is loss of echoes from the walls of the portal vein[20,26]. 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 di-

rect 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')[11].

Statistical Analysis

The distributions of MTHFR alleles and genotypes in studied group were checked by χ^2 test or Fisher's exact test. ANOVA and Student *t* test was used to assess the difference in averages between subjects with MTHFR heterozygous and homozygous A1298C polymorphism vs. wild MTHFR 1298AA genotype. Two-sided *p*-value < 0.05 was considered statistically significant. Higher quartiles of age, homocysteine and of high density lipoprotein (HDL) cholesterol were defined; thereafter, the associations of older age, HDL cholesterol, sex, and MTHFR polymorphisms were also assessed as Odds Ratio (ORs) to NAFLD, with 95% confidence intervals. Likelihood Ratio was assessed and sensitivity, specificity and predictivity were calculated.

Results

The study, after excluding C677T polymorphisms, considered 94 subjects: 26 with MTHFR Wild genotype (MTHFR1298AA) and 68 with MTHFR A1298C single polymorphism. Of these last, 35 were homozygous (MTHFR1298CC) and 33 were heterozygous (MHTFR1298 AC). The distributions of the genotypes of MTHFR 677CT and 1298AC polymorphisms observed did not differ significantly from those expected under Hardy-Weinberg equilibrium.

By ANOVA (Table I), the three MTHFR subgroups (wild and A1298C single polymorphism) were not different for age, gender, dietary profile and BMI. Also blood B12 vitamin, folic acid, albumin, haemoglobin and CRP were not significantly different in the three considered groups, and were all in the normal range. Significant differences were observed for AST, γGT, HOMA and BLS, that were greater in A1298C subjects. MTHFR Wild genotype (MTHFR 1298AA) vs. MTHFR 1298AC (heterozygous) subjects had less severe NAFLD (BLS: 0.54±0.76 vs. 1.12±1.14; $p < 0.029$), lower insulin resistance (HOMA-IR 2.12±1.12 vs. 3.20±2.35; $p < 0.036$) and lower AST and γGT (Table II). These differences were not significant between the A1298C

Table I. Characteristic of study population and differences between MTHFR genotypes.

	Total (94 subjects)	MTHFR wild genotype (26 subjects)	MTHFR 1298 AC (33 subjects)	MTHFR 1298 CC (35 subjects)	*p*
Age, years	55.65 ± 15.43	56.27 ± 16.42	55.03 ± 16.58	55.77 ± 13.93	0.954
BMI, kg/m^2	27.88 ± 5.17	27.74 ± 5.44	28.66 ± 5.96	27.24 ± 4.10	0.528
CRP, mg/dl	4.63 ± 9.81	2.56 ± 3.09	6.89 ± 14.58	4.03 ± 6.90	0.129
Blood glucose, mg/dl	93.94 ± 18.01	88.77 ± 11.43	96.70 ± 17.06	95.17 ± 22.09	0.216
Triglycerides, mg/dl	104.04 ± 51.36	109.77 ± 73.19	108.12 ± 44.71	95.93 ± 35.86	0.500
Total cholesterol, mg/dl	205.04 ± 51.61	193.25 ± 43.14	203.94 ± 37.33	201.39 ± 44.49	0.550
HDL cholesterol, mg/dl	55.97 ± 18.92	59.12 ± 20.85	55.20 ± 17.79	54.36 ± 18.73	0.603
LDL cholesterol, mg/dl	124.62 ± 39.49	112.18 ± 36.84	127.12 ± 32.36	131.50 ± 45.92	0.152
AST, U/L	24.69 ± 14.19	20.10 ± 6.97	29.92 ± 18.23	23.16 ± 12.52	**0.020**
ALT, U/L	17.10 ± 5.84	17.06 ± 4.52	19.23 ± 5.70	17.10 ± 5.84	0.197
Alkaline phosphatase, U/L	76.76 ± 25.56	68.38 ± 14.80	81.47 ± 29.01	78.54 ± 27.47	0.130
γGT, U/L	30.88 ± 26.98	23.79 ± 11.77	40.36 ± 40.25	27.21 ± 15.01	**0.037**
HOMA	2.56 ± 1.71	2.12 ± 1.12	3.20 ± 2.35	2.29 ± 1.11	**0.025**
Homocysteine, μmol/L	20.05 ± 4.40	19.74 ± 4.62	20.47 ± 5.09	19.89 ± 3.56	0.794
Albumin, g/dl	4.61 ± 0.36	4.63 ± 0.28	4.60 ± 0.41	4.60 ± 0.37	0.916
AMDS	30.37 ± 8.88	30.55 ± 8.72	29.87 ± 8.99	30.71 ± 8.55	0.918
BLS	0.77 ± 0.96	0.54 ± 0.76	1.12 ± 1.14	0.60 ± 0.81	**0.027**
Men, n	47	11	20	16	0.308*
Obesity, n	29	6	14	9	0.198*

BMI: Body Mass Index; CRP: C-Reactive Protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γGT: γ-Glutamyl Transpeptidase, AMDS: Adherence Mediterranean Diet Score; BLS, Bright Liver Score. *Pearson Chi-Square. Significant *p* are evidenced in bold fonts.

Table II.

t-test between MTHFR 1298AC heterozygous polymorphism vs. MTHFR Wild genotype			
	MTHFR Wild genotype (26 subjects)	MTHFR 1298 AC (33 subjects)	*p*
Age, years	56.27 ± 16.42	55.03 ± 16.58	0.776
BMI, kg/m^2	27.74 ± 5.44	28.66 ± 5.96	0.546
CRP, mg/dl	2.56 ± 3.09	6.89 ± 14.58	0.142
Blood glucose, mg/dl	88.77 ± 11.434	96.70 ± 17.060	**0.047**
Triglycerides, mg/dl	109.77 ± 73.19	108.12 ± 44.71	0.915
Total cholesterol, mg/dl	193.25 ± 43.14	203.94 ± 37.33	0.312
HDL cholesterol, mg/dl	59.12 ± 20.85	55.20 ± 17.79	0.439
LDL cholesterol, mg/dl	112.18 ± 36.84	127.12 ± 32.36	0.103
AST, U/L	20.10 ± 6.969	29.92 ± 18.236	**0.012**
ALT, U/L	17.06 ± 4.522	19.23 ± 5.705	0.118
Alkaline phosphatase, U/L	68.38 ± 14.80	81.47 ± 29.01	**0.041**
γGT, U/L	23.79 ± 11.77	40.36 ± 40.25	**0.047**
HOMA	2.12 ± 1.12	3.20 ± 2.35	**0.036**
Homocysteine, mcmol/L	19.74 ± 4.62	20.47 ± 5.09	0.575
Albumin, g/dl	4.63 ± 0.28	4.60 ± 0.41	0.704
Vit. B12, mcg/dl	632.24 ± 471.01	663.64 ± 427.01	0.790
Folic acid, ng/ml	9.91 ± 4.67	11.47 ± 4.74	0.211
AMDS	30.55 ± 8.72	29.87 ± 8.99	0.771
BLS	0.54 ± 0.76	1.12 ± 1.14	**0.029**
t-test between MTHFR 1298CC homozygous polymorphism vs. MTHFR Wild genotype			
	MTHFR Wild genotype (26 subjects)	MTHFR 1298 AC (33 subjects)	*p*
Age, years	56.27 ± 16.42	55.77 ± 13.93	0.899
BMI, kg/m^2	27.74 ± 5.44	27.24 ± 4.10	0.684
CRP, mg/dl	2.56 ± 3.09	4.03 ± 6.90	0.314
Blood glucose, mg/dl	88.77 ± 11.434	95.17 ± 22.094	0.183
Triglycerides, mg/dl	109.77 ± 73.19	95.93 ± 35.86	0.334
Total cholesterol, mg/dl	193.25 ± 43.14	205.04 ± 51.61	0.349
HDL cholesterol, mg/dl	59.12 ± 20.85	54.36 ± 18.73	0.354
LDL cholesterol, mg/dl	112.18 ± 36.84	131.50 ± 45.92	0.083
AST, U/L	20.10 ± 6.969	23.16 ± 12.522	0.266
ALT, U/L	17.06 ± 4.522	17.10 ± 5.841	0.976
Alkaline phosphatase, U/L	68.38 ± 14.80	78.54 ± 27.47	0.093
γGT, U/L	23.79 ± 11.77	27.21 ± 15.01	0.339
HOMA	2,12 ± 1.12	2.29 ± 1.11	0.566
Homocysteine, mcmol/L	19.74 ± 4.62	19.89 ± 3.56	0.891
Albumin, g/dl	4.63 ± 0.28	4.60 ± 0.37	0.695
Vit. B12, mcg/dl	632.24 ± 471.01	598.43 ± 375.81	0.756
Folic acid, ng/ml	9.91 ± 4.67	8.61 ± 4.70	0.286
AMDS	30.55 ± 8.72	30.71 ± 8.55	0.943
BLS	0.54 ± 0.76	0.60 ± 0.81	0.765

BMI: Body Mass Index; CRP: C-Reactive Protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γGT: γ-Glutamyl Transpeptidase, AMDS: Adherence Mediterranean Diet Score; BLS, Bright Liver Score. Significant p (no one is present in this table) are evidenced in bold fonts.

homozygous (CC) group vs. MTHFR Wild genotype (MTHFR 1298AA) (Table II).

By Odds Ratio (Figure 1) the presence of obesity (OR 14.063; CI 95% 4.323-45.746), insulin resistance (OR 3.360; CI 95% 1.368-8.253), higher γGT (OR 2.368; CI 95% 0.944-5.940), A1298C polymorphism, overall (OR 1.697; CI 95% 0.675-4.267) and more significantly as heterozygosis (OR 2.462; CI 95% 0.858-7.065), were all factors associated with greater Odds of NAFLD. The sensitivity of the A1298C MTHFR polymorphism positivity is 77.8%, with a posi-

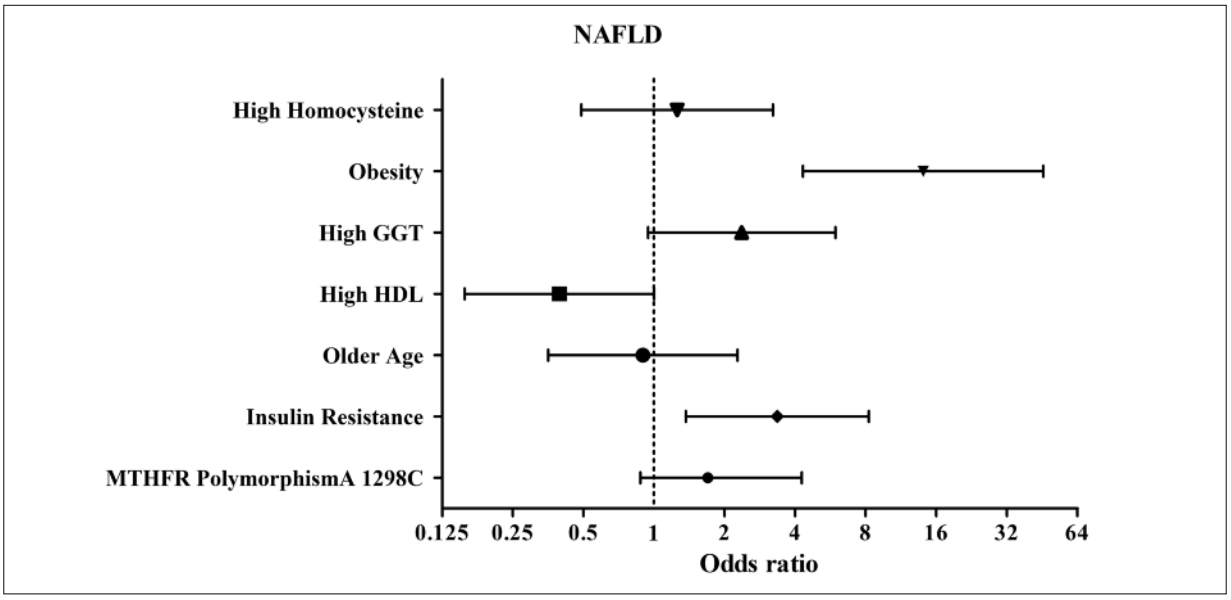

Figure 1. By Odds Ratio the presence of obesity (OR 14.063; CI 95% 4.323-45.746), insulin resistance (OR 3.360; CI 95% 1.368-8.253), higher γGT (OR 2.368; CI 95% 0.944-5.940), A1298C mutation, overall (OR 1.697; CI 95% 0.675-4.267) were all factors associated with a greater prevalence of NAFLD. Higher homocysteine and older age are not associated with any significant difference of NAFLD prevalence; higher HDL-cholesterol is associated with a lower prevalence of NAFLD.

tive likelihood ratio (LR+) of 1.155 [0.9 to 1.483] and a negative likelihood ratio (LR-) of 0.681 [0.345 to 1.341] the specificity is 32.7%. The positive predictive value (PPV = 51.5%), is small, as showed by the nearness of the two lines. Also the negative predictive value (NPV 61.5%) is present but quite low (Figure 2).

Discussion

From our results, MTHFR 1298AC gene polymorphisms can be a marker of greater susceptibility to NAFLD, even without a significant predictivity (Figure 2). Both MTHFR polymorphisms are exceedingly more prevalent in Mediterranean area[13] and since 85-90% of the inhabitants have one or the other polymorphism, these are actually the most common genotype in this area. Genetic variants of novel yet unconsidered putative candidate genes were identified in NAFLD[27]. Genetic, viral[28] and environmental risk factors for NAFLD interact and influence the severity of steatosis and oxidative stress, the cytokine milieu, the magnitude of the immune response, and/or the severity of fibrosis: several genes are associated with NAFLD and might play a role in its pathogenesis[29]. Ethnic variation in the prevalence of NAFLD are associated with genetically deter-

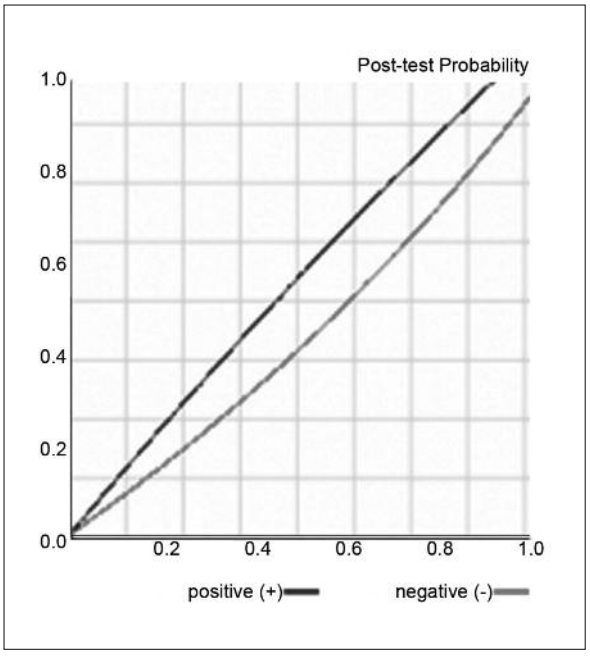

Figure 2. In its association with NAFLD, the sensitivity of the A1298C MTHFR polymorphism positivity is 77.8%, with a positive likelihood ratio (LR+) of 1.155 [0.9 to 1.483] and a negative likelihood ratio (LR-) of 0.681 [0.345 to 1.341] the specificity is 32.7%. The positive predictive value (PPV = 51.5%) is small, as showed by the nearness of the two lines. Also the negative predictive value (NPV 61.5%) is present but quite low. [Graph from Statistics Calculator. By Courtesy of CEBM].

mined differences in body fat distribution: this points to a strong component of genetic predisposition which underlies many of these differences[30,31]. C677T MTHFR polymorphism is associated with a greater risk of developing hepatocellular carcinoma in alcoholic cirrhosis[32] and of liver fibrosis progression in patients with recurrent hepatitis C, but not with a concurrent fatty liver promotion[33]. In the subjects we studied, the currently defined "wild" MTHFR genotype shows an homozygous prevalence of 26/263, i.e. less than 10.0%, so that a selective advantage of the other, very prevalent polymorphisms can be envisaged. It is recognized that the 677C > T mutation in MTHFR reduces colon cancer risk, perhaps by increasing 5,10-methylenetetrahydrofolate levels for DNA synthesis[34,35]. More recent data suggest that low plasma folate levels may be associated with lower risk of colorectal cancer, but that this association may not be due to folate intake; the inverse association between both MTHFR 677TT and for 1298CC genotypes with colorectal cancer risk is strongly confirmed[35]. These lower-risks for cancer genotypes are associated with lower circulating plasma folate levels. Nonetheless, the actual mechanisms remain elusive since plasma folate levels can variously reflect dietary intake, genetic influences, and other factors[36]. For MTHFR A1298C several epidemiological studies did not find any association of genotype with lower or higher breast cancer risk, possibly since the variant allele for MTHFR A1298C may have less impact on enzyme activity than the MTHFR C677T variant allele. It is also possible that the variant genotype for this polymorphism is only important in the presence of the variant genotype for the MTHFR 677 polymorphism[37].

Dietary profile is a critical determinant of homocysteine level: it was reported that a greater adherence to Mediterranean Diet, assessed by the Mediterranean Diet Score Questionnaire[20-22], is associated with lower levels of homocysteine[38]. Folic Acid and B12 vitamin were inside the normal ranges, without a significant difference in 1298AC polymorphism vs. the homozygous 1298AA or "wild" genotype. The explicit information that we are providing is that these subjects have a greater association, if not a risk, of NAFLD, associated with higher γGT, AST, HOMA-IR and BLS (bright liver score) when they also have the heterozygous 1298AC MTHFR polymorphism, not dependent by nutritional behavior differences. This association is much more noteworthy since there were no differences of BMI and of obesity, defined by current criteria (Tables I, II) between any group. This situation shares analogies with the composite effects of MTHFR polymorphism in cancer occurrence[34-36]. A selection or survival advantage for individuals with combined MTHFR 1793G > A and MTHFR 1298A > C genotypes, possibly owing to a mutually stabilizing effect on MTHFR enzyme activity, was suggested in a large cohort of kidney transplant recipients[39]. Even if no definite MTHFR polymorphism advantage for lifespan is currently described, beneficial effects of possible deleterious genes may have played a role in the development and maintenance of diabetes-obesity-fatty liver susceptible human populations[40]. Such genes could have provided the survival advantage that has allowed both the development and the successful establishment of species in sites of countries in which non-optimal climate and environment changes occur, such as borderline desert regions and other less affluent regions[40,41]. Obesity in different populations, such as elderly people and patients with cardiovascular diseases (CVD), like heart failure or coronary artery disease, is surprisingly not associated with a higher but with a lower mortality risk[42]: a tendency to weight loss with aging is observed and frailty is often the ineluctable association for older subjects. The A1298C allele frequency is high in our as in other population, but a direct association with specific diseases is not established[43]. Occurrence of fatty liver, very prevalent in our populations and suitable for a reliable non-invasive follow-up[44], cannot be regarded as a straightforward unfavorable condition[45,46], particularly in this subset. Lifestyle changes that are already the mainstay of treatment for patients with the metabolic syndrome and/or diabetes are, conceivably, the most reliable tools also for reducing steatosis, i.e. to counteract the clinical and histological progression both of "purely metabolic" and of HCV associated steatosis[47-48]. Greater fat liver content accounts for a decreased renal function in NAFLD patients: inter-related factors can be operating early also in the natural history of obesity-related kidney and liver disease[49], and what is attributable to liver, kidney or generalized vascular disease, with or without hyperhomocysteinemia, is difficult to ascertain and still controversial.

Conclusions

Both NAFLD and MTHFR 1298AC polymorphism are observed within a significant adher-

ence to current guidelines and healthy nutritional profiles. These are not preventing the occurrence of a condition, fatty liver, considered a sign of metabolic liver dysfunction. Moreover, MTHFR 1298AC gene heterozygous polymorphisms can be a marker predictive for greater NAFLD severity. Since this mutation occurs frequently in populations whit still relatively low prevalence of overall mortality and of atherosclerosis-associated disease, it could maintain its persistence by an heterozygosis advantage mechanism, within significant adherence to healthy nutritional profiles.

Appendix 1

The traditional Mediterranean diet prescribed is characterized by a high intake of vegetables, legumes, fruits and nuts, cereals, a high intake of olive oil, and 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 7 value of 0 was assigned to food items rarely or never consumed; (1) daily consumption of non-refined cereals and products (e.g., whole-grain bread, pasta, brown rice, and the like), fruits (4 to 6 servings/day), vegetables (2 to 3 servings/day), olive oil (as the main added lipid), and non-fat or low-fat dairy products (1 to 2 servings/day); (2) weekly consumption of fish, poultry, potatoes, olives, pulses, and nuts (4 to 6 servings/week), as well as more rarely eggs and sweets (1 to 3 servings/week), and monthly consumption of red meat and meat products (4 to 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 to 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 to 4 times/month; 2 = 5 to 8 times/month; 3 = 9 to 11 times/month; 4 = 12 to 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.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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