

# BMJ Open Association between complement C3 and the prevalence of metabolic-associated fatty liver disease in a Chinese population: a cross-sectional study

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## ABSTRACT

**Objectives** Recently studies demonstrated that adipose tissue can produce and release complement C3 and serum complement C3 levels were associated with diabetes mellitus, metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). Thus, we plan to investigate the association of complement C3 levels and the presence of metabolic-associated fatty liver disease (MAFLD).

**Design** Observational study with a cross-sectional sample.

**Setting** This study surveyed 4729 participants in Zhejiang province, China.

**Participants** 55 participants were excluded for acute infection and 1001 participants were excluded for lack of ultrasonography diagnoses and complete or partial absence of laboratory tests. The final sample size was 3673 participants.

**Outcome measures** Spearman correlation analysis was used to examine the correlations between complement C3 levels and variables. Binary logistic regression was carried out to evaluate the association between complement C3 levels and the presence of MAFLD after adjustment for demographic and biochemical variables. Mediation effects were used to explore whether insulin resistance (IR), hyperlipidaemia and obesity mediated the association between complement C3 and MAFLD.

**Results** Participants with MAFLD had higher complement C3 levels and complement C3 levels were closely associated with body mass index, waist circumference, alanine aminotransferase, aspartate aminotransferase,  $\gamma$ -glutamyl transpeptidase and homeostasis model assessment (HOMA)-IR. The presence of MAFLD increased with the increase of complement C3 levels and the presence of MAFLD were highest in the HOMA-IR  $\geq 2.5$  participants. We found the OR and CI of standardised C3 for MAFLD was 1.333 (1.185–1.500), each 1 SD increase in C3 would increase the presence of MAFLD by 33.3%, and obesity partly mediated the effect of complement C3 on the presence of MAFLD.

**Conclusions** The present results suggest that complement C3 can be used as a risk factor for the presence of MAFLD after adjustment for confounding variables and obesity may partly mediate the effect of complement C3 on the presence of MAFLD.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This study evaluated whether complement C3 can predict the presence of metabolic-associated fatty liver disease (MAFLD) in Chinese adults.
- ⇒ Regression analyses were applied to analyse the association between complement C3 and MAFLD.
- ⇒ This study based on a large population.
- ⇒ The diagnosis of hepatic steatosis was based on an ultrasonography examination.
- ⇒ This study was a retrospective cross-sectional study and cannot explain causal effects between complement C3 and MAFLD.

## INTRODUCTION

The diagnosis of non-alcoholic fatty liver disease (NAFLD) excluded alcohol intake, viral hepatitis and autoimmune hepatitis, but ‘excess alcohol’ intake was difficult to determine, and some studies have shown that alcohol increased the risk of liver disease, furthermore some gut microbes produce alcohol, which can also cause liver damage.<sup>1 2</sup> Sarin *et al*<sup>3</sup> indicated alcohol-related liver disease and hepatitis B/C-related liver disease developed more rapidly in the context of metabolic dysfunction and obesity. Eslam *et al*<sup>4</sup> pointed out the heterogeneity of major drivers and coexisting disease in NAFLD population is an important barrier to effective drug therapy, and response rates for the investigational drugs range from 20% to 40%, the difference compared with placebos was 10%–20%. To solve these issues, the panel of international experts recently proposed a change in nomenclature to a definition of fatty liver associated with metabolic dysfunction, and proposed new diagnostic criteria which did not exclude patients with alcohol intake or other chronic liver diseases in 2020.<sup>4 5</sup> The criteria were based on evidence

of hepatic steatosis in the presence of one or more of overweight/obesity, type 2 diabetes mellitus or evidence of metabolic dysregulation.<sup>1–6</sup> Thus, the metabolic-associated FLD (MAFLD) links FLD more closely with obesity and metabolic abnormality, and considered other etiologies of FLD.

The complement system was a complex immune network, the progress in recent years transformed the perception of complement an antimicrobial system to a regulator of immunity and tissue homeostasis.<sup>7–8</sup> The complexity of complement system was readily reflected by the multifaceted involvement of complement-driven networks in inflammatory, obesity, insulin resistance (IR), diabetes, neurodegenerative disorders and cancers.<sup>8,9</sup> Complement C3 is produced mainly by the liver and consisted of soluble and membrane-bound proteins, can modulate the immune system and promote biological activities, and produce C3a with local and systemic immunologic properties by the complement cascade.<sup>10,11</sup> Recent studies demonstrated that complement C3 and several other components of the complement pathway were mainly produced and released from adipose tissue, and then C3a was metabolised to acylation stimulating protein (ASP) which was a potent lipogenic hormone.<sup>10,12</sup> The close correlations between complement C3 levels and obesity/IR have been reported in some researches.<sup>10,12,13</sup> IR is the first step in a series of events that lead to obesity from simple to complex.<sup>10,13</sup> The large cohort studies found that complement C3 could independently predict the development of diabetes mellitus, pre-diabetes and metabolic syndrome.<sup>10,14</sup> The cross-sectional study of Xu *et al*<sup>15</sup> found NAFLD participants had higher serum complement C3 levels and complement C3 levels were positively associated with the presence and severity of NAFLD.

Lin *et al*<sup>16</sup> compared the characteristics of MAFLD and NAFLD, found MAFLD patients had higher body mass index (BMI) level, homeostasis model assessment (HOMA)-IR, lipid and liver enzymes, and indicated MAFLD definition was more practical for identifying patients with high risk of FLD progression than NAFLD definition. Thus, we performed a cross-sectional study based on a large Chinese population to investigate the association of complement C3 levels and the presence of MAFLD.

## METHODS

### Study population

This study was performed among adults who underwent their annual health examinations at the Health Care Centre in the First Affiliated Hospital of Medical College of Zhejiang University between July 2014 and November 2017. A total of 4729 participants were enrolled in this study. Fifty-five participants were excluded for self-reported acute infection within 2 weeks and 1001 participants were excluded for lack of ultrasonography diagnoses and complete or partial absence of laboratory tests. The final sample size was 3673 participants.

### Patient and public involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient-relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

### Diagnostic criteria

MAFLD is diagnosed based on an ultrasonography diagnosed hepatic steatosis and in the presence of one or more of overweight/obesity (BMI  $\geq 23$  in Asians), type 2 diabetes mellitus or evidence of metabolic dysregulation.<sup>5</sup> The hepatic steatosis was detected by ultrasonography techniques using a Toshiba Nemio 20 sonography machine with a 3.5 MHz probe (Toshiba, Tokyo, Japan). Hepatic steatosis was diagnosed according to characteristic echo patterns, such as diffuse hyperechogenicity compared with the kidneys, the gradual attenuation of far field ultrasound echo, vascular blurring and poor visualisation of intrahepatic structures.<sup>17</sup> The metabolic dysregulation was defined as the presence of at least two of the following at-risk criteria: (A) waist circumference (WC)  $\geq 90/80$  cm in men/women; (B) Blood pressure  $\geq 130/85$  mm Hg or specific drug treatment; (C) triglycerides (TG)  $\geq 1.70$  mmol/L or specific drug treatment; (D) high-density lipoprotein-cholesterol (HDL-C)  $< 1.0$  mmol/L for men and  $< 1.3$  mmol/L for women or specific drug treatment; (E) Pre-diabetes (ie, fasting glucose (Glu) levels 5.6–6.9 mmol/L, or 2 hour post-load Glu levels 7.8 to 11.0 mmol/L or glycated haemoglobin 5.7% to 6.4%); (F) HOMA-IR score  $\geq 2.5$  and (G) high-sensitivity C reactive protein (hsCRP) level  $> 2$  mg/L<sup>5</sup>.

### Assessment of demographic and biochemical variables

All participants underwent a physical examination that included demographic data, medical history, blood pressure measurement and health habit inventory. Systolic and diastolic blood pressures (SBP and DBP) were measured by an automated sphygmomanometer with the participants in the sitting position. BMI was calculated as measured weight (kg) divided by height squared ( $m^2$ ).

All venous blood samples were obtained in the morning after a 12-hour fast. The following biochemical parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), TG, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), total bilirubin (TB),  $\gamma$ -glutamyl transpeptidase (GGT), Glu, creatinine (Cr), uric acid (UA) and hsCRP were measured by a Hitachi Two D modules and one P module (DDP) autoanalyzer (Hitachi, Ibaragi, Japan) using assay-specific Roche reagents (Roche Diagnostics, Indianapolis, Indiana, USA), except for Shanghai Shensuoyoufu reagent (Shanghai, Shensuoyoufu, China) for hsCRP. Serum complement C3 levels were assessed using immune-turbidimetric assay by a Hitachi 008 autoanalyzer (Hitachi, Ibaragi, Japan) using Shanghai Zhicheng reagent (Zhicheng, Shanghai, China). Glycated haemoglobin (HbA1c) was determined by Tosoh HLC 723G8 (Tosoh

Bioscience, Tokyo, Japan) using Tosoh original reagent. Insulin (INS) were determined by electrochemiluminescence immunoassay using an ARCHITECT i4000 (Abbott Diagnostics, Abbott Park, Illinois, USA). IR was assessed by the HOMA-IR. HOMA-IR was calculated according to the following equation:  $\text{HOMA-IR (mIU}\cdot\text{mmol/L)} = \text{fasting INS (mIU/L)} \times \text{fasting Glu (mmol/L)} / 22.5$ .

### Statistical analysis

Continuous normally distributed variables were presented as mean $\pm$ SD and non-normally distributed variables were presented as median (25th–75th percentile). Categorical variables were expressed as percentages. Differences between/among groups were analysed by Student's t-test/one-way analysis of variance or the Mann-Whitney U test/Kruskal-Wallis H test for continuous variables, and the  $\chi^2$  test for categorical variables. Spearman correlation analysis was used to examine the correlations between C3 levels and demographic and laboratory variables. Binary logistic regression was carried out to evaluate the association between C3 levels and the presence of MAFLD after adjustment for demographic and biochemical variables. The mediated effect was calculated by using three regression equations. In the first step, the regression equation between complement C3 and MAFLD was calculated and the coefficient was set as c; in the second step, the regression equation between complement C3 and other variables were calculated and the coefficient was set as a; in the third step, after controlling the influence of C3, the regression equation between other variables and MAFLD were calculated and the coefficient of the variable was set as b. The ratio of mediating effect to total effect is calculated by  $M = (a \times b / c) \times 100\%$ . Mediation effects were used to explore whether IR, hyperlipidaemia and obesity mediated the association between complement C3 and MAFLD.  $P < 0.05$  (two tailed) were considered as statistically significant. All analyses were conducted by SPSS V.22 software (SPSS).

## RESULTS

### Baseline characteristics of the participants

A total of 3673 participants were divided into two groups: MAFLD group ( $n=1262$ ) and non-MAFLD group ( $n=2411$ ). The baseline characteristics of the 3673 participants are shown in [table 1](#). Participants with MAFLD were older and had higher BMI, WC, SBP, DBP, complement C3 levels, hepatic enzymatic variables (ALT, AST and GGT), lipid variables (TG, TC and LDL-C), kidney variables (Cr and UA), Glu, HbA1c, HOMA-IR, hsCRP, diabetes rates, smoke rates and drinking rates, along with lower HDL-C, female and physical exercise rates. The prevalence of diabetes and obesity (BMI  $>23$  kg/m<sup>2</sup>) were 16.2% and 93.3% in MAFLD participants, compared with only 5.3% and 53.4% in non-MAFLD participants.

### Correlation analyses between complement C3 and other variables

The correlation analyses revealed that complement C3 levels in MAFLD participants were correlated with age ( $r=-0.084$ ,  $p=0.003$ ), WC ( $r=0.159$ ,  $p<0.001$ ), BMI ( $r=0.196$ ,  $p<0.001$ ), SBP ( $r=0.168$ ,  $p=0.001$ ), DBP ( $r=0.133$ ,  $p<0.001$ ), ALT ( $r=0.201$ ,  $p<0.001$ ), AST ( $r=0.165$ ,  $p<0.001$ ), GGT ( $r=0.130$ ,  $p<0.001$ ), TB ( $r=-0.058$ ,  $p=0.040$ ), TG ( $r=0.140$ ,  $p<0.001$ ), TC ( $r=0.151$ ,  $p<0.001$ ), Cr ( $r=-0.114$ ,  $p<0.001$ ), LDL-C ( $r=0.165$ ,  $p<0.001$ ), UA ( $r=0.086$ ,  $p=0.002$ ), HbA1c ( $r=0.123$ ,  $p<0.001$ ), hsCRP ( $r=0.276$ ,  $p<0.001$ ), Glu ( $r=0.105$ ,  $p<0.001$ ), HOMA-IR ( $r=0.082$ ,  $p=0.003$ ). These results suggest that the above-mentioned variables may act as cofactors for the link between complement C3 and MAFLD.

### Subgroup analysis of C3 levels and MAFLD

We divided 3673 participants into 10 groups based on the deciles of C3, and found that the presence of MAFLD increased with the increase of complement C3 level ([figure 1](#)). This showed complement C3 was closely related to MAFLD. Further subgroup analysis was performed to identify possible cofactors. Serum complement C3 levels and the presence of MAFLD were significantly higher in obesity participants (BMI  $>23$  kg/m<sup>2</sup>), metabolic abnormalities participants (WC  $\geq 90/80$  cm, high blood pressure, high TG, high HOMA-IR, high hsCRP and low HDL-C), diabetes mellitus and pre-diabetes than in lean participants (BMI  $\leq 23$  kg/m<sup>2</sup>), metabolic normal participants and normal Glu metabolism participants. The complement C3 levels and the presence of MAFLD were highest (median 120 mg/dL and 65.7%) in the HOMA-IR  $\geq 2.5$  participants among all subgroups. We found that these three states (obesity, metabolic abnormalities and diabetes mellitus) were related to complement C3 and the presence of MAFLD.

### The association between complement C3 and the presence of MAFLD

Univariate regression analyses were performed to analyse the associations between complement C3/other variables and the presence of MAFLD in the 3673 participants ([table 2](#)). Data in [table 2](#) show that complement C3, age, gender, WC, BMI, SBP, DBP, ALT, AST, GGT, TB, TG, TC, HDL-C, LDL-C, Cr, UA, HbA1c, Glu, INS, HOMA-IR, hsCRP, drinking, physical exercise and smoking (all  $p<0.05$ ) could impact the presence of MAFLD. Then these variables were included in the binary logistic regression analysis for MAFLD. Since HOMA-IR was calculated by Glu and INS, Glu and INS were not included in binary logistic regression analysis so as not to increase the weight of these two variables. The results of the adjusted binary logistic regression analysis models are shown in [table 3](#). In the adjusted models, the OR and 95% CI for MAFLD was 1.015 (1.009 to 1.021) ([table 3](#), the OR of the covariates was in online supplemental table 1). Thus, each 1 unit increase in complement C3 level would increase the risk of the presence of MAFLD by 1.5% in all participants. To

**Table 1** Baseline characteristics of the study population

Characteristics	Non-MAFLD (n=2411)	MAFLD (n=1262)	P value
Age (year)	48.7±9.9	50.5±8.9	<0.001*
WC (cm)	83.2±8.2	92.7±7.6	<0.001*
BMI (kg/m <sup>2</sup> )	23.6±2.7	26.6±2.7	<0.001*
SBP (mm Hg)	125±18	135±17	<0.001*
DBP (mm Hg)	76±11	83±10	<0.001*
ALT (U/L)	16 (12–23)	26 (19–38)	<0.001#
AST (U/L)	19 (17–23)	22 (18–27)	<0.001#
GGT (U/L)	20 (14–34)	37 (24–58)	<0.001#
TB (μmol/L)	11 (8–14)	12 (9–15)	<0.001#
TG (mmol/L)	1.17 (0.87–1.68)	1.96 (1.43–2.87)	<0.001#
TC (mmol/L)	4.67 (4.13–5.28)	4.94 (4.38–5.54)	<0.001#
HDL-C (mmol/L)	1.28 (1.07–1.53)	1.05 (0.90–1.23)	<0.001#
LDL-C (mmol/L)	2.66 (2.23–3.16)	2.84 (2.37–3.35)	<0.001#
Cr (μmol/L)	71 (59–81)	75 (63–83)	<0.001#
UA (μmol/L)	311 (257–372)	379 (321–433)	<0.001#
Glu (mmol/L)	4.89±0.90	5.50±1.55	<0.001*
HbA1c (%)	5.5 (5.3–5.7)	5.7 (5.4–6.1)	<0.001#
hsCRP (mg/L)	1.0 (0.47–1.97)	1.5 (0.8–2.77)	<0.001#
INS (μU/mL)	6.5 (4.7–9.1)	10.8 (7.8–14.4)	<0.001#
HOMA-IR	1.39 (0.98–1.99)	2.52 (1.78–3.57)	<0.001#
C3 (mg/dL)	103 (92–115)	118 (107–129)	<0.001#
Smoke, n (%)	552 (22.9)	349 (27.7)	0.001\$
Drinking, n (%)	427 (17.7)	308 (24.4)	<0.001\$
Female, n (%)	1087 (45.1)	331 (26.2)	<0.001\$
Physical exercise, n (%)	832 (34.5)	346 (27.4)	<0.001\$
BMI >23, n (%)	1287 (53.4)	1178 (93.3)	<0.001\$
Diabetes, n (%)	128 (5.3)	205 (16.2)	<0.001\$

Continuous variables were presented as mean±SD or median (25th–75th percentile). The statistical significance of differences between the non-MAFLD group and MAFLD group were analysed by Student's t-test (\*), the Mann-Whitney U test (#) or the X<sup>2</sup> test (\$).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; C3, complement C3; Cr, creatinine; DBP, diastolic blood pressure; GGT, γ-glutamyl transpeptidase; Glu, glucose; HbA1c, glycosylated haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homoeostasis model assessment of insulin resistance; hsCRP, High sensitivity C reactive protein; INS, insulin; LDL-C, low-density lipoprotein cholesterol; MAFLD, metabolic associated fatty liver disease; SBP, systolic blood pressure; TB, total bilirubin; TC, total cholesterol; TG, triglyceride; UA, uric acid; WC, waist circumference.

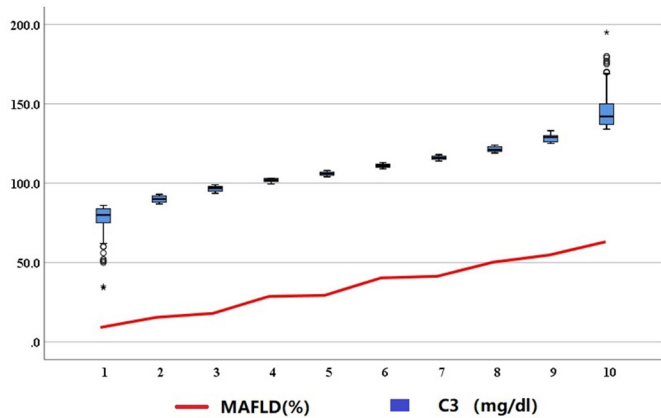
increase the ability of the models to identify risks for the presence of MAFLD, we standardised the complement C3 data, and found the OR and CI of the standardised complement C3 for MAFLD was 1.333 (1.185–1.500), each 1 SD increase in component level would increase the risk of the presence of MAFLD by 33.3% in all participants. This showed that complement C3 could be used as a risk factor for the presence of MAFLD.

#### Mediated effects of HOMA-IR/TG/BMI on the association between C3 and NAFLD

IR, hyperlipidaemia and obesity are variables of metabolic abnormalities in MAFLD. IR (HOMA-IR), hyperlipidaemia (TG) and obesity (BMI) and complement C3 were positively associated with the presence of MAFLD (table 2),

while complement C3 was positively correlated with HOMA-IR, TG, and BMI, suggesting a mechanistic link between complement C3 and MAFLD, possibly explained by HOMA-IR, TG, or BMI. To explore the internal relationships among HOMA-IR/TG/BMI, C3 and MAFLD, we conducted the mediated effects analysis to explore whether HOMA-IR/TG/BMI mediated the association between complement C3 and the presence of MAFLD. The contribution rate of mediated effects of BMI, TG, and HOMA-IR to the total effect (complement C3 on the presence of MAFLD) were 55.7%, 14.0% and 26.3% (all p<0.001), respectively, this showed obesity significantly partly mediated the effect of complement C3 on the presence of MAFLD compared with TG and HOMA-IR.





**Figure 1** The presence of MAFLD in the decile subgroups of complement C3 levels. Numbers from 1 to 10 represent the decile grouping of complement C3 (from 1st to 10th). MAFLD, metabolic-associated fatty liver disease. \* at 1st decile represent the lowest C3 level and \* at 10th decile represent the highest C3 level.

## DISCUSSION

Participants with MAFLD had higher complement C3 levels, BMI, WC, SBP, DBP, ALT, AST, GGT, TB, TG, TC, LDL-C, Cr, UA, Glu, INS, HbA1c, HOMA-IR, hsCRP, diabetes rates, smoke rates and drinking rates, but lower HDL-C and physical exercise rates, compared with that of participants without MAFLD. Serum complement C3 levels were closely associated with abdominal obesity (BMI and WC), liver enzymology markers (ALT, AST and GGT), and HOMA-IR by spearman correlation analyses. The presence of MAFLD increased with the increase of complement C3 level, and the presence of MAFLD were highest (median 120mg/dL and 65.7%) in the HOMA-IR  $\geq 2.5$  participants. We found the OR and CI of standardised complement C3 for MAFLD was 1.333 (1.185–1.500), each 1 SD increase in complement C3 levels would increase the risk of the presence of MAFLD by 33.3% by the adjusted binary regression models. Complement C3 can not only directly affect the presence of MAFLD, but also indirectly affect the presence of MAFLD through the mediated effects of obesity (accounted for 55.7%).

The complement system is an innate immune system, playing a key role in host homeostasis, the regulation of inflammation, and in the defence against pathogens.<sup>14</sup> The excessive activation of complement is associated with many autoimmune diseases.<sup>18</sup> Complement C3 is mainly produced by the liver and adipocytes/macrophages in adipose tissue and is a key element in three complement activation pathways (classical, alternative and lectin pathways), inflammation and metabolic effects.<sup>10 19 20</sup> Complement C3 is involved in metabolic disorders mainly by C3a and ASP.<sup>20</sup> C3a has INS-like effects and facilitates TG metabolism, and can trigger a cytokine/chemokine response and mediate IR.<sup>10 12</sup> ASP can stimulate synthesis and release of TG, this leads to excessive accumulation of fat in liver cells and disorder of lipid metabolism.<sup>21</sup>

**Table 2** Associations of C3 with the presence of MAFLD by univariate regression analyses

Characteristics	OR (95% CI)	P value
Age	1.019 (1.012 to 1.026)	<0.001
WC	1.167 (1.154 to 1.181)	<0.001
BMI	1.570 (1.518 to 1.624)	<0.001
SBP	1.033 (1.028 to 1.037)	<0.001
DBP	1.054 (1.047 to 1.061)	<0.001
ALT	1.048 (1.042 to 1.054)	<0.001
AST	1.046 (1.037 to 1.056)	<0.001
GGT	1.012 (1.010 to 1.014)	<0.001
TB	1.024 (1.012 to 1.037)	<0.001
TG	1.971 (1.827 to 2.127)	<0.001
TC	1.352 (1.253 to 1.459)	<0.001
HDL-C	0.074 (0.057 to 0.097)	<0.001
LDL-C	1.310 (1.196 to 1.434)	<0.001
Cr	1.015 (1.011 to 1.020)	<0.001
UA	1.008 (1.008 to 1.009)	<0.001
Glu	1.678 (1.552 to 1.814)	<0.001
HbA1c	1.977 (1.772 to 2.206)	<0.001
hsCRP	1.015 (1.002 to 1.027)	0.019
INS	1.223 (1.201 to 1.246)	<0.001
HOMA-IR	2.267 (2.107 to 2.440)	<0.001
C3	1.045 (1.040 to 1.049)	<0.001
Smoke	1.287 (1.102 to 1.504)	0.001
Drinking	1.499 (1.270 to 1.769)	<0.001
Female	0.433 (0.373 to 0.503)	<0.001
Physical exercise	0.717 (0.617 to 0.832)	<0.001
BMI >23	12.248 (9.679 to 15.498)	<0.001
Diabetes	3.459 (2.742 to 4.365)	<0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; C3, complement C3; Cr, creatinine; DBP, diastolic blood pressure; GGT,  $\gamma$ -glutamyl transpeptidase; Glu, glucose; HbA1c, glycosylated haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homoeostasis model assessment of insulin resistance; hsCRP, High sensitivity C reactive protein; INS, insulin; LDL-C, low-density lipoprotein cholesterol; MAFLD, metabolic-associated fatty liver disease; SBP, systolic blood pressure; TB, total bilirubin; TC, total cholesterol; TG, triglyceride; UA, uric acid; WC, waist circumference.

Adipocytes in addition to complement C3 can also produce complement C3 receptors, mild inflammation of adipose tissue leads to complement C3 activation, which may lead to further deterioration of metabolic complications in obese patients.<sup>19 22</sup> Some reports have shown that there is a strong relationship between C3/C3a/ASP and adipose tissue, cardiovascular disease, metabolic syndrome, diabetes, NAFLD and AFLD, implying that complement C3 may play a central role and maybe a powerful predictor or therapeutic target for FLD.<sup>10 15 19 23</sup>



**Table 3** Associations of C3 with the presence of MAFLD by multivariate regression analyses

Characteristics	OR (95% CI)	P value
Model 1	1.027 (1.022 to 1.032)	<0.001
Model 2	1.023 (1.018 to 1.028)	<0.001
Model 3	1.021 (1.016 to 1.026)	<0.001
Model 4	1.017 (1.011 to 1.022)	<0.001
Model 5	1.019 (1.013 to 1.025)	<0.001
Model 6	1.015 (1.009 to 1.021)	<0.001

Adjusted model 1: Age, WC, BMI, SBP, DBP, sex, drinking, physical exercise and smoke (baseline indexes); model 2: model 1+AST, ALT, GGT and TB liver markers); model 3: model 2+Cr and UA (kidney markers); model 4: model 3+TC, TG, HDL-C, and LDL-C (lipid markers); model 5: model 4+hsCRP; model 6: model 5+HOMA-IR and HbA1c.  
 ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cr, creatinine; DBP, diastolic blood pressure; GGT,  $\gamma$ -glutamyl transpeptidase; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; hsCRP, high-sensitivity C reactive protein; LDL-C, low-density lipoprotein cholesterol; MAFLD, metabolic-associated fatty liver disease; SBP, systolic blood pressure; TB, total bilirubin; TC, total cholesterol; TG, triglycerides; UA, uric acid; WC, waist circumference.

Our study showed that the serum complement C3 levels in MAFLD patients were significantly higher than that in non-MAFLD patients, and closely associated with obesity, higher TG and HOMA-IR, indicating that the synthesis and release of complement C3 in MAFLD patients were increased under the interaction of various factors. There are some relevant studies on NAFLD and AFLD.<sup>3 15</sup> The study of Rensen *et al*<sup>24</sup> indicated 74% of the NAFLD patients showed activated complement C3 and C4d were deposited predominantly around hepatocytes with macrovesicular steatosis, and were accompanied by hepatic neutrophil infiltration, increased interleukin (IL)-8/IL-6 expression, and numbers of apoptotic cells. Patients with Nonalcoholic steatohepatitis (NASH) had a higher presence of activated complement C3 deposition.<sup>7 24</sup> Zhong *et al*<sup>23</sup> found complement C3 promoted the accumulation of TG and contributed to liver injury and steatosis by regulating the production of tRNA-derived fragments glycine tRNA-derived fragments (*Gly-tRF*) in AFLD live. Wlazlo *et al*<sup>7</sup> found C3a were associated with liver fat content and hepatocellular injury in heavy alcohol consumers daily. Complement C3 deficiency has been shown to impart protective effects against ethanol-induced hepatic steatosis and inflammation.<sup>23</sup> Animal experiments also found important cross-talk between complement C3 and lipid regulators in alcohol-induced steatosis, and in C3<sup>-/-</sup> mice liver steatosis was strongly reduced after alcohol exposure.<sup>25 26</sup> This suggests that the complement C3 contributes to the development of alcohol-induced fatty liver. The inhibitors of C3 activation or Gly-tRF may provide a precise therapeutic approach for AFLD.

Our study also found that complement C3 levels were positively correlated with common indicators ALT and AST of liver injury, the presence of MAFLD increased with the increase of complement C3 level, and complement C3 was a risk factor for the presence of MAFLD. There are some studies that are close to our results. The study of Jia *et al*<sup>14</sup> first demonstrate that serum complement C3 levels were independently associated with a higher presence of NAFLD and AFLD in adult male population, the presence of NAFLD and AFLD in the highest quartile of complement C3 compared with the lowest quartile was 4.13 and 2.09 times, respectively. The Chinese cross-sectional study of Xu *et al*<sup>15</sup> found that serum complement C3 was independently associated with risk for NAFLD and complement C3 level is positively associated with prevalence and severity of NAFLD. Pan *et al*<sup>27</sup> revealed that complement C3 can be used as a surrogate biomarker of NAFLD in patients with chronic kidney disease with sensitivity 63.9% and specificity 70.1%. Ursini *et al*<sup>18</sup> revealed complement C3 can be used as a surrogate biomarker of NAFLD in rheumatoid arthritis patients with sensitivity 76% and specificity 64%. Therefore, the activation of the component in NAFLD patients was associated with disease occurrence and disease severity, and C3 can be a risk factor for the prevalence of MAFLD.

In addition, we found the presence of MAFLD was highest in the IR participants (HOMA-IR  $\geq 2.5$ ), and obesity, HOMA-IR and TG may partly mediate the effect of complement C3 on the presence of MAFLD by mediate effect. Then, we suggested a mechanistic link between complement C3 and MAFLD which might be explained by metabolic disorder. There are some similarities between these following studies and our study. Castellano-Castillo *D et al*<sup>12</sup> found complement C3 mRNA levels were associated with Glu metabolism and IR, while complement C3 methylation levels were associated with adiposity variables. Al-Domi *et al*<sup>9</sup> found C3 and IR were significantly higher in the obese group than that in the normal body weight, suggested complement C3 may be an independent marker of the chronic inflammatory process and may have a role in the progression of IR during obesity. Himoto *et al*<sup>28</sup> found complement C3 levels were significantly correlated with BMI, HOMA-IR and TG, and increased with the aggravation of liver steatosis and liver fibrosis in chronic hepatitis C patients, they suggested complement C3 levels may reflect obesity, IR and hepatic steatosis. Therefore, we speculated that C3 might be of great significance for the incidence and progression of MAFLD, and the regulation of complement C3, C3a and ASP levels might provide therapeutic targets for the control of hepatic steatosis in MAFLD patients.

Our study has several potential limitations. First, the diagnosis of hepatic steatosis was based on an ultrasonography examination, which may miss mild steatosis. Second, this study was a retrospective cross-sectional study not a prospective study. Third, our study was limited to Chinese Han adults, and our study may not be applicable to other ethnic groups and children. Therefore,

prospective, multicentre and multiracial clinical research need to be further carried out.

In conclusion, our results first demonstrated a significant correlation between complement C3 levels and MAFLD. Thus, complement C3 was a risk factor of the presence of MAFLD after adjustment for confounding variables and obesity may partly mediate the effect of complement C3 on the presence of MAFLD.

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