A novel index including SNPs for the screening of nonalcoholic fatty liver disease among elder Chinese
A population-based study
Huanhuan Yang, MM, Guochong Chen, PhD, Chunli Song, MM, Deming Li, MM, Qinghua Ma, MM, Guangliang Chen, PhD, Xinli Li, PhD,a,c

Abstract
Presently noninvasive methods were employed to the diagnosis of nonalcoholic fatty liver disease (NAFLD), including fatty liver index (FLI), hepatic steatosis index (HSI), product of fasting triglyceride and glucose levels (TyG), and single nucleotide polymorphism (SNP), whereas the accuracy of those indexes need to be improved. Our study aimed to investigate the feasibility of a new index: comprehensive index (CI), consisting of 6 serum biomarkers and anthropometric parameters through multivariate logistic regression analysis, to the earlier detection of NAFLD, and the diagnostic value of 5 SNPs [S1: rs2854116 of apolipoprotein C3 [APOC3], S2: rs4149267 of ATP-binding cassette transporter [ABCA1], S3: rs13702 of lipoprotein lipase [LPL], S4: rs738409 of protein 3 [patatin-like phospholipase domain containing protein 3 (PNPLA3)], S5: rs780094 of glucokinase regulatory protein gene (GCKR)] for NAFLD were also explored. Area under the receiver operating characteristic curves (AUROC) and Youden index (YI) were calculated to assess the diagnostic value. The AUROC of CI was higher than FLI, HSI, and TyG (CI: 0.897, FLI: 0.873, HSI: 0.855, TyG: 0.793). Therefore, CI might be a better index for the diagnosis of NAFLD. Although there had no statistical significance (P = 0.125), the AUROC and YI were increased when CI combined with rs2854116 (S1) (AUROC = 0.902, YI = 0.6844). The combination of CI with S1 showed even better diagnostic accuracy than CI, which suggests the potential value of rs2854116 for the diagnosis of NAFLD.

Abbreviations: ABCA1 = ATP-binding cassette transporter, ALT = alanine aminotransferase, APOC3 = apolipoprotein C3, AST = aspartate aminotransferase, AUROC = the area under the receiver operating characteristic curve, BMI = body mass index, CI = comprehensive index, CI = confidence interval, CT = computerized tomography, DBP = diastolic blood pressure, DNTP = deoxy-ribonucleoside triphosphate, EDTA = ethylenediaminetetraacetic acid, FLI = fatty liver index, FPG = fasting plasma glucose, GCKR = glucokinase regulatory protein gene, H MRS = H magnetic resonance spectroscopy, HC = hip circumference, HCC = hepatocellular carcinoma, HDL-C = high-density lipoprotein cholesterol, HR = heart rate, HSI = hepatic steatosis index, LDL-C = low-density lipoprotein cholesterol, LPL = lipoprotein lipase, MRI = magnetic resonance imaging, NAFLD = nonalcoholic fatty liver disease, OR = odds ratio, PCR = polymerase chain reaction, PNPLA3 = patatin-like phospholipase domain containing protein 3, r-GGT = γ-glutamyl transferase, S1 = rs2854116, S2 = rs4149267, S3 = rs13702, S4 = rs738409, S5 = rs780094, SBP = systolic blood pressure, SNP = single nucleotide polymorphism, T2D = type 2 diabetes, TC = total cholesterol, TG = triglyceride, TyG = product of fasting triglyceride and glucose levels, UA = uric acid, WC = waist circumference, WHR = waist-to-hip ratio, YI = Youden index.

Keywords: comprehensive index, diagnosis, nonalcoholic fatty liver disease, sensitivity, serum biomarkers, single nucleotide polymorphism, specificity

1. Introduction
Nonalcoholic fatty liver disease (NAFLD) has become an emerging healthy problem worldwide.[1] With the increase of its prevalence, NAFLD affected 15% to 40% of the general population at present.[2] As the most common of liver disease, NAFLD was coincident with other liver diseases, such as steatohepatitis, fibrosis, liver cirrhosis, and liver failure.[3,4] In addition, NAFLD was associated with metabolic syndrome (MetS), type 2 diabetes (T2D), and cardiovascular disease.[5–7] Thus, the earlier detection and diagnosis of NAFLD is essential. Liver biopsy was considered to be the “criterion standard” for the diagnosis of NAFLD,[8] whereas the features of invasiveness, high cost, and the possibility of potential complications induced by biopsy procedure limited its employment. Computerized tomography (CT), magnetic resonance imaging (MRI), and H magnetic resonance spectroscopy (H MRS) were regarded as noninvasive, and more accurate methods in the diagnosis of NAFLD.[9] However, the high cost, the requirement of the infrastructure, and related knowledge also limited their use. Ultrasound was employed...
to the diagnosis of NAFLD widely now, because it is relatively economical and convenient in contrast to other diagnostic methods. Meanwhile, several new diagnostic indexes, such as fatty liver index (FLI), hepatic steatosis index (HSI), the product of fasting triglyceride and glucose levels (TyG), and liver fat scores have developed recently, whereas the accuracy and reliability of those above indexes needs to be improved based on the area under the receiver operating characteristic curves (AUROC). Many studies have reported the role of serological indicators in the prediction and diagnosis of related diseases. Therefore, we consider to build a new index which would consist of serological indicators and anthropometric parameters and it would show a higher AUROC, sensitivity, and specificity than the existing ones.

Although the exact pathogenesis of NAFLD is not clarified completely, increasing evidence supported the role of single nucleotide polymorphism (SNP) in the occurrence and development of NAFLD, especially SNPs for those genes involving in metabolic disorders, including insulin resistance and atherosclerosis. Genome-wide association studies for NAFLD have identified some novel susceptibility genes, including PNPLA3 rs738409, and GCKR rs780094. The effects of PNPLA3 on NAFLD have been reported in numerous studies among people of different races. And a meta-analysis had proved that there had significant association between GCKR rs780094 and risk of NAFLD among both Asian and non-Asian populations. A research of southern Indian people had reported that APOC3 rs2854116 was significantly associated with NAFLD (P = 0.001) and elevated serum triglycerides (TGs) in NAFLD patients. Another one provides the evidence of APOC3 rs2854116 genotype was a independent factor (hazard ratio: 3.93; 95% confidence interval: 1.30–11.84; P = .013) predicting hepatocellular carcinoma (HCC) development. Accumulating evidence indicates that the efflux transport of bioactive lipids, including cholesterol and phospholipids, was mediated by ABCA1 in various types of tissues and cells. Rs4149267 of ABCA1 was a predictor of high-density lipoprotein cholesterol (HDL-C) in both Sacramento and Beltsville population, and HDL-C was recognized as a risk factor of NAFLD. LPL rs13702 modulates lipid traits through disruption of a micro-RNA-410 seed site and was associated with various metabolic disorders, including insulin resistance and atherosclerosis. However, there had few researches explored the possibility of these SNPs in the diagnosis of NAFLD. Therefore, this study would to explore the diagnostic value of these SNPs.

FLI, HSI, and TyG had employed the following biochemical indicators to the diagnosis of NAFLD, including TG, body mass index (BMI), γ-glutamyl transferase (r-GGT), and waist circumference (WC); however, there had many other parameters that had strong correlation with NAFLD, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), low-density lipoprotein cholesterol (LDL-C), HDL-C, sex, age, height, weight, hip circumference (HC), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), fasting blood glucose (FPG), uric acid (UA), waist-to-hip ratio (WHR), and AST/ALT, whether these parameters could be used to the diagnosis of NAFLD need to be investigated. Thus, our present study aimed to clarify the possibility of a new comprehensive index (CI), combing with serum biomarkers and anthropometric parameters, to diagnose NAFLD. And the value of SNPs in the diagnosis of NAFLD was also investigated by being added to the new index CI.

2. Materials and methods

2.1. Subjects

This cross-sectional, community-based study was conducted in XiangCheng District, Suzhou, China on July to October in 2016. We numbered all the 5363 elderly people of XiangCheng District and randomly selected 1100 subjects. Finally, 918 subjects were enrolled in the present study, which included 310 (33.8%) men and 608 (66.2%) women, with the mean age of 73.78 ± 4.78 and 71.12 ± 5.67 years, respectively. The 918 participants were randomly assigned into 2 groups randomly, it contained 459 subjects of each group, the training group was used to build the model, and the validation group to validate the accuracy of the model (Fig. 1). Diagnosis of NAFLD was conducted according to the “Guidelines for management of nonalcoholic fatty liver disease: an updated and revised edition”. Subjects conserved the following characteristics were excluded from the study: population with the history of alcohol abuse (ethanol intake >140g/wk for men or >70g/wk for women); population with the following diseases: Wilson disease, drug hepatitis, alcohol-related liver disease, autoimmune hepatitis, and other related diseases; liver cirrhosis or HCC; and other concomitant disease. In addition, T2D was defined as a self-reported medical history of T2D, or FPG ≥126mg/dL (about 7mmol/L) according to the 2010 American Diabetes Association criteria.

2.2. Measurement of biochemical and anthropometric parameters

Peripheral blood was drawn after a 12-hour fast and divided into 2 portions: 1 portion was collected with ethylene diamine tetraacetic acid and used for DNA extraction, the remaining portion of blood sample was separated to obtain serum and used for determination of the following parameters: hepatic enzymes
(including ALT, AST, r-GGT), lipid profile (including TG, TC, HDL-C, LDL), FPG, and UA. Anthropometric parameters, including height, weight, BMI, WC, and HC were also measured. All the biochemical makers and anthropometric parameters were performed by experienced physicians. Examination of abdominal ultrasounds was blindly conducted by experienced physicians. WC was measured at a level midway between the lower rib margin and iliac crest with the tape all around the body in the horizontal position. BMI was calculated as weight (kg) divided by height (m) squared.

2.3. Genomic DNA extraction and genotyping

Peripheral blood samples were collected in K3-ethylenediamine-tetraacetic acid tubes from the participants. Genomic DNA was extracted from the blood using DNA purification kit (Spin Columns). The purity of DNA was measured by spectrophotometer and the integrity of DNA was determined by 0.7% agarose gel electrophoresis.

The gene polymorphism of SNPs was genotyped by ligase detection reaction method. The polymerase chain reaction (PCR) solution contained 1 μL of DNA, 1.5 μL of 10× buffer, 1.5 μL of MgCl2, 0.3 μL of deoxy-ribonucleoside triphosphate (Fermentas, Canada), 0.3 μL of Taq polymerase (Fermentas), 0.15 μL of primers, and 15 μL of water. PCR conditions included an initial denaturation at 94°C for 3 minutes, and 35 cycles each consisting of 30 seconds at 94°C, 30 seconds at 55°C, and 90 seconds at 72°C. The final denaturation was at 72°C for 3 minutes. The connection reaction solution contained: 3 μL of PCR products, 1 μL of 10× Taq DNA ligase buffer, 0.125 μL of Taq DNA ligase (40 U/μL), 0.01 μL of Probe (10p), and 10 μL of water. The connection conditions included 30 cycles each consisting of 30 seconds at 94°C and 3 minutes at 56°C. Approximately 8 μL loading buffer was added to 1 μL of the reaction products and denaturation at 95°C for 3 minutes. And DNA was sequenced by gene sequence (3730XL, ABI Inc., Foster City, CA 94404, USA) after ice water bath. Primer and probe sequences, number, and size of PCR restriction fragment length polymorphism bands are shown in Table 1.

2.4. Equations that are referred to in the text

(1) FLI = \[\frac{\text{BMI}(cm)}{\text{Ht}(cm)} \times \frac{\text{WC}(cm)}{\text{HC}(cm)} = \frac{\text{BMI}}{\text{Ht}} \times \frac{\text{WC}}{\text{HC}} \]

(2) HSI = \[8 \times \frac{\text{ALT}}{\text{AST}} + \text{BMI} \times (2 + \text{T2D}) \times (2 + \text{female}) \]

(3) TyG = ln(TG × FPG/2) \[\text{[mmol/L]} \]

All the units of the parameters included in Eq. (1) and Eq. (2) are shown in Table 2, whereas the unit of TG and FPG in Eq. (3) was mg/dL. Informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision,
2008) as reflected in a priori approval by the institution’s human research committee.

2.5. Statistical analysis

SPSS version 18.0 and MedCalc version 11.4 were employed to conduct statistical analysis. The training group was conducted to build the new formula. Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed as counts and percentages. Age, height, weight, WC, HC, SBP, DBP, heart rate, TC, LDL-C, HDL-C, and UA were analyzed using t test for normal distributional data. Mann-Whitney U test was used to calculate ALT, AST, and AST/ALT because of their non-normal distribution. For those categorical variables including sex and degrees of WHR, BMI, and FPG were analyzed using chi-square test. Multivariate binary logistic regression was conducted to select the potential risk factors for NAFLD. The variables that had statistical difference between NAFLD group and control group would be entered into multivariate analysis. The regression model was established by forward selection method to eliminate multicollinearity. The odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were presented.

In validation group, AUROC, sensitivity, and specificity were conducted by the “ROC curve analysis” function of MedCalc, Youden index (YI) was calculated as sensitivity added by specificity and then minus one. These indexes would be compared among CI, FLI, HSI, and TyG. Comparison of AUROC was conducted by z test, z = |AUROC1 – AUROC2|/sqrt(SE1² + SE2²). A P value < .05 was considered to be statistically significant.

3. Results

3.1. Basic characteristic of the subjects

As displayed in Table 2, the participants of the training group were divided into 2 subgroups based on the results of hepatic ultrasounds: NAFLD group (n=97, 21.1%) and health group (n=362, 78.9%). Women presented higher prevalence of NAFLD than men, and they were 23.9% and 15.8% respectively. There had significant differences (P < .05) in the biochemical indexes and anthropometric parameters between NAFLD and health group except the height, SBP, DBP, and heart rate. Age, AST/ALT, and HDL-C were significantly higher in health group than in NAFLD group. And the other parameters were higher in NAFLD group. The prevalence of NAFLD was positively related to WHR, BMI, and FPG, and there were statistical differences among 3 categories of WHR, as well as BMI and FPG.

Comparing with the lowest category of WHR, the prevalence of NAFLD in the highest category increased from 5.9% to 30.0%, BMI and FPG had the similar tendency as WHR. With the increase of BMI and FPG from the lowest to the highest category, the prevalence of NAFLD ranged from 9.0% to 50.0% for BMI, and 17.1% to 38.9% for FPG.

3.2. Multivariate analysis to select potential variables associated with NAFLD

Multivariate logistic regression analysis was carried out to select potential predictors of NAFLD and the adjusted ORs were also calculated. Results of Table 3 suggest the tighter association of weight, WC, BMI, AST/ALT, TG, and FPG with NAFLD.

The coefficients of CI were calculated through the regression coefficients B. Based on the results of multivariate logistic regression analysis, a formula to calculate CI was given as follows:

\[ \text{CI} = -0.063 \times \text{Weight} + 0.065 \times \text{WC} + 0.315 \times \text{BMI} - 2.165 \times \text{AST/ALT} + 0.935 \times \text{TG} + 0.276 \times \text{FPG} - 11.236 \]

3.3. Allele frequencies and genotypes distribution in NAFLD group and control group

Allele frequencies and genotypes distribution of S1 (APOC3 rs2854116), S2 (ABCA1 rs4149267), S3 (LPL rs13702), S4 (PNPLA3 rs738409), and S5 (GCKR rs780094) polymorphisms were detected (Table 4). The distributions of all genotypes were compatible with Hardy-Weinberg equilibrium. There had no statistical significance between the minor allele and the major allele of these SNPs between NAFLD and health group. There had no significant difference among 3 genotypes in the SNPs (P > .05), except for the difference between AA and AG genotype in S2 (P = .006).

Although PNPLA3 rs738409 and GCKR rs780094 had been shown to be associated with NAFLD in other populations, there had no statistical significance between the minor allele and the major allele in this study.

3.4. The comparison between CI and other indexes

As shown in Table 5, comparing with other 3 indexes FLI, TyG, and HSI, CI had the highest AUROC (AUROC=0.897), then was 0.873 for FLI, and the lowest was TyG’s, and there had significant difference when TyG and HSI compared with CI respectively. Although it has no statistical significance, the AUROC of CI was obviously higher than that of FLI. CI also had

| Table 3 |
| Variables associated with nonalcoholic fatty liver disease under multivariate analysis by forward selection. |
| Variables | β | SE | P | OR | 95% CIs |
| Weight (cm) | -0.063 | 0.027 | .020 | 0.939 | 0.890–0.990 |
| WC (cm) | 0.065 | 0.030 | .033 | 1.067 | 1.005–1.133 |
| BMI | 0.315 | 0.097 | .001 | 1.370 | 1.133–1.656 |
| AST/ALT | -2.165 | 0.485 | <.001 | 0.115 | 0.044–0.297 |
| TG (mmol/L) | 0.095 | 0.192 | <.001 | 2.548 | 1.749–3.713 |
| FPG (mmol/L) | 0.276 | 0.125 | .028 | 1.318 | 1.031–1.684 |
| Constant | -11.236 | 2.262 | |

P < .05 was considered statistically significant.

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, FPG = fasting blood-glucose, OR = odds ratio, TG = triglyceride, WC = waist circumference.
the highest YI in contrast to other 3 indexes, which suggested the best accuracy and reliability of CI for the diagnosis of NAFLD. The sensitivity and specificity of CI were 89.66/76.20, the sensitivity was ranked only second to FLI and the specificity to HSI. Although FLI had the highest sensitivity, its specificity was much less than others. HSI had the highest specificity, but its sensitivity was the lowest among the 4 indexes (Fig. 2, Table 5).

When index CI combined with SNPs respectively, the AUROC of “CI+S1” was 0.902, and of “CI+S2”, “CI+S3,” “CI+S4,” and “CI+S5” were all 0.897. Although the difference had no statistical significance, it was higher than that of CI after added S1. “CI+S1” showed the highest YI and specificity, but its sensitivity was lower than others. “CI+S2” and “CI+S3” also showed higher sensitivity than CI, whereas both their specificity were lower. “CI+S4” and “CI+S5” were not better the CI in all ways (Fig. 3, Table 5).

### Table 5

| Gene | Control group (n=725) | NAFLD group (n=193) | Nonadjusted | Adjusted |
|------|----------------------|--------------------|-------------|----------|
|      |                      |                    | P  | OR (CIs 95%) | P  | OR (CIs 95%) |
| S1: APOC3 rs2854116 | AA | 135 (37.3) | 39 (40.2) | .667 | 0.900 (0.556–1.457) | .714 | 0.912 (0.558–1.492) |
|      | AG | 177 (48.9) | 46 (47.4) | .802 | 1.391 (0.836–2.313) | .831 | 1.335 (0.800–2.235) |
|      | GG | 50 (13.8) | 12 (12.4) | .616 | 0.831 (0.403–1.713) | .750 | 0.888 (0.426–1.848) |
| S2: ABCA1 rs4149267 | AA | 200 (55.2) | 41 (42.3) | .874 | 1.930 (1.199–3.034) | .915 | 1.931 (1.160–3.203) |
|      | AG | 133 (36.7) | 52 (53.6) | .006 | 1.390 (0.822–2.327) | .006 | 1.391 (0.823–2.326) |
|      | GG | 29 (8.9) | 4 (4.1) | .479 | 0.673 (0.224–2.017) | .536 | 0.706 (0.233–2.131) |
| S3: LPL rs13702 | AA | 256 (71.3) | 67 (69.1) | .227 | 1.362 (0.825–2.248) | .181 | 1.141 (0.851–2.531) |
|      | AG | 82 (22.7) | 29 (29.9) | .901 | 1.756 (1.033–2.981) | .906 | 1.763 (1.038–2.981) |
|      | GG | 22 (6.1) | 1 (1.0) | .901 | 1.756 (1.033–2.981) | .906 | 1.763 (1.038–2.981) |
| S4: PNPLA3 rs738409 | GG | 129 (35.6) | 30 (30.9) | .218 | 1.398 (0.820–2.385) | .251 | 1.372 (0.799–2.353) |
|      | CC | 123 (34.9) | 40 (41.2) | .855 | 1.055 (0.592–1.883) | .801 | 1.078 (0.601–1.934) |
|      | CC | 110 (30.4) | 27 (28.0) | .855 | 1.055 (0.592–1.883) | .801 | 1.078 (0.601–1.934) |
| S5: GCKR rs780094 | AA | 118 (32.6) | 30 (30.9) | .701 | 1.105 (0.664–1.838) | .766 | 1.081 (0.646–1.808) |
|      | AG | 178 (49.2) | 50 (51.5) | .969 | 1.013 (0.520–1.974) | .842 | 0.933 (0.475–1.834) |
|      | GG | 66 (18.2) | 17 (17.5) | .969 | 1.013 (0.520–1.974) | .842 | 0.933 (0.475–1.834) |

4. Discussion

Our present study aimed to investigate the possibility for the diagnosis of NAFLD by a new index CI among population older than 60 years, which was established by serum biochemical indexes and anthropometric parameters, meanwhile, we firstly suggested the diagnostic value of SNPs (S1: APOC3 rs2854116, S2: ABCA1 rs4149267, S3: LPL rs13702, S4: PNPLA3 rs738409, S5: GCKR rs780094) in NAFLD.

Based on the analysis of the related risk factors for NAFLD, we established the regression model by binary logistic regression to calculate the coefficient of CI, which contained 6 independent risk factors (weight, WC, BMI, AST/ALT, TG, FPG). Further analysis showed that the index CI conserved highest AUROC and YI in contrast to other 3 indexes FLI, SLI, and TyG, which were the noninvasive methods employed to the diagnosis of NAFLD presently. Although there had increasing researches suggested the role of SNP in the occurrence and development of NAFLD, there are only few studies had evaluated the value of the genotype in the validation group.

### Table 6

| The usefulness of the comprehensive index in validation group. | AUROC | P  | SE | Criterion | Sensitivity % | Specificity% | YI  |
|-------------------------------------------------------------|--------|----|----|-----------|---------------|-------------|-----|
| CI                                                          | 0.897  | .018 | .016 | >-1.4403  | 89.66         | 76.20       | 0.6586 |
| FLI                                                         | 0.873  | .020 | .021 | >-32.479  | 77.01         | 79.22       | 0.5623 |
| HS                                                         | 0.855  | .003 | .021 | >-3.4279  | 81.29         | 66.12       | 0.4841 |
| TyG                                                        | 0.793  | <.001 | .026 | >1.2804   | 82.29         | 66.12       | 0.4841 |
| CI+S1                                                      | 0.902  | .123 | .019 | >0.4305   | 93.10         | 66.27       | 0.5937 |
| CI+S2                                                      | 0.897  | .794 | .018 | >0.1261   | 94.25         | 69.88       | 0.6413 |
| CI+S3                                                      | 0.897  | .794 | .018 | >0.1432   | 91.95         | 73.80       | 0.6575 |
| CI+S4                                                      | 0.897  | .757 | .018 | >0.1579   | 89.66         | 75.60       | 0.6526 |
| CI+S5                                                      | 0.897  | .915 | .018 | >0.1618   | 89.66         | 76.20       | 0.6586 |

P < .05 was considered statistically significant.

**AUROC** = area under the ROC curve, **FLI** = fatty liver index, **HS** = hepatic steatosis index, **S1** = rs2854116 in apolipoprotein C3 (APOC3), **S2** = rs4149267 in ATP-binding cassette transporter (ABCA1), **S3** = rs13702 in lipoprotein lipase (LPL), **S4** = rs738409 in patatin-like phospholipase domain containing protein 3 (PNPLA3), **S5** = rs780094 in glucokinase regulatory protein gene (GCKR). TyG = product of fasting triglyceride and glucose levels, YI = Youden index.
detect NAFLD. According to our previous work, we introduced 5 SNPs that had a tighter relationship with NAFLD: the APOC3 rs2854116, ABCA1 rs4149267, LPL rs13702, PNPLA3 rs738409, and GCKR rs780094. In the regression model, the AUROC was higher than CI when the first SNP was combined with CI. All these results supported the potential value of CI or CI combined with APOC3 rs2854116 in the detection of NAFLD patients.

Published research has shown that >20% of NAFLD patients may develop cirrhosis during their lifetime, and 30% to 40% of the cirrhosis patients may suffer from liver-related mortality within a 10-year period. Furthermore, strong evidence suggests that the prevalence of NAFLD has paralleled with obesity, T2D, MetS, and the development and progression of cardiovascular disease. Therefore, the earlier detection of NAFLD patients seems to be important, not only in the management of NAFLD but also in pervasion the progression from cirrhosis to HCC, and its related complications.

Liver biopsy is considered to be the "criterion standard" for diagnosis of NAFLD, whereas the possibility of intraperitoneal bleeding and death (1 in 10,000) and much higher rates of pain and other discomfort limits its use. HRS, CT, and MRI are now employed to diagnose NAFLD, but those examination were often used for research purposes or for special or diagnosed patients. Ultrasound has been evaluated as a noninvasive method for the diagnosis of NAFLD presently. The result of a meta-analysis suggested that ultrasound is an accurate, reliable imaging technique for the detection of fatty liver. Comparing with liver biopsy, the overall sensitivity and specificity of ultrasound for the detection of moderate-severe fatty liver were 84.8% (95% confidence interval: 79.5%–88.9%) and 93.6% (87.2%–97.0%), respectively. However, it could be more economical and convenient in the diagnosis of NAFLD for large populations by the index CI presented in our study, which contained serum variables and anthropometric parameters, both of which were easy to determine, and the determination was cheaper, accessible and noninvasive, and there had studies demonstrated the potentiality of indexes composed of serum biomarkers in the diagnosis of NAFLD and NASH, such as FLI, HSI, and TyG. While the CI had higher AUROC and YI in contrast to FLI, HSI, and TyG. Although the FLI had higher sensitivity, it was easy to induce misdiagnosis because of its low specificity, and the HSI was easy to omit NAFLD patients because of its low sensitivity. Fortunately, the index CI showed higher level in both sensitivity and specificity. In view of the role of SNP in the pathogenesis of NAFLD, we combined the SNP with CI to diagnose NAFLD, what’s interesting was the better sensitivity and specificity of CI combing with APOC3 rs2854116, companing with the higher AUROC and YI, for the diagnosis of NAFLD in contrast to CI. Those data supported the potential value of the SNP combing with CI for screening NAFLD.

Nevertheless, our study also had limitations. Firstly, liver biopsy was not performed in the diagnosis of NAFLD instead of ultrasound, which also conserved better accuracy, reliability for the detection of fatty liver. Secondly, the index CI can only be used for the diagnosis of preliminary NAFLD, and cannot distinguish the simple fatty liver, hepatic steatosis, cirrhosis, or HCC. Thirdly, all participants included in our study were aged older than 60 years and recruited from the same city of China, and more research work are required to generalize our results to different ethnics and general population in future.

In conclusion, our results firstly investigated the possibility of the diagnostic value of a new index CI for NAFLD based on our present results, which was formulated by weight, WC, BMI, AST/ALT, TG, and FPG; furthermore, CI combing with SNP rs2854116 in APOC3 also showed the better diagnostic value than CI alone; thus, CI combing with rs2854116 in APOC3 might be a better method for the diagnosis of NAFLD in the future.
Author contributions
Conceptualization: X. Li.
Data curation: H. Yang, G. Chen, D. Li, Q. Ma, G. Chen.
Formal analysis: H. Yang, G. Chen, X. Li.
Funding acquisition: X. Li.
Investigation: G. Chen, C. Song, D. Li.
Methodology: H. Yang, C. Song, D. Li, G. Chen.
Project administration: Q. Ma, X. Li.
Resources: Q. Ma, X. Li.
Supervision: G. Chen, X. Li.
Validation: C. Song.
Visualization: C. Song.
Writing – original draft: H. Yang.
Writing – review & editing: X. Li.

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