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Original Article

Methylenetetrahydrofolate Reductase *Gene rs1801131* and *rs1801133* Polymorphisms were Associated with Susceptibility to Coronary Artery Disease and Nonalcoholic Fatty Liver Disease

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Abstract

Background and objectives: Methylenetetrahydrofolate Reductase (*MTHFR*) is the critical enzyme in folate and 1-carbon metabolism. *MTHFR* polymorphisms may result in increased homocysteine levels, and be associated with abnormal lipid metabolism in the liver. This study aims to explore the association between the gene polymorphisms of *MTHFR rs1801131* and *rs1801133* and the susceptibility of nonalcoholic fatty liver disease (NAFLD) and coronary artery disease (CAD).

Methods: This case-control study included 103 NAFLD patients, 176 CAD patients, 94 patients with NAFLD complicated with CAD, and 183 healthy controls. Basic clinical information was collected, and all participants were genotyped using polymerase chain reaction. Data were analyzed by SPSS 26.0.

Results: The genotype distribution of *MTHFR rs1801131* had no significant difference in the four groups (NAFLD, CAD, NAFLD+CAD, and Healthy controls) (all $P > 0.05$). The genotype distribution of *MTHFR rs1801133* was significantly different in the four groups ($P_0 = 0.014$), while the allele distribution was not significant ($P_0 = 0.139$). In both the dominant model (TT vs CT+CC) and co-dominant model (TT+CC vs CT), the genotype distribution of *rs1801133* was statistically significant between the CAD and NAFLD+CAD, healthy controls and NAFLD+CAD, and NAFLD and NAFLD+CAD groups (all $P < 0.05$). In the NAFLD+CAD group, fasting plasma glucose (FPG) levels of different genotypes of carriers were statistically different (TT vs CT+CC: $P = 0.047$, TT+CC vs CT: $P = 0.002$).

Conclusions: The C allele of *MTHFR rs1801133* was a risk factor for NAFLD+CAD. The CT genotype of *MTHFR rs1801133* was associated with FPG level in patients with NAFLD complicated with CAD.

Keywords: Methylenetetrahydrofolate reductase; Nonalcoholic fatty liver disease; Coronary artery disease; Polymorphism; Lipids metabolism.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAD, coronary artery disease; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptadase; Hcy, homocysteine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MTHFR, methylenetetrahydrofolate reductase; NAFLD, nonalcoholic fatty liver disease; OR, odd ratio; TC, total cholesterol; TG, triglyceride; T2DM, Type 2 diabetes; 95% CI, 95% confidence interval.

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Introduction

Nonalcoholic Fatty Liver Disease (NAFLD) is a clinicopathological syndrome characterized by excessive deposition of fat in liver cells, which is closely related to insulin resistance and genetic susceptibility and is caused by alcohol and other clear liver damage factors.[1,2] The risk factors for NAFLD include a high-fat diet, a high-calorific diet, a sedentary lifestyle, insulin resistance, and metabolic syndrome,[3–5] which are all risk factors for cardiovascular disease.[2,6] Coronary artery disease (CAD) refers to coronary artery atherosclerosis caused by lumen stenosis or occlusion, result-

ing in myocardial ischemia, hypoxia, or necrosis caused by heart disease. Cardiovascular diseases have become the main cause of death globally, with more than 17.6 million deaths in 2016, and the number is expected to grow to more than 23.6 million by 2030.[7] NAFLD and CAD are complex diseases resulting from the presence of susceptibility genes combined with environmental exposure.

MTHFR rs1801133 and *rs1801131* are the most common genetic mutations of methylenetetrahydrofolate reductase (*MTHFR*).[8] PolyPhen was used to predict the effect of the SNP site on proteins, the results showed that *rs1801131* and *rs1801133* may lead to impaired protein function, which may affect the function of *MTHFR*. *MTHFR* is the critical enzyme in folate 1-carbon and homocysteine (Hcy) metabolism.[9,10] It has been reported that increased serum Hcy levels may affect intracellular fat metabolism and promote liver fat infiltration, leading to NAFLD.[11] Studies by Xie Jun *et al.*[10] showed that a history of high Hcy is an independent risk factor for cardiovascular and cerebrovascular diseases. Increased circulating levels of homocysteine accelerate atherosclerosis through several mechanisms.[10,11] Some studies support the association of polymorphisms with susceptibility to NAFLD and CAD,[8,12,13] while others do not.[14–16]

This study aims to explore the association between *MTHFR gene rs1801131* and *rs1801133* polymorphisms and the susceptibility of NAFLD and CAD.

Subjects and methods

Study subjects

This case-control study was approved by the Qingdao Hospital Ethics Committee (Approval NO. 2017-20), and was based on the principles of the Declaration of Helsinki and its appendices.[17] All the subjects were informed and signed an informed agreement upon joining this study. From June 2018 to June 2019, a total of 556 patients from Qingdao Municipal Hospital participated in the study, including 103 NAFLD patients, 176 CAD patients, 94 patients with NAFLD complicated with CAD (NAFLD+CAD), and 183 healthy controls. The NAFLD patients were diagnosed according to the Guidelines of prevention and treatment of nonalcoholic fatty liver disease (2018),[18] while the CAD patients were diagnosed according to the Guidelines for Diagnosis and Treatment of Stable Coronary Heart Disease.[19] None of the patients with abnormal blood glucose content in this study was diagnosed with diabetes.

Biochemical analyses

Basic clinical information was collected such as sex, age, height, and weight. The body mass index (BMI) could be calculated by mass (kg)/height (m^2). Fasting blood was taken from the subjects to test their biochemical parameters, such as alanine aminotransferase (ALT), aspartate aminotransferase, fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), gamma-glutamyl transpeptadase (GGT), alkaline phosphatase (ALP), total bilirubin.

Genomic DNA extraction and genotyping

Whole blood genomic DNA was extracted (blood genomic DNA extraction kit; Beijing Bomiao Biotechnology Co. Ltd, Beijing, China) and stored at 20°C. *MTHFR rs1801133* and *rs1801131* were genotyped by the polymerase chain reaction combined with sequencing, and specific steps were described in the references.[20] Primer sequence of *MTHFR* was as follows: *rs1801133*, 5′-ACGTTGGATGCTTGAAGGAGAAGGTGTCTG-3′ and 5′-ACGTTGGATGACACGTTGGATGCTTCACAAAGCGGAAGAATG-3′; *rs1801131*, 5′-ACGTTGGATGTGAAGAGCAAGTCCCCCAAG-3′and5′-ACGTTGGATGCCGAGAGGTAAAGAACGAAG-3′. *MTHFR rs1801131* showed that there were three genotypes: AA, CC, and AC, and *MTHFR rs1801133* showed that there were three genotypes: TT, CC, and CT.

Statistical analysis

The data were analyzed using SPSS version 26.0. Pearson's χ^2 test was used to analyze the Hardy-Weinberg balance. Genotypes, allele frequencies, and other qualitative data comparisons were tested by Pearson's χ^2 test. After normality tests, continuous variables were expressed as mean ± standard deviation or median (interquartile range) for normal and abnormal distributed parameters, respectively. The measurement data were tested by the *t*-test and Wilcoxon rank sum test. The association between SNPs and the risk of NAFLD and CAD was estimated by computing odds ratios (ORs) and 95% confidence interval (95% CI). $P < 0.05$ was statistically significant.

Results

Demographic and clinical characteristics

The general clinical data and biochemical indicators were compared in Table 1. The NAFLD patients had higher BMI values and serum levels of FPG, ALT, GGT, TC, TG, and LDL than the healthy controls (all $P < 0.05$), with the two groups matched for gender (all $P > 0.05$); The CAD patients had higher BMI values and serum levels of FPG, ALT, GGT, and ALP than the healthy controls, besides, the serum level of TC, HDL, and LDL in CAD patients was significantly lower compared to the healthy controls (all $P < 0.05$), and the two groups were matched for gender and age (all $P > 0.05$); The NAFLD+CAD patients had higher BMI values and serum levels of FPG, ALT, GGT, and ALP than the healthy controls, besides, the serum level of HDL and LDL in CAD patients was significantly lower compared to the healthy controls (all $P < 0.05$).

Genotypes and alleles distributions of MTHFR rs1801131 and rs1801133

The distribution of *MTHFR rs1801131* and *rs1801133* polymorphisms in healthy controls was consistent with the Hardy-Weinberg equilibrium (*rs1801131*: $\chi^2 = 0.094$, $P = 0.954$; *rs1801133*: $\chi^2 = 0.482$, $P = 0.786$). There was no significant difference in the genotype distribution and allele frequency of *rs1801131* among the four groups (NAFLD, CAD, NAFLD+CAD, and Healthy controls) (all $P > 0.05$) (Table 2).

The genotype distribution of *rs1801133* was statistically different among the four groups (NAFLD, CAD, NAFLD+CAD, and Healthy controls) ($P = 0.014$), while the allele distribution was the same among the 4 groups ($P = 0.139$). Moreover, there were significant differences in the allele distribution of *rs1801133* between the NAFLD+CAD and CAD groups ($P_2 = 0.021$). The genotypes of the three groups (NAFLD, CAD, and Healthy controls) were statistically different from those of the NAFLD+CAD group (all $P < 0.05$) (Table 3).

Analysis of MTHFR rs1801133 genotype model

Analysis of the *MTHFR rs1801133* genotypes model showed

Table 1. Association of non-genetic variables in the study subjects

	Healthy controls (n = 183)	NAFLD (n = 103)	CAD (n = 176)	NAFLD+CAD (n = 94)	P_0
Male/Female	104.00/79.00	69.00/34.00	116.00/60.00	68.00/26.00[#]	0.055
Age, y	47.00 (40.00, 57.00)	43.00 (38.00, 45.00)[#]	66.00 (59.20, 75.75)	63.00 (57.00, 68.00)[#]	<0.001
BMI, kg/m^2	23.60 ± 3.19	26.24 ± 2.56[#]	24.59 ± 3.22[#]	25.08 ± 2.67[#]	<0.001
FPG, mmol/L	4.57 (4.06, 5.05)	4.85 (4.52, 5.21)[#]	5.21 (4.55, 6.43)[#]	5.42 (4.80, 6.07)[#]	<0.001
ALT, U/L	19.02 (13.36, 26.58)	22.67 (18.30, 39.44)[#]	21.85 (14.98, 32.22)[#]	22.67 (15.36, 32.45)[#]	<0.001
AST, U/L	20.87 (18.84, 25.04)	22.20 (18.77, 26.21)	22.34 (17.08, 34.49)	21.50 (16.80, 32.10)	0.515
GGT, U/L	22.43 (16.45, 30.44)	30.09 (20.19, 45.27)[#]	27.35 (18.75, 41.58)[#]	26.11 (18.25, 43.93)[#]	<0.001
ALP, U/L	69.31 (55.98, 83.91)	67.36 (57.40, 79.17)	82.71 (64.59, 107.38)[#]	82.50 (70.99, 98.06)[#]	<0.001
TC, mmol/L	5.00 (4.20, 5.64)	5.44 (4.96, 5.99)[#]	4.48 (3.77, 5.35)[#]	4.25 (3.83, 5.51)	<0.001
TG, mmol/L	1.21 (0.90, 1.94)	1.49 (1.08, 2.20)[#]	1.36 (0.99, 1.86)	1.35 (0.94, 2.08)	0.177
HDL, mmol/L	1.28 (1.07, 1.51)	1.22 (1.08, 1.35)	1.01 (0.85, 1.16)[#]	1.05 (0.88, 1.19)[#]	<0.001
LDL, mmol/L	3.06 (2.64, 3.54)	3.27 (2.82, 3.59)[#]	2.69 (2.07, 3.30)[#]	2.51 (2.14, 3.37)[#]	<0.001

[#]NAFLD, CAD and NAFLD+CAD were compared with Healthy controls respectively, $P < 0.05$, which was statistically significant; P_0: Healthy controls vs NAFLD vs CAD vs NAFLD+CAD. ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CAD, coronary artery disease; FPG, fasting plasma glucose; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; TC, total cholesterol; TG, Triglyceride.

that the genotype distribution was statistically significant under the dominant model (TT vs CT+CC) and the co-dominant model (TT+CC vs CT) (all $P < 0.05$). After adjusting for age, BMI, and gender, there was no statistical significance between the NAFLD and NAFLD+CAD groups. (TT vs CT+CC: $P_1 = 0.074$, TT+CC vs CT: $P_1 = 0.881$), but there remained a statistical difference in other groups (all $P < 0.05$) (Table 4).

Association of MTHFR rs1801131 and rs1801133 gene polymorphism with clinical parameters characteristics in all subjects

The clinical data of all participants were compared between carriers and non-carriers of the *rs1801131* allele A, and the differences

Table 2. Distributions of the *MTHFR rs1801131* genotypes and alleles in the study groups

	NAFLD+CAD	NAFLD	CAD	Healthy controls	P_0	P_1	P_2	P_3
Genotypes								
AA	79 (73.8)	80 (76.1)	139 (77.7)	138 (74.2)	0.684	1.000	0.957	0.278
CC	2 (1.9)	1 (1.0)	0 (0)	3 (1.6)				
AC	26 (24.3)	24 (22.9)	40 (22.3)	45 (24.2)				
Alleles								
C	30 (14.0)	26 (12.4)	40 (11.2)	51 (13.7)	0.695	0.917	0.650	0.300
A	184 (86.0)	184 (87.6)	318 (88.8)	321 (86.3)				

P_0: NAFLD+CAD vs NAFLD vs CAD vs Healthy controls P_1: NAFLD+CAD vs Healthy controls P_2: NAFLD vs Healthy controls P_3: CAD vs Healthy controls; $P < 0.05$ was statistically significant. CAD, coronary artery disease; MTHFR, methylenetetrahydrofolate reductase; NAFLD, nonalcoholic fatty liver disease.

Table 3. Distributions of the *MTHFR rs1801133* genotypes and alleles in the study groups

	NAFLD+CAD	NAFLD	CAD	Healthy controls	P_0	P_1	P_2	P_3
Genotypes								
TT	16 (16.0)	35 (33.0)	64 (36.2)	63 (33.7)	0.014	0.009	0.001	0.002
CC	13 (13.0)	16 (15.1)	23 (13.0)	29 (15.5)				
CT	71 (71.0)	55 (51.9)	90 (50.8)	95 (50.8)				
Alleles								
T	103 (51.5)	125 (59.0)	218 (61.6)	221 (59.1)	0.139	0.128	0.021	0.081
C	97 (48.5)	87 (41.0)	136 (38.4)	153 (40.9)				

P_0: NAFLD+CAD vs NAFLD vs CAD vs Healthy controls P_1: NAFLD+CAD vs NAFLD P_2: NAFLD+CAD vs CAD P_3: NAFLD+CAD vs Healthy controls; $P < 0.05$ was statistically significant. CAD, coronary artery disease; MTHFR, methylenetetrahydrofolate reductase; NAFLD, nonalcoholic fatty liver disease.

Table 4. Comparison of *MTHFR rs1801133* genotypic distribution under different gene models

	NAFLD	NAFLD +CAD	OR	95%CI	P_1	CAD	NAFLD +CAD	OR	95%CI	P_2	Healthy controls	NAFLD +CAD	OR	95%CI	P_3
Recessive model															
TT+CT	90	87	1.190	(0.541–2.619)	0.666	154	87	1.000	(0.482–2.072)	0.999	158	87	1.228	(0.607–2.485)	0.567
CC	16	13				23	13				29	13			
Dominant model															
TT	35	16	2.588	(1.324–5.061)	0.005	64	16	2.973	(1.605–5.507)	0.001	63	16	2.667	(1.443–4.932)	0.002
CT+CC	71	84				113	84				124	84			
Dominant model[a]															
TT	35	16	1.391	(0.198–9.786)	0.740	64	16	3.192	(1.678–6.071)	<0.001	63	16	3.423	(1.623–7.222)	0.001
CT+CC	71	84				113	84				124	84			
Co-dominant model															
TT+CC	51	29	2.270	(1.276–4.038)	0.005	87	29	2.367	(1.403–3.992)	0.001	93	29	2.371	(1.412–3.982)	0.001
CT	55	71				90	71				95	71			
Co-dominant model[a]															
TT+CC	51	29	0.880	(0.165–4.691)	0.881	87	29	2.468	(1.433–4.251)	0.001	93	29	2.584	(1.372–4.867)	0.003
CT	55	71				90	71				95	71			

[a]Binary logistic regression models with an adjustment for age, gender, and body mass index (BMI); Dominant model: TT vs CT+CC recessive model: TT+CT vs CC co-dominant model: TT+CC vs CT; P_1: NAFLD+CAD vs NAFLD P_2: NAFLD+CAD vs CAD P_3: NAFLD+CAD vs healthy controls. CAD, coronary artery disease; MTHFR, methylenetetrahydrofolate reductase; NAFLD, nonalcoholic fatty liver disease.

Table 5. Correlation analysis between *rs1801133* genotypes and non-genetic variables in the NAFLD+CAD group under the dominant model

	TT	CC+CT	Statistics (t/z)	*P*
Age, y	62.63 ± 7.80	61.79 ± 7.57	0.405	0.687
BMI, kg/m^2	25.67 ± 2.24	24.99 ± 2.66	0.958	0.341
FPG, mmol/L	5.13 (4.79, 5.41)	5.58 (4.82, 6.52)	−1.984	0.047
ALT, U/L	22.50 (13.14, 39.48)	22.71 (16.03, 32.77)	−0.188	0.851
AST, U/L	22.93 (17.29, 41.75)	21.60 (16.92, 31.52)	−0.498	0.618
GGT, U/L	29.93 (19.48, 46.60)	25.64 (18.12, 42.51)	−0.672	0.501
ALP, U/L	83.21 (76.90, 102.59)	80.69 (69.09, 95.69)	−0.846	0.397
TC, mmol/L	4.25 (3.96, 5.57)	4.22 (3.78, 5.46)	−0.155	0.877
TG, mmol/L	1.34 (1.00, 1.87)	1.32 (0.95, 2.11)	−0.141	0.888
HDL, mmol/L	1.06 (0.98, 1.18)	1.02 (0.85, 1.19)	−1.020	0.308
LDL, mmol/L	2.55 (2.21, 3.36)	2.50 (2.02, 3.39)	−0.028	0.977
TBIL, umol/L	13.05 (9.93, 14.30)	14.15 (10.73, 17.10)	−1.133	0.257

P < 0.05 was statistically significant. ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CAD, coronary artery disease; FPG, fasting plasma glucose; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; TC, total cholesterol; TBIL, total bilirubin; TG, triglyceride.

were not statistically significant ($P > 0.05$).

The clinical data of healthy controls, NAFLD, and CAD patients were compared between the homozygous (TT+CC) and heterozygous (CT) genotypes of the *rs1801133*, and the differences were not statistically significant ($P > 0.05$). In the NAFLD+CAD group, FPG levels of different genotypes were statistically different (Dominant model: $P = 0.047$, Co-dominant model: $P = 0.002$) (Tables 5 and 6).

Discussion

The findings of the present study provide a comprehensive understanding of the correlation between *MTHFR rs1801131* and *rs1801133* polymorphism in *MTHFR* and the susceptibility to NAFLD and CAD in China.

As mentioned in the introduction, *MTHFR*, as a key enzyme, is involved in the occurrence and development of NAFLD and CAD diseases by regulating Hcy metabolism. *MTHFR* polymorphisms may be closely related to NAFLD and CAD susceptibility. Although some studies have shown that the *rs1801131* genotype is associated with CAD susceptibility,[13–15] other studies showed that *MTHFR rs1801131* polymorphism had no significant relationship with CAD.[15,21,22] There are also inconsistent results in studies on the correlation between *rs1801131* polymorphisms and NAFLD susceptibility.[21,23,24] In the Turkish and Italian populations, *rs1801131* polymorphism was significantly associated with NAFLD,[23,24] while in the Chinese population, *rs1801131* polymorphism was not associated with NAFLD.[21] In this study, no corre-

Table 6. Correlation analysis between *rs1801133* genotypes and non-genetic variables in the NAFLD+CAD group under the co-dominant model

	TT+CC	CT	Statistics (t/z)	*P*
Age, y	62.17 ± 8.20	61.82 ± 7.36	0.212	0.833
BMI, kg/m^2	24.90 ± 2.41	25.18 ± 2.68	−0.485	0.629
FPG, mmol/L	5.01 (4.57, 5.51)	5.64 (4.89, 6.74)	−3.073	0.002
ALT, U/L	18.15 (14.94, 33.09)	22.84 (16.63, 32.89)	−0.574	0.566
AST, U/L	21.52 (18.64, 54.64)	21.88 (16.83, 28.83)	−0.593	0.554
GGT, U/L	25.44 (17.94, 36.64)	27.09 (18.49, 44.92)	−0.479	0.632
ALP, U/L	82.40 (70.25, 98.11)	83.72 (70.19, 96.39)	−0.406	0.684
TC, mmol/L	4.27 (3.75, 5.70)	4.21 (3.79, 5.42)	−0.266	0.790
TG, mmol/L	1.37 (0.95, 2.06)	1.31 (0.96, 2.13)	−0.562	0.574
HDL, mmol/L	1.03 (0.89, 1.16)	1.04 (0.85, 1.20)	−0.167	0.867
LDL, mmol/L	2.61 (2.08, 3.35)	2.46 (2.03, 3.40)	−0.034	0.973
TBIL, umol/L	13.00 (9.95, 14.75)	14.30 (10.80, 17.70)	−1.516	0.130

P < 0.05 was statistically significant. ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CAD, coronary artery disease; FPG, fasting plasma glucose; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; TC, total cholesterol; TBIL, total bilirubin; TG, triglyceride.

lation was found between *rs1801131* polymorphism and NAFLD and CAD susceptibility ($P > 0.05$). Allele A frequencies in this study (86.3%) were consistent with the Chinese Beijing population (A 81.6%).[25] This difference may be due to regional, lifestyle, and ethnic differences. Qingdao's economy is relatively developed: the local people enjoy good nutrition and eat more seafood.

The correlation between *rs1801133* polymorphism and NAFLD and CAD susceptibility is also controversial. For *MTHFR rs1801133*, T allele frequencies in this study (59.1%) were consistent with the Chinese Tianjin population (T 56%)[26] and differed from the Chinese Beijing population (T 41.3%).[25] Some studies showed that the T allele of *rs1801133* gene polymorphism was a risk factor for CAD.[25,27] while some Chinese studies showed that the CT genotype might be the susceptibility factor of CAD patients.[21] A Meta-analysis conducted by Sun *et al.* revealed that *MTHFR rs1801133* gene polymorphism was implicated in susceptibility to NAFLD.[8] Literature shows that the genotype frequency of *MTHFR rs1801133* varies greatly by race.[28,29] Our study showed that *MTHFR rs1801133* gene polymorphism was not associated with the risk of CAD or NAFLD, however, *MTHFR rs1801133* polymorphism was associated with the risk of NAFLD complicated with CAD. There are no other studies on the correlation between polymorphism and susceptibility to NAFLD and CAD. According to our results, for healthy people, NAFLD, and CAD patients, *rs1801133* polymorphism was associated with the risk of NAFLD combined with CAD disease. In this study, different gene models were used to analyze the genotype distribution of *rs1801133* polymorphism. In the codominant model, the CT genotype of *MTHFR rs1801133* was a risk factor for NAFLD combined with CAD, while in the dominant model, the CT+CC genotype was a risk factor for NAFLD combined with CAD. This is not completely consistent with other studies on NAFLD or CAD. Considering the complexity of the disease and the absence of relevant references, the rationality of the results of this study cannot be denied.

MTHFR rs1801133 could affect the total serum Hcy level, which might affect the risk of Type 2 diabetes (T2DM).[30] *MTHFR rs1801133* polymorphism was found to be significantly associated with T2DM.[31,32] Different meta-analyses showed a significant relationship between *rs1801133* polymorphism and T2DM.[33,34] Elevated FPG (≥7.0 mmol/L) is currently used to diagnose T2DM.[9]

In this study, the CT genotype and CC+CT genotype of *MTHFR rs1801133* were associated with an increased FPG level in NAFLD+CAD patients (both $P < 0.05$). Given that *rs1801133* polymorphisms were strongly associated with diabetes risk, it was reasonable to influence FPG levels in the patients with NAFLD complicated with CAD. This study has its limitations in that all samples were only collected in Qingdao, China, which has regional limitations. Compared with the south of China, the taste in food of the Qingdao area is heavy; People there like to eat pickled food, the dietary structure protein fat content is higher, and people generally eat more. Qingdao produces seafood, and the seafood intake is higher than in other areas. Also, the diagnosis of fatty liver relied on ultrasound examinations and liver biopsy was not performed.

Conclusion

In conclusion, the CT genotype and CC+CT genotype of *MTHFR rs1801133* were the risk factors for NAFLD combined with CAD. The CT genotype of *MTHFR rs1801133* was associated with the up-regulation of FPG levels in patients with NAFLD combined with CAD.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (XYN and ZY); subjects collection (SH and LCM); acquisition and analysis of data (SH and ZZZ); drafting of the manuscript (SH and ZZZ); the revision of the manuscript (LSS, XYN, and ZY). Huan Song and Zhenzhen Zhao contributed equally to the article and are first authors, while Yongning Xin and Yong Zhou are corresponding authors. All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical statement

This study was approved by the Ethics Committee of Qingdao Municipal Hospital before participation (Approval NO. 2017-20). All the subjects have signed written informed consent.

Data sharing statement

The data used in support of the findings of this study are available from the corresponding author at xinyongning@163.com upon request.

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