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A novel index including SNPs for the screening of nonalcoholic fatty liver disease among elder Chinese

A population-based study

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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 ($P = .123$), 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, CIs = confidence interval, CT = computerized tomography, DBP = diastolic blood pressure, DNTP = deoxy-ribonucleoside triphosphate, EDTA = ethylene diamine tetraacetic 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

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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,[4] 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.[10–12] 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 lipid handling, insulin signaling, and oxidative stress, for example, the patatin-like phospholipase domain containing protein 3 (*PNPLA3*), glucokinase regulatory protein gene (*GCKR*), apolipoprotein C3 (*APOC3*), ATP-binding cassette transporter (*ABCA1*), lipids and lipoprotein lipase (*LPL*), peroxisome proliferator activated receptors α and γ (*-α and PPAR-γ*) etc.[13–18] Genome-wide association studies for NAFLD have identified some novel susceptibility genes, including *PNPLA3 rs738409*, and *GCKR rs780094*.[19,20] The effects of *PNPLA3* on NAFLD have been reported in numerous studies among people of different races.[21–23] 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.[24] 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.[25] And 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.[26] 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.[27]*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.[28]*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.[29,30] 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.

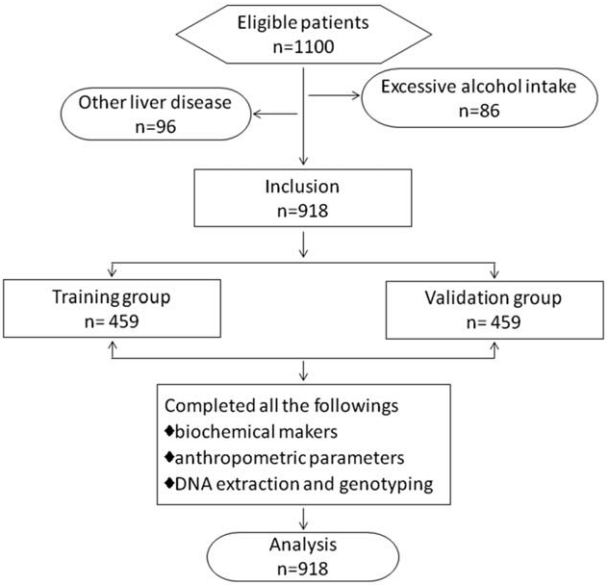

Figure 1. Flow chart for participants selection.

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".[31] Subjects conserved the following characteristics were excluded from the study: population with the history of alcohol abuse (ethanol intake >140 g/wk for men or >70 g/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 ≥126 mg/dL (about 7 mmol/L) according to the 2010 American Diabetes Association criteria.[32]

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.[11] 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-ethylenediaminetetraacetic 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 $MgCl_2$, 0.3 μL of deoxy-ribonucleoside triphosphate (Fermentas, Canada), 0.3 μL of Taq polymerase (Fermentas), 0.15 μL/strip 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/strip of Probe (10p), and 10 μL 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 sequencer (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) $\mathrm{FLI} = \frac{e^{0.953\times\log(\mathrm{TG})+0.139\times\mathrm{BMI}+0.718\times\log(\mathrm{GGT})+0.053\times\mathrm{WC}-15.745}}{1+e^{0.953\times\log(\mathrm{TG})+0.139\times\mathrm{BMI}+0.718\times\log(\mathrm{GGT})+0.053\times\mathrm{WC}-15.745}} \times 100$[12];

(2) $\mathrm{HSI} = 8 \times \frac{\mathrm{ALT}}{\mathrm{AST}} + \mathrm{BMI}(+2 \text{ if T2D}; +2 \text{ if female})$[33];

(3) $\mathrm{TyG} = \ln(\mathrm{TG} \times \mathrm{FPG}/2)$[34];

Table 2

Clinical characteristics of controls and non-alcoholic fatty liver disease patients.

Variables	Health group (n = 362)	NAFLD group (n = 97)	P
Men (n, %)	133 (84.2)	25 (15.8)	.043
Women (n, %)	229 (76.1)	72 (23.9)	.043
Age (yr)	72.53 ± 5.67	70.95 ± 4.73	.006
Height (cm)	154.82 ± 7.85	154.05 ± 7.07	.395
Weight (kg)	54.71 ± 9.46	61.30 ± 8.22	<.001
WC (cm)	84.94 ± 8.41	92.16 ± 6.60	<.001
HC (cm)	90.55 ± 6.21	95.20 ± 6.44	<.001
SBP (mm Hg)	143.63 ± 21.66	145.59 ± 19.55	.421
DBP (mm Hg)	77.78 ± 11.11	79.87 ± 10.66	.098
HR (per min)	75.84 ± 11.37	76.89 ± 11.72	.424
WHR			
<0.90	95 (94.1)	6 (5.9)	<.001
0.90∼	239 (75.2)	79 (24.8)	
≥1.05	28 (70.0)	12 (30.0)	
BMI (kg/m^2)			<.001
<24	232 (91.0)	23 (9.0)	
24∼	83 (61.9)	51 (38.1)	
≥28	17 (50.0)	17 (50.0)	
ALT (U/L)	16.39 ± 9.75	24.07 ± 14.35	<.001
AST(U/L)	23.31 ± 9.40	26.29 ± 13.51	.025
AST/ALT	1.58 ± 0.52	1.18 ± 0.33	<.001
TC (mmol/L)	4.71 ± 0.90	4.98 ± 0.94	.011
TG (mmol/L)	1.25 ± 0.56	2.03 ± 1.14	<.001
LDL-C (mmol/L)	2.69 ± 0.71	2.90 ± 0.75	.008
HDL-C (mmol/L)	1.48 ± 0.39	1.24 ± 0.27	<.001
FPG (grade)			<.001
<6.10 (%)	311 (82.9)	64 (17.1)	
6.10–7.00 (%)	29 (60.4)	19 (39.6)	
≥7.00 (%)	22 (61.1)	14 (38.9)	
UA (μmol/L)	305.46 ± 81.61	324.58 ± 72.36	.037

Data presented as Mean ± standard deviation (SD) or number of cases (%).Comparing with control group, $P < 0.05$ was considered statistically significant.

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, DBP = diastolic blood pressure, FPG = fasting blood glucose, HC = hip circumference, HDL-C = high-density lipoprotein cholesterol, HR = heart rate, LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride, UA = uric acid, WC = waist circumference, WHR = waist-to-hip ratio.

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,

Table 1

Primer sequence and restriction enzymes for the analysis of single nucleotide polymorphisms.

Gene	SNP	Polymorphism	Sense primer/antisense primer	Amplicon size (bp)	Primer	Sequences
APOC3	rs2854116	[A/G]	GTGAGGGGCTTCTTCAGACT/ AAACCCAGAGATGGAGGTGC	157bp	A699-S6-TR	TTCAGTAAATTGATTTGATCATTTCTTTTTTTTTTT
ABCA1	rs4149267	[A/G]	TGTGCAATTAGAGGAGCAAGG/ TCCTCCTGTAGCATTTTCCTG	211bp	A699-S7-TR	TCAAGAGTCATCAAAATAATTTTGA
LPL	rs13702	[A/G]	GCTCCATTTACACATCCACAC/ TGATTTGTTGTTGGCATCCCC	210bp	A699-S10-TA	TTTTTCTTTCCATCATATGTGTGGATGCA
PNPLA3	rs738409	[A/G]	TAGCAGAGAAAGCCGACTTAC/ TAACCTACTCTGTGCAAAGGG	214bp	A699-S6-TG	TTTTTTTTTTTTTTTTTTAACATCT AGTTCTTCCCTTTGCAGG
GCKR	rs780094	[A/G]	TCATTCCACTAAACCACAGGC/ TAGGCTTGTTGAGAACTCCTG	210bp	A699-S4-TG	AGACGGGAAAGGTTTTACATTTTGTTTTGTTTTTAC

ABCA1 = ATP-binding cassette transporter, APOC3 = apolipoprotein C3, *GCKR* = glucokinase regulatory protein gene, *LPL* = lipoprotein lipase, *PNPLA3* = patatin-like phospholipase domain containing protein 3, SNP = single nucleotide polymorphism.

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, $z = |\text{AUROC1} - \text{AUROC2}|/\text{sqrt}\ (\text{SE1}^2 + \text{SE2}^2)$. 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 β. Based on the results of multivariate logistic regression analysis, a formula to calculate CI was given as follows:

$$\begin{aligned}\text{CI} = &-0.063 \times \text{Weight} + 0.065 \times \text{WC} + 0.315 \times \text{BMI} \\ &- 2.165 \times \text{AST/ALT} + 0.935 \times \text{TG} + 0.276 \times \text{FPG} \\ &- 11.236\end{aligned}$$

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)	0.935	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.

Table 4

Genotypes distribution in healthy control and nonalcoholic fatty liver disease patients.

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)		1		1
AG	177 (48.9)	46 (47.4)	.667	0.900 (0.556–1.457)	.714	0.912 (0.558–1.492)
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)		1		1
AG	133 (36.7)	52 (53.6)	.006	1.907 (1.199–3.034)	.006	1.931 (1.206–3.092)
GG	29 (8.0)	4 (4.1)	.479	0.673 (0.224–2.017)	.536	0.705 (0.233–2.131)
S3: LPL rs13702						
AA	258 (71.3)	67 (69.1)		1		1
AG	82 (22.7)	29 (29.9)	.227	1.362 (0.825–2.249)	.181	1.414 (0.851–2.351)
GG	22 (6.1)	1 (1.0)	.091	0.175 (0.023–1.322)	.096	0.179 (0.023–1.359)
S4: PNPLA3 rs738409						
GG	129 (35.6)	30 (30.9)				
CG	123 (34.0)	40 (41.2)	.218	1.398 (0.820–2.385)	.251	1.372 (0.799–2.353)
CC	110 (30.4)	27 (27.8)	.855	1.055 (0.592–1.883)	.801	1.078 (0.601–1.934)
S5: GCKR rs780094						
AA	118 (32.6)	30 (30.9)		1		1
AG	178 (49.2)	50 (51.5)	.701	1.105 (0.664–1.838)	.766	1.081 (0.646–1.808)
GG	66 (18.2)	17 (17.5)	.969	1.013 (0.520–1.974)	.842	0.933 (0.475–1.834)

$P < .05$ was considered statistically significant.

ABCA1 = ATP-binding cassette transporter, Adjusted OR = adjusted for sex, age, APOC3 = apolipoprotein C3, *GCKR* = glucokinase regulatory protein gene, *LPL* = lipoprotein lipase, OR = odds ratio, *PNPLA3* = patatin-like phospholipase domain containing protein 3, SNP = single nucleotide polymorphisms.

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).

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*) 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

Table 5

The usefulness of the comprehensive index in validation group.

	AUROC	*P*	SE	Criterion	Sensitivity %	Specificity%	YI
CI	0.897		0.018	>-1.4403	89.66	76.20	0.6586
FLI	0.873	.123	0.019	>0.4305	93.10	66.27	0.5937
HIS	0.855	.003	0.021	>32.4479	77.01	79.22	0.5623
TyG	0.793	<.001	0.026	>1.2804	82.29	66.12	0.4841
CI+ *S1*	0.902	.182	0.018	>0.2253	86.21	82.23	0.6844
CI+ *S2*	0.897	.794	0.018	>0.1261	94.25	69.88	0.6413
CI+ *S3*	0.897	.794	0.018	>0.1432	91.95	73.80	0.6575
CI+ *S4*	0.897	.757	0.018	>0.1579	89.66	75.60	0.6526
CI+ *S5*	0.897	.915	0.018	>0.1618	89.66	76.20	0.6586

$P < .05$ was considered statistically significant.

AUROC = area under the ROC curve, CI = comprehensive index, FLI = fatty liver index, HSI = hepatic steatosis index, S1 = rs2854116 in apolipoprotein C3 (APOC3), S2 = rs4149267 in ATP-binding cassette transporter (*ABCA1*), S3 = rs13702 in lipids and lipoprotein lipase (*LPL*), S4 = rs738409 in patatin-like phospholipase domain containing protein 3 (*PNPLA3*), S5 = rs780094 in glucokinase regulatory protein gene (*GCKR*), TyG = the product of fasting triglyceride and glucose levels, YI = Youden index.

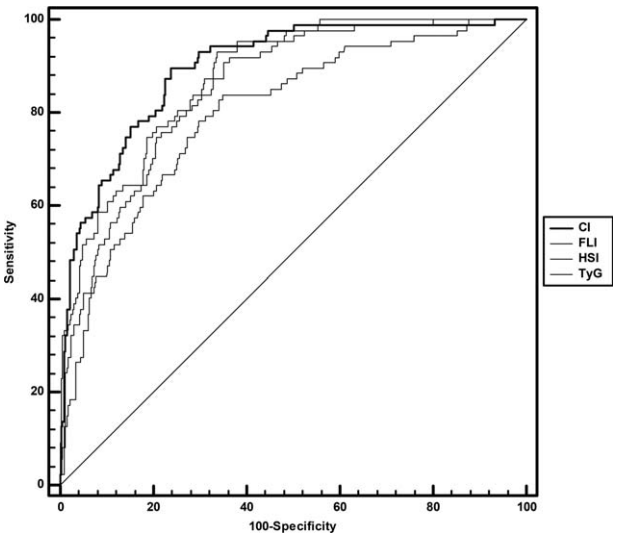

Figure 2. Comparing the area under the receiver-operating characteristic curves between the comprehensive index (CI) and the other 3 indexes to predict nonalcoholic fatty liver disease. The diagonal line represents detection achieved by chance alone (AUROC = 0.50), the ideal AUROC is 1.00. Receiver operating characteristic (ROC) curve analyses of CI, FLI, HSI, and TyG to predict NAFLD. The uppermost diagonal line was for CI. CI = comprehensive index, FLI = fatty liver index, HIS = hepatic steatosis index, TyG = product of fasting triglyceride and glucose levels.

detection of NAFLD. According to our previous work, we introduced 5 SNPs that had tighter relationship with NAFLD, the *APOC3 rs2854116*, *ABCA1 rs4149267*, *LPL rs13702*, *PNPLA3 rs738409*, and *GCKR rs780094* into the regression model, and the AUROC was higher than CI when the first SNP was combined with CI. All those results supported the potential value of CI, or CI combing with *APOC3 rs2854116* in the detection of NAFLD patients.

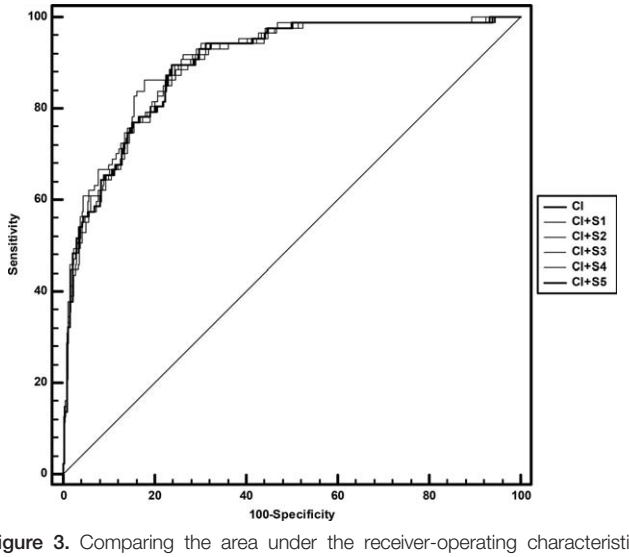

Figure 3. Comparing the area under the receiver-operating characteristic curves between the comprehensive index (CI) and "CI+*3S*" to predict nonalcoholic fatty liver disease. Receiver operating characteristic (ROC) curve analyses of comprehensive index (CI) and "CI+*3S*" (CI combined with 3 *SNPs rs2854116* in *APOC3*, *rs4149267* in *ABCA1*, *rs13702* in *LPL*) to predict NAFLD. The bold diagonal line was for CI.

Published research had 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.[35] Furthermore, strong evidence suggests that the prevalence of NAFLD has paralleled with obesity, T2D, MetS, and the development and progression of cardiovascular disease.[36] 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.[37] H MRS, 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.[38,39] The result of a meta-analysis suggested that ultrasound is an accurate, reliable imaging technique for the detection of fatty liver.[40] 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.[40,41] 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*, companying 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

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Supervision: G. Chen, X. Li.
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Visualization: C. Song.
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