Genes & Diseases (2019) 6, 88–95

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

FULL LENGTH ARTICLE

The methylenetetrahydrofolate reductase genotype 677CT and non-alcoholic fatty liver disease have a synergistic effect on the increasing homocysteine levels in subjects from Chongqing, China

Xiaolin Wang [a], Yongli Zhou [b], Mingjun Zhang [a], Yonghong Wang [a,*], Bo Qin [c,]**

[a] *Health Management Center, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China*
[b] *Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China*
[c] *Department of Infectious Diseases, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China*

Received 3 February 2018; accepted 17 July 2018
Available online 29 July 2018

KEY WORDS
Gene polymorphism;
Homocysteine;
Methylenetetrahydrofolate reductase;
Nonalcoholic fatty liver disease;
Synergistic effect

Abstract The methylenetetrahydrofolate reductase (MTHFR) genotypes 677CT and 677TT are associated with elevated serum homocysteine (Hcy) levels by means of lowering the activity of MTHFR, and the increase in serum Hcy may be linked to increased susceptibility to non-alcoholic fatty liver disease (NAFLD). However, there are contradictory reports of the relationship among the MTHFR 677CT gene polymorphism, Hcy, and NAFLD. Therefore, the aim of this study was to identify potential associations and interactions of either Hcy levels or the MTHFR 677CT gene polymorphism with the susceptibility to NAFLD in a Chinese population. The association between the MTHFR 677 CT gene polymorphism and Hcy levels was determined in 243 subjects with NAFLD and 388 healthy subjects without NAFLD using polymerase chain reaction-restriction fragment length polymorphism analysis and high-performance liquid chromatography. In subjects with NAFLD, there was no statistical difference in the genotypic and allelic frequencies of the MTHFR 677 CT gene polymorphism, while serum Hcy levels were

* Corresponding author. Department of Physical Examination, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing, 400016, China.
** Corresponding author. Department of Infectious Diseases, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing, 400016, China.
E-mail addresses: wyh0232983@163.com (Y. Wang), qinbo33@hotmail.com (B. Qin).
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2018.07.003
2352-3042/Copyright © 2018, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

significantly higher in subjects with NAFLD. Furthermore, these results strongly suggest that the MTHFR 677CT gene polymorphism and NAFLD have a potential synergistic effect on Hcy elevation, although the MTHFR 677CT gene polymorphism was not correlated with NAFLD in a Chinese population.
Copyright © 2018, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

With the continued improvement in living standards and overall health status, the spectrum of chronic liver disease has been changed dramatically from chronic hepatitis B infection to non-alcoholic fatty liver disease (NAFLD), according to a community-based random sample of large and medium-sized cities in China, where the average prevalence of NAFLD is about 15% (range, 6.3%—27%).[1] Previous studies have reported that metabolic syndrome and its components (i.e., obesity, dyslipidemia, hypertension, hyperglycemia, and so on) are closely associated with NAFLD.[2—4]

Hyperhomocysteinemia (HHcy) can result in vascular disease,[5] promote the secretion of inflammatory cytokines,[6] induce oxidative stress and endoplasmic reticulum stress,[7] and change the state of blood coagulation and platelet function.[8] 5-Methyltetrahydrofolate is the main substrate for transforming homocysteine (Hcy) to methionine and the methylenetetrahydrofolate reductase (MTHFR) genotypes 677CT and 677TT can lower the activity of the MTHFR, resulting in elevated Hcy levels,[9] while the frequencies of the MTHFR 677CT and 677TT genotypes in China are comparatively higher than in other countries.[10]

According to a case—control study including 2370 subjects with NAFLD and 4833 healthy subjects, serum Hcy levels were identified significantly higher in patients with NAFLD vs. controls,[11] and another study shows higher Hcy levels existed in NAFLD patients and were correlated with the severity of insulin resistance.[12] But, serum Hcy levels were not statistically different between patients with biopsy-proven NAFLD and controls after adjustment for BMI, waist circumference, gender, age, and log (HOMA-IR).[13] So, the association between serum Hcy levels and the prevalence of NAFLD is inconclusive. At the same time, contradictory results regarding the potential correlation between the MTHFR 677CT gene polymorphism and NAFLD have been reported.[14—16] Therefore, the first aim of the present study was to confirm the association between MTHFR 677CT gene polymorphism and the prevalence of NAFLD. Furthermore, we also want to explore interactions between the MTHFR 677CT genotype and NAFLD with Hcy levels in Chongqing, China.

Methods

Study population

All subjects were recruited from the Health Management Center of the First Affiliated Hospital of Chongqing Medical University (Chongqing, China). Random data tables (SAS ver. 9.4 software; SAS Institute, Cary, NC, USA) were used to select 814 subjects who underwent annual physical evaluations from January 2016 to July 2017. The inclusion criteria were age >20 years and no serious chronic or infectious disease, while the exclusion criteria were incomplete data, a definite history of alcohol use (>30 g for males and >20 g for females), positive results for the surface antigen of the hepatitis B virus or anti hepatitis C virus, autoimmune hepatitis, Wilson's disease, hemochromatosis, any chronic liver disease, and malignancy. Finally, 631 subjects were included for analysis: 243 subjects with NAFLD and 388 without. Prior to subject recruitment, the study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (protocol No. 2017-036). Informed consent was obtained from all subjects.

Baseline data collection

Body weight, height, and waist circumference (WC) were measured by experienced physicians who had received specialized training. Before being measured, the subjects were requested to remove excessive clothing and stand barefoot. Height and WC were accurate to 0.1 cm and body weight to 0.1 kg, and body mass index (BMI) was calculated as weight (kg)/height (m^2).

Blood samples were collected from the antecubital vein after overnight fasting. The following biochemical parameters were assessed: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (γ-GT), fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), uric acid (UA), white blood cell (WBC) count, and fasting serum insulin (INS). Genomic DNA was isolated from peripheral blood leukocytes by the salting-out method.[17] Serum Hcy levels were measured by high-performance liquid chromatography. The presence of the MTHFR 677CT polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism analysis.[18] All measurements were performed with auto analyzers in the Clinical Science Experiment Center of the First Affiliated Hospital of Chongqing Medical University. The clinical laboratory was in compliance with the accreditation criteria for the quality and competence of medical laboratories (ISO15189, NO. ML00036).

Diagnostic criteria

In this study, TC > 5.72 mmol/L was defined as high, TG > 1.70 mmol/L was defined as high, HDL-C < 0.90 mmol/L was defined as low, LDL-C > 3.10 mmol/L was defined as high, ALT >50 U/L was defined as high, AST >40 U/L was defined as high, γ-GT > 60 U/L was defined as high, and FPG >5.6 mmol/L was defined as high. Hyperuricemia was defined as UA ≥ 7 mg/dL (420 μmol/L) in men and ≥6 mg/dL (360 μmol/L) in women. HHcy was defined as > 10 μmol/L.[10] Abdominal obesity was defined according to the National Cholesterol Education Program—Adult Treatment Panel III criteria, WC ≥ 90 cm for men ≥80 cm for women. This study adopted the definition of overweight/obese recommended by the guidelines for prevention and control of overweight and obesity in Chinese adults, in which overweight was defined as BMI = 24—27.9 and obese as BMI ≥ 28.[19] Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as: HOMA-IR = FPG (mmol/L) × INS (mU/L)/22.5.[20] With ultrasound imaging, fatty liver was diagnosed with well-established criteria, including intrahepatic echo contrast, liver parenchymal brightness, deep beam attenuation, and vascular blurring.[21] Ultrasonographic images of fatty liver were evaluated by two experienced radiologists.

Statistical analysis

All statistical analyses were performed with SAS ver. 9.4 software. Categorical variables are presented as numbers and percentages, while continuous data are expressed as the mean ± standard deviation. Inter-group differences were identified using the chi-square test or Fisher's exact test for categorical variables and the *t*-test for continuous variables. If there are some asymmetrically distributed variables, the asymmetrically distributed variables are presented as median (interquartile range). Besides, non-parametric Mann—Whitney U and Kruskal—Wallis tests were employed for asymmetrically distributed variables to compare the difference between two groups. Genotype and allele distributions were assessed with the Fisher's exact test. Compliance with the Hardy—Weinberg equilibrium was determined using the chi-square test. Multiple logistic regression analysis was performed to determine the association of NAFLD with the odds of clinical and biochemical indexes. Furthermore, the relative excess risk due to interaction (RERI) synergy index (S) and the corresponding 95% confidence interval (CI) were computed to assess the interaction effect between the MTHFR 677CT gene polymorphism and risk factors of NAFLD. If no interaction effect was found, then S = 1 and RERI = 0; if a positive interaction was found, then S > 1 and RERI >0; and if negative interaction was found, then S < 1 and RERI <0. The attributable proportion due to the interaction was defined as the proportion of all cases that can be attributed to the interaction between two factors.[22] The interaction effect between the gender,MTHFR 677CT gene polymorphism and NAFLD with Hcy level was assessed by factorial analysis. A probability (*p*) value of <0.05 was considered statistically significant.

Results

Population characteristics and laboratory evaluation

The demographic and clinical data of NAFLD patients and controls are shown in Table 1. Levels of BMI, WC, γ-GT, AST, ALT, WBC, TC, TG, LDL-C, FPG, UA, Hcy, INS, and HOMA-IR were greater in the NAFLD group than in the control group,

Table 1 Clinical and biochemical levels of subjects among the NAFLD group and control group.

Variable	NAFLD group (*n* = 243)	Control group (*n* = 388)	*p* value
Age, years, M (Q1～Q3)	50.00 (44.00—54.00)	51.00 (44.00—58.00)	0.262
BMI, kg/m2, M (Q1～Q3)	26.40 (24.98—28.96)	23.32 (21.63—25.31)	<0.001
WC,cm, (X ± S)	90.22 ± 7.9	81.02 ± 7.76	<0.001
γ-GT, U/l, M (Q1～Q3)	38.00 (23.00—58.00)	22.00 (15.00—36.5.)	<0.001
ALT, U/l, M (Q1～Q3)	30.00 (22.00—45.00)	21.00 (16.00—30.25)	<0.001
AST, U/l, M (Q1～Q3)	24.00 (20.00—31.00)	22.00 (19.00—27.00)	0.007
WBC,10^9/L, M (Q1～Q3)	6.25 (5.27—7.47)	5.73 (4.83—6.70)	<0.001
TC, mmol/L, M (Q1～Q3)	5.08 (4.43—5.81)	4.83 (4.3—5.46)	0.002
TG, mmol/L, M (Q1～Q3)	2.08 (1.41—3.08)	1.23 (0.98—1.77)	<0.001
HDL-C, mmol/L, M (Q1～Q3)	1.26 (1.09—1.45)	1.42 (1.23—1.71)	<0.001
LDL-C, mmol/L, M (Q1～Q3)	3.28 (2.77—3.92)	3.06 (2.62—3.63)	<0.001
FPG, mmol/L, M (Q1～Q3)	5.70 (5.20—6.60)	5.30 (5.00—5.70)	0.001
UA, μmol/L, M (Q1～Q3)	378.00 (322.00—440.00)	313.50 (263.00—383.00)	<0.001
Hcy, μmol/l, M (Q1～Q3)	10.10 (8.40—12.20)	9.40 (7.65—11.60)	0.015
INS, uIU/ml, M (Q1～Q3)	8.25 (5.76—11.81)	4.79 (3.47—6.73)	<0.001
HOMA-IR, M (Q1～Q3)	2.14 (1.54—3.24)	1.16 (0.81—1.71)	<0.001

Abbreviations: NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; WC, waist circumference; γ-GT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cell; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; UA, uric acid; Hcy, homocysteine; INS, fasting serum insulin. M(Q1～Q3) median (interquartile range), *p*-Value < 0.05 was considered statistically significant.

while HDL-C concentrations were lower in the NAFLD group as compared to the control group.

As shown in Table 2, the prevalence of male, overweight/obese, abdominal obesity, high γ-GT, high ALT, high AST, high WBC, high TC, high TG, high LDL-C, high FPG, HHcy, and hyperuricemia was significantly greater in the NAFLD group than in the control group.

Risk factors of NAFLD filtrated by multiple logistic regression analysis

As shown in Fig. 1, the features related to male (OR = 1.66; 95% CI = 1.017–2.718), overweight/obese (OR = 2.74; 95% CI = 1.662–4.527), abdominal obesity (OR = 3.50; 95% CI = 2.146–5.699), high ALT (OR = 3.31; 95% CI = 1.31–8.364), high WBC (OR = 3.14; 95% CI = 1.011–9.726), high TG (OR = 2.98; 95% CI = 1.948–4.545), high FPG (OR = 2.07; 95% CI = 1.36–3.147), and hyperuricemia (OR = 1.85; 95% CI = 1.159–2.961) were assumed to be significant predictors of NAFLD, while age, high γ-GT, high AST, high TC, low HDL-C, high LDL-C, HHcy, MTHFR 677CT (CT vs. CC), and MTHFR 677CT (TT vs. CC) were not.

Frequency of the MTHFR 677CT polymorphism was not related with susceptibility of NAFLD

The allelic and genotypic frequencies of the MTHFR 677CT polymorphism in the NAFLD and control groups are summarized in Fig. 2. There were no statistical differences in the genotypic and allelic frequencies of the MTHFR 677CT polymorphism between the NAFLD and control groups. The genotype distributions of all polymorphisms in the NAFLD and control groups were in accordance with the Hardy–Weinberg equilibrium ($\chi^2 = 3.96$, $p = 0.14$).

MTHFR 677CT polymorphism and serum Hcy level had no interaction effect on NAFLD susceptibility

Table 3 shows the interaction effects of the MTHFR6 77CT gene polymorphism related to sex, overweight/obese, abdominal obesity, high ALT, high WBC, high TG, high FPG, and hyperuricemia on NAFLD risk. The corresponding RERI was 1.40 (95% CI = −0.295–3.086; $p = 0.039$) for the risk of NAFLD among subjects with the MTHFR 677CT or 677TT genotype and overweight/obese, suggesting a marginally additive significant interaction, where the interaction effects between the MTHFR 677CT or 677TT genotype and overweight/obese accounted for 27% (95% CI = −0.018–0.551) of all NAFLD subjects. The corresponding RERI was 4.33 (95% CI = −0.877–9.539; $p = 0.044$) for the risk of NAFLD among subjects with the MTHFR 677CT or 677TT genotype and high ALT, suggesting a marginally additive significant interaction, where the interaction effects between the MTHFR 677CT or 677TT genotype and high ALT accounted for 68% (95% CI = 0.274–1.08) of all NAFLD subjects. But The corresponding RERI was 0.18 (95% CI = −1.0581–0.695; $p = 0.734$) for the risk of NAFLD among subjects with the MTHFR 677CT or 677TT genotype and HHcy.

Table 2 Clinical and biochemical characteristics of subjects among the NAFLD group and control group.

Parameter	NAFLD group ($n = 243$)	Control group ($n = 388$)	p value
Age,*n* (%)			
≤45	70 (28.81)	109 (28.09)	0.846
>45	173 (71.19)	279 (71.91)	
Gender,*n* (%)	171 (70.37)	214 (55.15)	<0.01
Overweight/Obesity	204 (83.95)	163 (42.01)	<0.01
Abdominal obesity, *n* (%)	178 (73.25)	124 (31.96)	<0.01
High γ-GT,*n* (%)	53 (21.81)	44 (11.34)	<0.01
High ALT,*n* (%)	46 (18.93)	17 (4.38)	<0.01
High AST,*n* (%)	22 (9.05)	15 (3.87)	<0.01
High WBC,*n* (%)	14 (5.76)	7 (1.80)	<0.01
High TC,*n* (%)	67 (27.57)	69 (17.78)	<0.01
High TG,*n* (%)	151 (62.14)	103 (26.55)	<0.01
Low HDL-C,*n* (%)	14 (5.76)	14 (3.61)	0.201
High LDL-C,*n* (%)	144 (59.26)	182 (46.91)	<0.01
High FPG,*n* (%)	126 (51.85)	121 (31.19)	<0.01
Hyperuricemia,*n* (%)	94 (38.68)	62 (15.98)	<0.01
HHcy,*n* (%)	122 (50.21)	164 (42.27)	0.059
MTHFR677CT, *n* (%)			
CC	90 (37.04)	138 (35.57)	0.518
CT	118 (48.56)	204 (52.58)	
TT	35 (14.4)	46 (11.86)	

Abbreviations: γ-GT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cell; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; UA, uric acid; HHcy, hyperhomocysteinemia; MTHFR, methylenetetrahydrofolate reductase. *p*-Value < 0.05 was considered statistically significant.

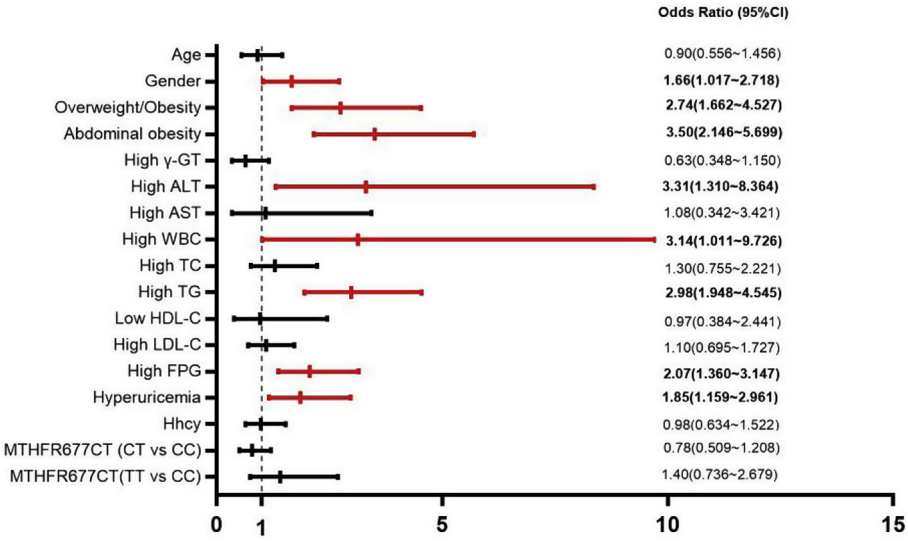

Fig. 1 Multiple logistic regression model with NAFLD as the dependent variable.

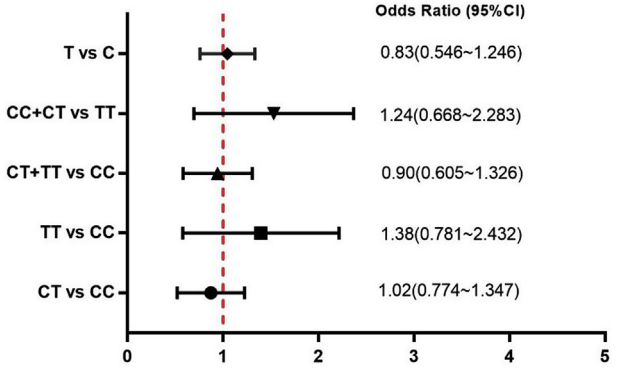

Fig. 2 Odds of allelic and genotypic frequencies of the MTHFR 677CT gene polymorphism to the NAFLD.

MTHFR 677CT gene polymorphism and NAFLD had a synergistic effect on the increasing homocysteine levels

Factorial analysis was used to assess the association between the MTHFR 677CT gene polymorphism and NAFLD and Hcy level. The results are presented in Table 4. Both MTHFR 677CT gene polymorphism ($p < 0.001$) and gender ($p < 0.001$) had significant effects on Hcy level. Further analysis found that MTHFR 677CT gene polymorphism and NAFLD had synergistic effects on the elevation in Hcy levels after adjustment for gender ($p < 0.05$).

Discussion

The prevalence of NAFLD is increasing continuously in recent society.[2] As predictive factors of NAFLD are unclear now. The present study had filtrated some risk factors of NAFLD by multiple logistic regression analysis (Figs. 1 and 2). Our results showed that factors such as male, abdominal obesity, high ALT, high WBC, high TG, high FPG and hyperuricemia may be related with NAFLD susceptibility (Fig. 1). NAFLD has been associated with various parameters, including BMI,[23] WC,[24] lipid,[25] and uric acid levels,[26] among others. The results of the present study revealed that the factors of male, overweight/obese, abdominal obesity, high ALT, high WBC, high TG, high FPG, and hyperuricemia were associated with an increased susceptibility of NAFLD.

Results of our study showed that the MTHFR 677CT gene polymorphism was not associated with NAFLD (Fig. 2). Contradictory results regarding the potential correlation between the MTHFR 677CT gene polymorphism and NAFLD have been reported, previous studies have found that the MTHFR 677TT genotype was associated with NAFLD.[15,27,28] For example, a meta-analysis conducted by Sun et al of 785 cases and 1188 controls revealed an association between the MTHFR 677CT gene polymorphism and NAFLD susceptibility, suggesting that the MTHFR 677 TT genotype is more likely to be linked with NAFLD susceptibility.[16] Another study showed increased prevalence of homozygote MTHFR 677CT mutation in patients with NAFLD compared with healthy controls.[15] However, other studies suggested that there was no significant difference in the frequency of the MTHFR 677CT gene polymorphism between NAFLD patients and healthy subjects.[14,29,30] The present study is the first description of the MTHFR 677CT gene polymorphism with NAFLD patients residing in Southwest Chinese. In this study, the MTHFR 677CT gene polymorphism was not related with NAFLD susceptibility and the MTHFR 677 TT and the MTHFR 677 CT genotypes were not risk factors for NAFLD. The effect of MTHFR677CT gene polymorphism in the susceptibility of NAFLD may be associated with different populations, different geographical areas or different disease states.[27,28] Our results founded that the MTHFR 677CT gene polymorphism has no necessary connection with NAFLD. Therefore, the relationship between the MTHFR 677CT gene polymorphism and NAFLD should be confirmed in large-scale sample studies of different populations, geographical areas and different disease states.

Table 3 Interaction effects of the MTHFR677CT gene polymorphism with risk factors on NAFLD susceptibility.

Exposure	RR (95%CI)	RERI (95% CI)	AP (95% CI)	S (95% CI)	*p* Value
MTHFR677CT	0.68 (0.387—1.191)	0.48 (-0.225—1.183)	0.30 (-0.168—0.774)	5.79 (0—87217.683)	0.169
Gender	1.42 (0.820—2.464)				
MTHFR677CT & Gender	1.58 (0.956—2.608)				
MTHFR677CT	0.52 (0.26—1.025)	1.4 (-0.295—3.086)	0.27 (-0.018—0.551)	1.49 (0.88—2.531)	0.039
Overweight/Obesity	4.32 (2.492—7.491)				
MTHFR677CT & Overweight/Obesity	5.23 (2.967—9.227)				
MTHFR677CT	0.78 (0.447—1.354)	0.24 (-2.188—2.671)	0.05 (-0.433—0.529)	1.06 (0.562—2.014)	0.497
Abdominal obesity	4.98 (2.803—8.865)				
MTHFR677CT & Abdominal obesity	5.00 (2.992—8.371)				
MTHFR677CT	0.81 (0.564—1.150)	4.33 (-0.877—9.539)	0.68 (0.274—1.08)	5.05 (0.575—44.419)	0.044
High ALT	2.26 (0.873—5.866)				
MTHFR677CT & High ALT	6.40 (2.922—14.017)				
MTHFR677CT	0.93 (0.665—1.311)	−3.65 (-17.927—10.626)	−1.38 (-7.159—4.409)	0.31 (0.014—6.877)	0.517
High WBC	6.37 (0.701—57.963)				
MTHFR677CT & High WBC	2.66 (0.931—7.568)				
MTHFR677CT	0.94 (0.575—1.525)	−0.16 (-2.516—2.203)	−0.04 (-0.593—0.519)	0.95 (0.477—1.911)	0.970
High TG	4.50 (2.546—7.94)				
MTHFR677CT & High TG	4.28 (2.594—7.047)				
MTHFR677CT	1.30 (0.820—2.077)	−1.56 (-3.541—0.421)	−0.64 (-1.483—0.194)	0.48 (0.225—1.008)	0.054
High FPG	3.67 (2.100—6.431)				
MTHFR677CT & High FPG	2.42 (1.473—3.976)				
MTHFR677CT	0.82 (0.550—1.226)	1.13 (-0.722—2.977)	0.34 (-0.133—0.812)	1.94 (0.552—6.828)	0.177
Hyperuricemia	2.38 (1.295—4.360)				
MTHFR677CT & Hyperuricemia	3.33 (1.979—5.586)				
MTHFR677CT	0.95 (0.608—1.495)	−0.18 (-1.058—0.695)	−0.14 (-0.828—0.543)	0.60 (0.079—4.548)	0.734
HHcy	1.50 (0.871—2.583)				
MTHFR677CT & HHcy	1.27 (0.814—1.989)				

Abbreviations: ALT, alanine aminotransferase; WBC, white blood cell; TG, triglycerides; FPG, fasting plasma glucose; MTHFR, methylenetetrahydrofolate reductase; HHcy, hyperhomocysteinemia; OR, odds ratio; RR, relative risk; CI, confidence interval; RERI, relative excess risk due to interaction; AP, attributable proportion due to interaction; S, synergy index. *p*-Value < 0.05 was considered statistically significant.

Table 4 The interaction between gender, MTHFR677CT gene polymorphism and NAFLD on Hcy level.

Variation sources	DOF	Anova sum of squares	mean square	F value	Pr > F
Gender	1	3.34599199	3.34599199	166.65	<0.0001
MTHFR677CT	2	2.49310464	1.24655232	62.08	<0.0001
NAFLD	1	0.01040359	0.01040359	0.52	0.4719
Gender*MTHFR677CT	2	0.44542077	0.22271039	11.09	<0.0001
Gender*NAFLD	1	0.00304818	0.00304818	0.15	0.6969
MTHFR677CT*NAFLD	2	0.13303007	0.06651503	3.31	0.0371
Gender*MTHFR67*NAFLD	2	0.11441002	0.05720501	2.85	0.0587

Recent researches found serum Hcy levels may be associated with NAFLD susceptibility.[12,13] MTHFR 677CT and 677TT can lower the activity of the MTHFR, resulting in elevated Hcy levels.[9] In this study, we had also analyzed relationship between serum Hcy levels as well as MTHFR 677CT gene polymorphism with NAFLD. However, serum Hcy levels were significantly higher among NAFLD subjects (Table 2), But further analysis found that MTHFR 677CT polymorphism and serum Hcy level had no interaction effect on NAFLD (Table 3). Up to now, the relationship between serum Hcy levels and the susceptibility of NAFLD is inconclusive. One study shows higher Hcy levels existed in NAFLD patients and were correlated with the severity of insulin resistance,[12] the mechanisms may be relevant to alter intracellular lipid metabolism and promote hepatic fat accumulation in subjects with HHcy.[31] But another study showed after adjustment for risk factors of NAFLD such as BMI, WC, gender, age, and log (HOMA-IR), there was no relationship between HHcy and the NAFLD susceptibility.[13] Therefore, the interaction effect of MTHFR 677CT polymorphism and serum Hcy level on NAFLD also need to be confirmed by animal experiments.

To clarify the possible internal relationship between among MTHFR677CT gene polymorphism, NAFLD and serum Hcy levels. We speculated that MTHFR 677CT gene polymorphism and NAFLD may be related to elevated Hcy levels. In order to prove our conjecture, we made an factorial analysis of three factors (Table 4). After adjustment for gender, our results showed the MTHFR 677CT gene polymorphism and NAFLD have a synergistic effect on the elevated level of Hcy. which may be related to dietary deficiency in methyl donors and cofactors (methionine, choline, folate, B vitamins, etc.) leading to NAFLD and liver damage.[15] Liver injury in NAFLD was also related to changes in the expression levels of one-carbon metabolism genes and Hcy levels.[32] Therefore, levels of folate and vitamin B12, together with the MTHFR 677CT gene polymorphism and Hcy levels, should be regularly evaluated in NAFLD patients to devise personalized nutrient supplementation of methyl-group donors.

There were several limitations to this study. First, this study was primarily based on retrospective analysis; therefore, the causal relationship between NAFLD and Hcy could not be absolutely confirmed. Second, the study participants were recruited from the Department of Physical Examination and more concerned about their health, as compared with those who were not. Consequently, there may have been a selection bias in the present study. Finally, the diagnosis of NAFLD was only based on ultrasound observations.

In conclusion, NAFLD and HHcy have become common diseases. Our present study revealed that the MTHFR 677CT gene polymorphism and Hcy was not associated with the increased risk of NAFLD. Furthermore, we founded the MTHFR 677CT genotype and NAFLD had a synergistic effect on elevating Hcy levels. Our results may provide some new aspects about the solid connection between MTHFR 677CT gene polymorphism,serum Hcy levels and NAFLD.

Author contributions

- Conceived the study design: Xiaolin Wang, Yonghong Wang and Bo Qin.
- Diagnosis and selection of study participants: Yongli Zhou.
- Contributed reagents/materials/analysis tools: Xiaolin Wang and Yongli Zhou.
- Analyzed the data and wrote the manuscript: Xiaolin Wang, Yongli Zhou and Mingjun Zhang.
- All authors provided critical feedback and helped shape the research and analysis, and approved the final version of the manuscript.

Conflicts of interest

The authors have no conflict of interest to declare.

Acknowledgments

This study was funded by a grant from the ChongQing Science and Technology Commission (grant no. cstc2015jcsf10012-03). We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

References

1. Guangyu C, Jiangao F. Research progress of Chinese fatty liver disease epidemiology. *Chin J Practical Intern Med*. 2013;33(9):738—741.
2. Du T, Yu X, Yuan G, et al. Combined influence of nonalcoholic fatty liver and body size phenotypes on diabetes risk. *Cardiovasc Diabetol*. 2015;14:144.
3. Lee MK, Park HJ, Jeon WS, et al. Higher association of coronary artery calcification with non-alcoholic fatty liver disease than with abdominal obesity in middle-aged Korean men: the Kangbuk Samsung Health Study. *Cardiovasc Diabetol*. 2015;14:88.

4. Vanni E, Bugianesi E, Kotronen A, et al. From the metabolic syndrome to NAFLD or vice versa? *Dig Liver Dis*. 2010;42(5):320—330.
5. Hu S, Ren L, Wang Y, et al. Homocysteine-lowering therapy and early functional outcomes of ischemic patients with H-type hypertension: a retrospective analysis of CNSR. *Australas Phys Eng Sci Med*. 2015;38(4):785—791.
6. Zanin RF, Bergamin LS, Morrone FB, et al. Pathological concentrations of homocysteine increases IL-1beta production in macrophages in a P2X7, NF-kB, and erk-dependent manner. *Purinergic Signal*. 2015;11(4):463—470.
7. Tripathi M, Zhang CW, Singh BK, et al. Hyperhomocysteinemia causes ER stress and impaired autophagy that is reversed by Vitamin B supplementation. *Cell Death Dis*. 2016;7(12):e2513.
8. Malinowska J, Kolodziejczyk J, Olas B. The disturbance of hemostasis induced by hyperhomocysteinemia; the role of antioxidants. *Acta Biochim Pol*. 2012;59(2):185—194.
9. Qin X, Li J, Cui Y, et al. MTHFR C677T and MTR A2756G polymorphisms and the homocysteine lowering efficacy of different doses of folic acid in hypertensive Chinese adults. *Nutr J*. 2012;11:2.
10. Jianping Li XL, Yong Huo, Nanfang Li. LeiSong H-type hypertension diagnosis and treatment experts consensus. *Chin J Hepertens*. 2016;24(2):123—127.
11. Dai H, Wang W, Tang X, et al. Association between homocysteine and non-alcoholic fatty liver disease in Chinese adults: a cross-sectional study. *Nutr J*. 2016;15(1).
12. Hu Y, Liu J, Dong X, et al. Clinical study of serum homocysteine and non-alcoholic fatty liver disease in euglycemic patients. *Med Sci Mon Int Med J Exp Clin Res*. 2016;22:4146—4151.
13. Polyzos SA, Kountouras J, Anastasilakis AD, et al. Association between circulating irisin and homocysteine in patients with nonalcoholic fatty liver disease. *Endocrine*. 2015;49(2):560—562.
14. Serin E, Guclu M, Atac FB, et al. Methylenetetrahydrofolate reductase C677T mutation and nonalcoholic fatty liver disease. *Dig Dis Sci*. 2007;52(5):1183—1186.
15. Kasapoglu B, Turkay C, Yalcin KS, et al. MTHFR 677C/T and 1298A/C mutations and non-alcoholic fatty liver disease. *Clin Med*. 2015;15(3):248—251.
16. Sun MY, Zhang L, Shi SL, et al. Associations between Methylenetetrahydrofolate Reductase (MTHFR) Polymorphisms and Non-Alcoholic Fatty Liver Disease (NAFLD) Risk: A Meta-Analysis. *PLoS One*. 2016;11(4):e0154337.
17. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16(3):1215.
18. Hanson NQ, Aras O, Yang F, et al. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. *Clin Chem*. 2001;47(4):661—666.
19. Working group on obesity problem in China (WGDC). The guidelines on the prevention and management of overweight and obesity in adults: *China*. *Acta Nutrimenta Sinica*. 2004;26(1):1—4.
20. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412—419.
21. Needleman L, Kurtz AB, Rifkin MD, et al. Sonography of diffuse benign liver disease: accuracy of pattern recognition and grading. *AJR Am J Roentgenol*. 1986;146(5):1011—1015.
22. VanderWeele TJ, Knol MJ. A tutorial on interaction. *Epidemiol Meth*. 2014;(1):3.
23. Li L, Liu DW, Yan HY, et al. Obesity is an independent risk factor for non-alcoholic fatty liver disease: evidence from a meta-analysis of 21 cohort studies. *Obes Rev*. 2016;17(6):510—519.
24. Park SK, Ryoo JH, Choi JM, et al. The risk of abdominal obesity according to the degree of non-alcoholic fatty liver disease in Korean men. *J Kor Med Sci*. 2016;31(3):410—416.
25. Zhang L, Zhang Z, Li Y, et al. Cholesterol induces lipoprotein lipase expression in a tree shrew (Tupaia belangeri chinensis) model of non-alcoholic fatty liver disease. *Sci Rep*. 2015;5:15970.
26. Gong S, Song J, Wang L, et al. Hyperuricemia and risk of nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol*. 2016;28(2):132—138.
27. Sazci A, Ergul E, Aygun C, et al. Methylenetetrahydrofolate reductase gene polymorphisms in patients with nonalcoholic steatohepatitis (NASH). *Cell Biochem Funct*. 2008;26(3):291—296.
28. Orlovskiy V, Kuchma N, Murenets N, et al. C677t and A1298c allele polymorphism gene of methylenetetrahydrafolatereductase in patients with nonalcoholic fatty liver disease and type 2 diabetes. *Georgian Med News*. 2015;(247):38—43.
29. de Carvalho SC, Muniz MT, Siqueira MD, et al. Plasmatic higher levels of homocysteine in non-alcoholic fatty liver disease (NAFLD). *Nutr J*. 2013;12:37.
30. Franco Brochado MJ, Domenici FA, Candolo Martinelli Ade L, et al. Methylenetetrahydrofolate reductase gene polymorphism and serum homocysteine levels in nonalcoholic fatty liver disease. *Ann Nutr Metab*. 2013;63(3):193—199.
31. Obeid R, Herrmann W. Homocysteine and lipids: S-Adenosyl methionine as a key intermediate. *FEBS (Fed Eur Biochem Soc) Lett*. 2009;583(8):1215—1225.
32. Kohlmeier M, da Costa KA, Fischer LM, et al. Genetic variation of folate-mediated one-carbon transfer pathway predicts susceptibility to choline deficiency in humans. *Proc Natl Acad Sci U S A*. 2005;102(44):16025—16030.