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

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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...
Introducion

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%). Previous studies have reported that metabolic syndrome and its components (i.e., obesity, dyslipidemia, hypertension, hyperglycemia, and so on) are closely associated with NAFLD. Furthermore, we also want to explore interactions between the MTHFR 677CT gene polymorphism and the prevalence of 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.

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 (m²).

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. 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. 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 > 400 μmol/L in men and ≥6 mg/dL (360 μmol/L) in women. HHcy was defined as > 10 μmol/L.10 Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as: HOMA-IR = [fasting glucose (mmol/L) x fasting insulin (μU/mL)] / 22.5. With ultrasound imaging, fatty liver was diagnosed 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) x 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.25 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,

| 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 (15.00–36.50) | <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,10⁹/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, μU/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 = 3.11; 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.

The allelic and genotypic frequencies of the MTHFR 677CT polymorphism in the NAFLD and control groups are summarized in Table 3. 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 ($\chi^2 = 3.96$, $p = 0.14$).

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 ($\chi^2 = 3.96$, $p = 0.14$).

### 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 (%)      |                       |                         | <0.01   |
| Male               | 171 (70.37)           | 214 (55.15)             |         |
| Female             | 72 (29.63)            | 164 (44.85)             |         |
| Overweight/Obesity|                       |                         | <0.01   |
| Overweight         | 204 (83.95)           | 204 (83.95)             |         |
| Obese              | 94 (38.68)            | 70 (18.21)              | <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 (82.21)             | <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 (%)  |                       |                         | 0.518   |
| CC                 | 90 (37.04)            | 138 (35.57)             |         |
| 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.
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. As predictive factors of NAFLD are unclear now. The present study had filtered 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, WC, lipid, and uric acid levels, 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. 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 677TT genotype is more likely to be linked with NAFLD susceptibility. Another study showed increased prevalence of homozygote MTHFR 677CT mutation in patients with NAFLD compared with healthy controls. 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. The present study is the first description of the MTHFR 677CT gene polymorphism with NAFLD patients residing in South-west 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. 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.
| 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 (2.701–15.763) |               |             |            |         |
| 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, hyper-homocysteinemia; 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.
Recent researches found serum Hcy levels may be associated with NAFLD susceptibility.\(^9\)\(^{12,13}\) 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 and 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,\(^14\) 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 MTHFR677CT gene polymorphism, serum Hcy levels 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.

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