Association of LDLR rs1433099 with the Risk of NAFLD and CVD in Chinese Han Population

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Abstract

Background and Aims: Recent genome-wide association studies have shown that low-density lipoprotein receptor (LDLR) rs1433099 polymorphism is associated with cardiovascular disease (CVD) risk in many countries. However, the association of LDLR rs1433099 with CVD in China has not been reported yet. There are no studies on LDLR rs1433099 and non-alcoholic fatty liver disease (NAFLD) as well. The purpose of this study was to investigate whether LDLR rs1433099 is related to CVD or NAFLD in the Chinese population. Methods: LDLR rs1433099 polymorphism was genotyped in 507 individuals, including 140 healthy controls, 79 NAFLD patients, 185 CVD patients, and 103 patients with NAFLD combined with CVD. The expression of LDLR was tested by the sequence detection system, and clinical parameters were assessed by biochemical tests and physical examination. Results: The genotype distribution of LDLR rs1433099 was not statistically different among the NAFLD group, the CVD group, the combined group, and the healthy control group (p > 0.05). There was no significant correlation of LDLR rs1433099 genotypic distribution or allele frequency and the risk of NAFLD, CVD or NAFLD combined with CVD (p > 0.05). In the CVD group, T allele carriers had higher alkaline phosphatase and gamma-glutamyl transpeptidase than non-carriers (p < 0.05). Conclusions: Our study demonstrated that the LDLR rs1433099 polymorphism is not a risk factor of NAFLD. The LDLR rs1433099 polymorphism may increase the risk of CVD through a mechanism involving alkaline phosphatase and gamma-glutamyl transpeptidase.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is becoming the most common chronic liver disease. The prevalence of NAFLD is constantly increasing, rising from 15% in 2005 to 25% in 2018. Approximately 27% of adults in Asia suffer from NAFLD, the rate of which is even higher in the Middle East and South America, with an estimated prevalence of 32% and 31% respectively. It was estimated in 2016 that the annual burden of NAFLD-related cases was $103 billion in the USA and 35 billion in four European countries per year. NAFLD consists of a broad spectrum of fatty liver disease, ranging from simple fatty infiltration in >5% of hepatocytes (steatosis), fatty infiltration plus inflammation, fibrosis, and ultimately cirrhosis, ending with liver failure and hepatocellular carcinoma. NAFLD is in close relationship with type 2 diabetes mellitus, obesity, and metabolic syndrome. Younossi et al. estimated that of all patients that have developed non-alcoholic steatohepatitis in the USA, 82% are obese, 48% have type 2 diabetes mellitus, 82% get hyperlipidemia, 76% are diagnosed with metabolic syndrome, and 70% suffer from hypertension. Biopsy remains the gold-standard for assessing the progression of NAFLD, but its side effects keep many patients away, especially in the early stage of fatty liver disease. The most commonly used biomarker of chronic liver disease to evaluate the function of the liver is alanine transaminase (ALT), while it has a low specificity.

With the development of the genome-wide association studies, many gene loci modulating metabolism have been demonstrated to influence the risk of diseases. PNPLA3 and TM6SF2 were of the first genes to be related to NAFLD. The low-density lipoprotein receptor (LDLR) is a widely distributed transmembrane glycoprotein regulating cholesterol homeostasis. Cells can internalize lipoprotein ligands, including chylomicrons, low-density lipoprotein (LDL), intermediate-density lipoprotein, or very-LDL mediated by LDL, facilitating cholesterol utilization. The gene for LDLR is located at 19p13.1–13.3 and spans 45 kb, including 18 exons and 17 introns. Early studies showed that mutations in LDLR can cause familial hypercholesterolemia, an autosomal dominant...
disorder characterized by severe hypercholesterolemia.11,12 C44857T (rs1433099) is a single nucleotide polymorphism within the 5′ region of the 3′ untranslated region of LDLR.12 Polasecki et al.13 found that carriers of the T allele at the C44857T locus had significantly lower levels of LDL-C, suggesting it as a decisive pathogenic factor of NAFLD and cardiovascular disease (CVD). However, the relationship between LDLR rs1433099 and NAFLD is still unknown. It remains unclear whether LDLR rs1433099 affects CVD risk in the Chinese population. Our study aimed to investigate whether LDLR rs1433099 is associated with NAFLD or CVD in the Chinese population.

Methods

Subjects

This study was permitted by the hospital ethical committee of Qingdao Municipal Hospital (Qingdao, China), following the principles of the Declaration of Helsinki and its appendices.14 The study selected 367 in-patients of Qingdao Municipal Hospital from January 2018 to September 2019, including 79 NAFLD patients (NAFLD group), 185 CVD patients (CVD group), and 103 patients with NAFLD combined with CVD (NAFLD combined with CVD group). The combined group were selected from the Department of Gastroenterology; CVD patients and NAFLD combined group were selected from the Department of Cardiology. At the same time, 140 healthy controls were selected from the Health Examination Center of Qingdao Municipal Hospital. All individuals were unrelated, ethnically Chinese Han adults. The diagnosis of NAFLD met the standards of the “Guidelines for Prevention and Treatment of Non-alcoholic Fatty Liver Disease”15 and was confirmed by B-ultrasonography. CVD was diagnosed by coronary angiography of the coronary artery or its branches. Excluded were patients with alcoholic hepatitis, viral or autoimmune hepatitis, drug-induced hepatitis, acute fatty liver of pregnancy, and other causes of liver disease, as well as aortic dissection, atrial fibrillation, rheumatic immune disease, cardiomyopathy, aortic arteritis, etc. that may cause secondary CVD. Healthy controls were confirmed by biochemical indicators combined with findings from ultrasound examination.

Specimen and data collection

After 12 hours of fasting, 8 mL of venous blood was collected routinely into two EDTA anti-coagulant tubes (designated as A and B respectively), with 4 mL in each. Tube A was used to detect biochemical indexes, including ALP, aspartate transaminase (AST), fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT). Tube B was centrifuged and stored at −80 °C for genotyping. The basic information, such as name, sex, and age of the subjects, was gathered by a standard questionnaire. Height and body mass was measured with professional instruments, and the body mass index (BMI) was calculated.

Genotyping

Genomic DNA was isolated from peripheral blood using a purification kit (Bio Miao Biological Technology, Beijing, China). The rs1433099 polymorphism of LDLR was detected by polymerase chain reaction (PCR) using the following primers designed and synthesized for LDLR polymorphism: 5′-ACGGTTGATGTAAGTGACCTCCATCCCCAG-3′, 5′-AGGGTGATGGAAGTGACCTCCATCCCCAG-3′. PCR amplification was performed under the following conditions: 5 min at 94 °C, then 45 cycles before denaturation at 94 °C for 20 s, annealing at 56 °C for 30 s, and elongation for 1 min at 72 °C. For direct DNA sequencing, the ABI Prism sequence detection system ABI veriti-384 (Foster City, CA, USA) was applied for the assay of LDLR genotypes. The average genotype call rate was above 95% and the genotype concordance rate of blind replicates was above 99%.

Statistical analysis

Statistical analysis was carried out using SPSS Statistics software, version 24.0 (IBM Corp., Armonk, NY, USA). When comparing the general clinical data among the four groups, the count data were compared by the χ2 test. The measurement data, not in accordance with normality and homogeneity of variance by Kolmogorov-Smirnov test, were expressed as median (quartile), compared by Kruskal-Wallis H test. For indexes with statistical differences by rank-sum test, the results were corrected by Bonferroni correction. The χ2 test was used to analyze whether the distribution of LDLR rs1433099 genotypes conformed to the law of Hardy-Weinberg genetic equilibrium to avoid the lack of population representativeness. The χ2 test was used to analyze the differences of LDLR rs1433099 genotype distribution and allele frequency among the four groups. Logistic regression analysis was performed to analyze the relationship between polymorphism and disease risk. Student’s t-test, Kruskal-Wallis test, and rank-sum test were used to evaluate the association of LDLR rs1433099 genotypic distribution with clinical characteristics. A p-value of <0.05 was considered statistically significant.

Results

Clinical characteristics of the individuals

We investigated 507 individuals in total. Table 1 shows the clinical characteristics and serum lipid levels of the subjects as well as comparisons of groups in sex (χ2 test) and other clinical parameters (Kruskal-Wallis test). NAFLD vs. control group: BMI, ALT, TG, GGT, and FBG of the NAFLD group are lower than those of the control group, while age is lower than that of the control group (Z=3.053–7.418, p<0.05). CVD vs. control group: age, ALT, TC, GGT, ALP, and FBG of the CVD group are higher than those of the control group, while TG, HDL-C, and LDL-C are lower than those of the control group (Z=2.828–10.768, p<0.05). Combined group vs. control group: age, BMI, ALT, TC, GGT, ALP, and FBG of the combined group are higher than those of the control group, while TG, HDL-C, and LDL-C are lower than those of the control group (Z=3.065–11.713, p<0.05). Combined group vs. NAFLD group: age, ALP, and FBG of the combined group are higher than those of the NAFLD group, while TG, HDL-C, and LDL-C are lower than those of the NAFLD group (Z=3.685–9.803, p<0.05).

LDLR rs1433099 genotypes and allele distribution

The genotype distribution of the LDLR rs1433099 corre-
**Table 1. Clinical characteristics of individuals in the four study groups**

| Group          | Sex, M/F | Age, years | BMI, kg/m² | ALT, U/L | AST, U/L | TG, mmol/L | TC, mmol/L | HDL-C, mmol/L | LDL-C, mmol/L | GGT, U/L | ALP, U/L | FBG, mmol/L |
|----------------|----------|------------|------------|----------|----------|------------|------------|---------------|--------------|----------|----------|------------|
| Control        | 60/80    | 52.5 (42–59)| 23.71 (21.26–25.95) | 17.79 (12.51–23.68) | 20.75 (17.71–23.84) | 5.11 (4.60–5.72) | 1.12 (0.87–1.57) | 1.29 (1.05–1.50) | 3.06 (2.65–3.42) | 19.90 (14.96–30.26) | 70.26 (58.08–83.81) | 4.51 (4.00–5.15) |
| NAFLD          | 47/33    | 43 (39–45) | 25.26 (23.78–26.77) | 20.14 (15.04–22.02) | 22.63 (16.83–23.76) | 4.27 (3.78–5.55) | 1.39 (0.94–2.17) | 1.05 (0.85–1.16) | 2.93 (2.12–3.40) | 27.19 (19.14–44.29) | 82.90 (68.86–107.72) | 5.23 (4.55–6.75) |
| CVD            | 120/65   | 67 (60–76) | 24.54 (22.52–26.72) | 1.99 (1.73–2.36) | 1.12 (0.95–1.35) | 4.47 (3.78–5.87) | 1.33 (0.96–1.83) | 1.01 (0.85–1.16) | 2.66 (2.08–3.33) | 21.84 (17.09–33.68) | 81.09 (73.51–98.01) | 5.48 (4.81–6.47) |
| Combined       | 79/57    | 62 (56–67) | 26.26 (23.44–28.09) | 1.99 (1.73–2.36) | 1.12 (0.95–1.35) | 4.47 (3.78–5.87) | 1.33 (0.96–1.83) | 1.01 (0.85–1.16) | 2.66 (2.08–3.33) | 21.84 (17.09–33.68) | 81.09 (73.51–98.01) | 5.48 (4.81–6.47) |

**Table 2. Association of LDLR rs1433099 genotypic distribution and allele frequency with the risk of NAFLD and CVD**

| Group          | Combined | CVD | NAFLD | Combined vs. Control | CVD vs. Control | NAFLD vs. Control | Combined vs. NAFLD |
|----------------|----------|-----|-------|----------------------|----------------|------------------|-------------------|
| p1             | <0.001   | 0.001 | 0.001 | 0.001                | 0.001         | 0.001            | 0.001             |
| p2             | <0.001   | 0.001 | 0.001 | 0.001                | 0.001         | 0.001            | 0.001             |
| p3             | <0.001   | 0.001 | 0.001 | 0.001                | 0.001         | 0.001            | 0.001             |
| p4             | <0.001   | 0.001 | 0.001 | 0.001                | 0.001         | 0.001            | 0.001             |

**Table 3. Association of LDLR rs1433099 genotypic distribution with clinical characteristics**

Table 3 shows the unconditional logistic regression model analysis for genotypes and alleles of rs1433099. There is no significant correlation between LDLR rs1433099 genotypic distribution or allele frequency for the risk of NAFLD, CVD or NAFLD combined with CVD (p>0.05). We observed no significant difference after adjustment for age, sex, and BMI (p>0.05).

**Table 4. Association of LDLR rs1433099 genotypic distribution with clinical characteristics**

Table 4 shows clinical characteristics in LDLR rs1433099 T carriers and non-carriers. Statistical analysis indicates no significant difference between the T-carriers and non-carriers among all subjects (p>0.05). Further analysis among the three genotypes suggests no statistical difference as well (Table 5; p>0.05). Analysis of clinical characteristics of LDLR rs1433099 in each group shows that T-carriers have higher ALP and GGT than non-carriers in the CVD group (Table 6; p<0.05).

**Discussion**

LDLR is a cell-surface receptor that removes excessive LDL from plasma and maintains the circulating cholesterol level. LDLR is closely related to metabolic syndrome. In the whole population, 0.2–0.5% of people have heterozygous mutations in LDLR.

Recently, international experts reached a consensus recommending a change in name from NAFLD to metabolic dysfunction-associated fatty liver disease, emphasizing it as a consequence of metabolic syndrome. Lipotoxicity is the initial factor in NAFLD development. Former studies showed that LDLR rs1433099 mutation can induce dyslipidemia, suggesting that it may influence the risk of NAFLD. We explored the relationship between LDLR rs1433099 and NAFLD for the first time. But this research shows no association for LDLR rs1433099 polymorphism with the incidence of NAFLD.

LDLR also has a close relationship with the development of atherosclerosis. Abnormal LDLR alleles in the human manifest as familial hypercholesterolemia, with dramatically increased risk of CVD. The severity of atherosclerosis is in correlation with the level and activity of liver LDLR.

Previously, Anand et al. conducted the INTERHEART case-control study, which included 8,795 individuals of European, South Asian, Arab, Iranian, and Nepalese origin. The investigators found that LDLR rs1433099 is associated with a lower apolipoprotein B/A1 ratio, an indicator proportional to the narrowness of coronary artery (p=0.0022). No direct correlation between LDLR rs1433099 and myocardial infarction was found. Takeuchi et al. investigated the relationship of LDLR rs1433099 and the risk of CVD in Japan from 12,066 individuals. Their study indicated a strong association.
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Table 2. Correlation of the rs1433099 polymorphism in the LDLR gene with NAFLD and CVD

|                  | Control, n=140 | NAFLD, n=79 | CVD, n=185 | Combined, n=103 |
|------------------|---------------|-------------|------------|----------------|
| Genotypes       |               |             |            |                |
| CC               | 70 (50.00%)   | 43 (54.43%) | 96 (51.89%) | 62 (60.19%)    |
| CT               | 62 (44.29%)   | 30 (37.97%) | 78 (42.16%) | 33 (32.04%)    |
| TT               | 8 (5.71%)     | 6 (7.59%)   | 11 (5.95%)  | 8 (7.77%)      |
| Alleles         |               |             |            |                |
| C                | 202 (72.14%)  | 116 (73.42%)| 270 (72.97%)| 157 (76.21%)   |
| T                | 78 (27.86%)   | 42 (26.58%) | 100 (27.03%)| 49 (23.79%)    |

Abbreviations: NAFLD, non-alcoholic fatty liver disease; CVD, cardiovascular disease.

Table 3. LDLR rs1433099 genotypes, alleles, and risk of NAFLD and CVD

|                | NAFLD vs. control | CVD vs. control | Combined vs. control | Combined vs. NAFLD |
|----------------|-------------------|----------------|----------------------|--------------------|
| Genotypes     |                   |                |                      |                    |
| CC            | 1                 | 0.529          | 0.735                | 0.116              |
| CT+TT         | 0.84 (0.48–1.46)  | 0.93 (0.60–1.44)| 0.66 (0.40–1.11)    | 0.79 (0.44–1.43)   |
| Allele        |                   |                |                      |                    |
| C             | 1                 | 0.774          | 0.81                 | 0.313              |
| T             | 0.94 (0.60–1.46)  | 0.96 (0.68–1.36)| 0.81 (0.53–1.22)    | 0.862 (0.535–1.389)|

Adjusted Genotypes

|                | NAFLD vs. control | CVD vs. control | Combined vs. control | Combined vs. NAFLD |
|----------------|-------------------|----------------|----------------------|--------------------|
| CC            | 1                 | 0.273          | 0.450                | 0.198              |
| CT+TT         | 0.68 (0.34–1.35)  | 0.80 (0.45–1.42)| 0.67 (0.37–1.23)    | 0.43 (0.07–2.64)   |
| Allele        |                   |                |                      |                    |
| C             | 1                 | 0.722          | 0.340                | 0.344              |
| T             | 0.91 (0.53–1.55)  | 0.81 (0.52–1.26)| 0.79 (0.49–1.28)    | 0.571 (0.136–2.401)|

*All p-values are Bonferroni corrected.

Table 4. Clinical characteristics of LDLR rs1433099 T carriers and non-carriers

|                  | CC, n=271       | CT+TT, n=236 | p  |
|------------------|----------------|-------------|----|
| Sex, M/F         | 154/117        | 150/86      | 0.123|
| Age, years       | 57.84±14.02    | 57.44±13.68 | 0.743|
| BMI, kg/m²       | 24.97 (22.53–27.14) | 24.67 (22.86–26.47) | 0.385|
| ALT, U/L         | 21.71 (15.49–30.21) | 20.92 (14.80–31.92) | 0.952|
| AST, U/L         | 21.19 (18.01–25.83) | 22.02 (18.17–27.59) | 0.375|
| TG, mmol/L       | 4.99±1.18      | 4.87±1.20   | 0.252|
| TC, mmol/L       | 1.30 (0.96–1.86) | 1.26 (0.90–1.76) | 0.434|
| HDL-C, mmol/L    | 1.10 (0.94–1.31) | 1.12 (0.94–1.29) | 0.962|
| LDL-C, mmol/L    | 2.90 (2.33–3.45) | 2.94 (2.28–3.46) | 0.683|
| GGT, U/L         | 25.21 (17.97–38.14) | 26.74 (18.50–42.04) | 0.526|
| ALP, U/L         | 74.59 (61.30–91.52) | 79.38 (64.15–97.37) | 0.052|
| FBG, mmol/L      | 4.91 (4.55–5.72) | 4.97 (4.49–5.98) | 0.530|

Abbreviations: BMI, body mass index; ALT, alanine transaminase; AST, aspartate aminotransferase; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; FBG, fasting blood glucose.
Numerous studies have revealed the association of CVD with ALP and GGT. A cross-sectional research study including 5,995 individuals found that elevated serum ALP is correlated with ALP and GGT. A Korean study involving 3,091 participants indicated an independently positive relationship of ALP with carotid-femoral pulse wave velocity, a surrogate marker for arterial stiffness. Many studies have shown that GGT is correlated with traditional risk factors, such as TC, LDL-C, glucose, insulin, BMI, etc. Further studies showed that higher GGT may increase cardiovascular mortality, and is an independent predictor for future cardiovascular mortality. Even within the normal range, higher GGT is associated with CVD risk factors, suggesting GGT as a superior marker for predicting CVD risk.

In conclusion, this study addressed that there was no association between LDLR rs1433099 polymorphism with the risk of CVD in the CVD group. We can conclude that LDLR rs1433099 polymorphism may increase the risk of CVD through ALP and GGT, from this first related research study in China; moreover, these findings are consistent with previous findings.

Our study has the following limitations. First, selection bias may exist since subjects comprised only a small sample size of patients in Qingdao district. Second, this study was confined to Chinese Han population in northern China, possibly with racial and geographical bias. Third, this study did not grade the severity of NAFLD patients. Further studies on a large-scale population of subjects and of different ethnicity are needed to estimate the impact of LDLR rs1433099 on CVD and NAFLD patients. Further research on the role of LDLR rs1433099 in CVD might help to enhance the application of future therapeutic strategies and interventions.

**Conclusions**

In conclusion, this study addressed that there was no association between LDLR rs1433099 polymorphism and incidence of NAFLD, for the first time. The LDLR rs1433099 T allele was found to significantly affect serum ALP and GGT in the CVD group. We can assume that LDLR rs1433099 polymorphism may influence the risk of CVD by ALP and GGT. The variant may be a risk factor in the early stage. Further studies on a large-scale population of subjects and of different ethnicity are needed to estimate the impact of LDLR rs1433099 on CVD and NAFLD patients. Further research on the role of LDLR rs1433099 in CVD might help to enhance the application of future therapeutic strategies and interventions.

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**Table 5. Analysis of clinical characteristics for LDLR rs1433099 C/T genotypes**

|                  | CC, n=271 | CT, n=203 | TT, n=33 | p     |
|------------------|-----------|-----------|----------|-------|
| Sex, M/F         | 154/117   | 127/76    | 23/10    | 0.225 |
| Age, years       | 59 (46–67) | 59 (46–66) | 60 (51–68) | 0.556 |
| BMI, kg/m²       | 24.87±3.35 | 24.70±3.24 | 24.85±2.95 | 0.850 |
| ALT, U/L         | 21.71 (15.49–30.21) | 20.88 (15.21–32.33) | 21.67 (13.28–30.21) | 0.947 |
| AST, U/L         | 21.19 (18.01–25.83) | 22.04 (18.15–28.09) | 21.19 (17.95–25.43) | 0.653 |
| TG, mmol/L       | 4.99±1.18 | 4.88±1.19 | 4.78±1.30 | 0.466 |
| TC, mmol/L       | 1.30 (0.96–1.86) | 1.25 (0.90–1.79) | 1.41 (0.92–1.67) | 0.601 |
| HDL-C, mmol/L    | 1.10 (0.94–1.31) | 1.12 (0.95–1.29) | 1.01 (0.88–1.32) | 0.678 |
| LDL-C, mmol/L    | 2.90 (2.33–3.45) | 2.96 (2.29–3.45) | 2.93 (2.22–3.64) | 0.918 |
| GGT, U/L         | 25.21 (17.97–38.14) | 26.53 (18.49–42.97) | 29.46 (18.51–38.91) | 0.818 |
| ALP, U/L         | 74.59 (61.30–91.52) | 80.28 (63.92–98.34) | 78.06 (65.18–92.93) | 0.148 |
| FBG, mmol/L      | 4.91 (4.55–5.72) | 4.95 (4.47–5.84) | 5.00 (4.63–6.51) | 0.552 |

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**Table 6. Analysis of clinical characteristics in LDLR rs1433099 T carriers and non-carriers of each group**

|                    | PControl* | PNAFLD* | PCVD* |
|--------------------|-----------|---------|-------|
| Age, years         | 0.608     | 0.847   | 0.532 |
| BMI, kg/m²         | 0.512     | 0.456   | 0.701 |
| ALT, U/L           | 0.376     | 0.276   | 0.524 |
| AST, U/L           | 0.497     | 0.267   | 0.349 |
| TG, mmol/L         | 0.563     | 0.477   | 0.157 |
| TC, mmol/L         | 0.522     | 0.701   | 0.957 |
| HDL-C, mmol/L      | 0.582     | 0.619   | 0.595 |
| LDL-C, mmol/L      | 0.170     | 0.400   | 0.392 |
| GGT, U/L           | 0.179     | 0.929   | 0.002#|
| ALP, U/L           | 0.181     | 0.370   | 0.024#|
| FBG, mmol/L        | 0.488     | 0.228   | 0.312 |

*Significance of each group comparing CT+TT and CC individuals.

#T carriers have higher ALP and GGT in the CVD group.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; CVD, cardiovascular disease; BMI, body mass index; ALT, alanine transaminase; AST, aspartate aminotransferase; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; FBG, fasting blood glucose.
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Conflict of interest
The authors have no conflict of interests related to this publication.

Author contributions
Design and data interpretation (YNX, YH, YSZ), manuscript writing (YH, SSL), critical revision of the manuscript (YSS, SSL, GXC, LLC). All authors reviewed and commented on the manuscript and approved the final version.

Data sharing statement
All data generated or analyzed in this study are available from the corresponding author for the reasonable request.

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