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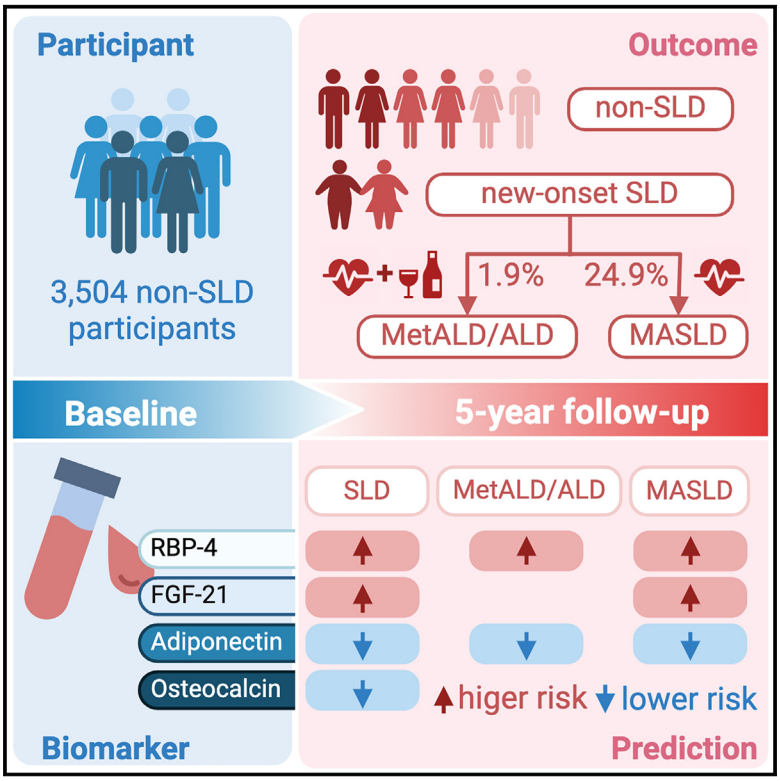
Associations between multiple metabolic biomarkers with steatotic liver disease subcategories: A 5-year Chinese cohort study

Graphical abstract



Highlights

- RBP-4, FGF-21, adiponectin, and osteocalcin enhance risk prediction for SLD
- Adiponectin shows the best predictive performance for SLD
- Elevated FGF-21 and RBP-4 emphasize metabolic and alcohol management, respectively

Authors

Hongli Chen, Siyu Chen, Dan Liu, ..., Li Wei, Xuhong Hou, Weiping Jia

Correspondence

houxuhong@sjtu.edu.cn (X.H.), wpjia@sjtu.edu.cn (W.J.)

In brief

Chen et al. find in a Chinese cohort study that four biomarkers—RBP-4, FGF-21, adiponectin, and osteocalcin—enhance the risk prediction for steatotic liver disease. Adiponectin shows the best predictive performance, while elevated RBP-4 and FGF-21 indicate the need to focus on alcohol intake control and metabolic management, respectively.

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Associations between multiple metabolic biomarkers with steatotic liver disease subcategories: A 5-year Chinese cohort study

Hongli Chen,[1,6] Siyu Chen,[2,6] Dan Liu,[1,6] Yebei Liang,[3] Huating Li,[1] Yuqian Bao,[1] Zhijun Zhu,[4] Keqing Dong,[4] Wen Li,[5] Liang Feng,[5] Di Cheng,[1] Fusong Jiang,[1] Li Wei,[1] Xuhong Hou,[1,*] and Weiping Jia[1,7,*]

[1]Department of Endocrinology and Metabolism, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghhai Clinical Center for Diabetes, Shanghai Key Clinical Center for Metabolic Disease, Shanghai 200233, China

[2]Department of Endocrinology and Metabolism, The Fourth Affiliated Hospital of Soochow University (Suzhou Dushu Lake Hospital), Suzhou 215000, China

[3]Department of Geriatrics, The First Affiliated Hospital of Fujian Medical University, Fuzhou 350005, China

[4]General Practitioner Teams, Community Health Service Center of Nicheng, Shanghai 201306, China

[5]Department of Ultrasound in Medicine, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200233, China

[6]These authors contributed equally

[7]Lead contact

*Correspondence: houxuhong@sjtu.edu.cn (X.H.), wpjia@sjtu.edu.cn (W.J.)

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SUMMARY

The effectiveness of established biomarkers for non-alcoholic fatty liver disease (NAFLD) within the updated framework of steatotic liver disease (SLD) remains uncertain. This cohort study examines the association of four metabolic biomarkers—retinol-binding protein 4 (RBP-4), fibroblast growth factor 21 (FGF-21), adiponectin, and osteocalcin—with SLD and its subtypes: metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction with alcohol-related liver disease (MetALD)/alcohol-related liver disease (ALD). Among 3,504 Chinese participants aged 55–70, 938 (26.8%) have developed SLD over 5 years, including 871 with MASLD and 67 with MetALD/ALD. The findings indicate that models incorporating RBP-4, FGF-21, adiponectin, and osteocalcin improve predictive accuracy for SLD beyond conventional models. Notably, adiponectin emerges as the most versatile marker, while elevated baseline levels of FGF-21 or RBP-4 indicate specific needs for metabolic or alcohol-related interventions, respectively, supporting tailored precision medicine strategies.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver conditions ranging from hepatic steatosis to steatohepatitis, fibrosis, and cirrhosis, affecting approximately one-quarter of adults worldwide.[1] Recently, the NAFLD nomenclature consensus group underscored the need for unified global nomenclature to improve disease management strategies and advance related research. Consequently, a new term—steatotic liver disease (SLD)—has been proposed. SLD serves as an umbrella term that includes the redefined NAFLD successor, metabolic dysfunction-associated steatotic liver disease (MASLD), as well as expanded classifications linked to NAFLD, specifically metabolic dysfunction and alcohol-related liver disease (MetALD) and alcohol-related liver disease (ALD).[2] Emerging studies indicate that different SLD subtypes exhibit distinct prognoses.[3–6] For example, the fibrosis[5] and cardiovascular disease[3,6] burden in the MetALD group was higher than that in the MASLD group. However, it remains unclear whether existing NAFLD biomarkers exhibit similar predictive performance for SLD with metabolic dysfunction and its subtypes, including MASLD, MetALD, and ALD.[2]

We previously reported that retinol-binding protein 4 (RBP-4),[7,8] fibroblast growth factor 21 (FGF-21),[9,10] adiponectin,[11] and osteocalcin[12–14] are effective biomarkers for multiple cardiometabolic diseases, including NAFLD, diabetes, and chronic kidney disease, based on findings from population-level studies. Similar outcomes have been reported by other researchers.[15–19] Moreover, in recent years, these biomarkers have been investigated as therapeutic targets in drug development, leading to a reduction in disease burden.[20–22] However, it remains unclear whether they are associated with novel SLD subcategories characterized by distinct etiologies, such as MASLD and MetALD/ALD.

To address this knowledge gap, we aimed to assess the heterogeneous roles, potential mediating roles, and additional predictive value of RBP-4, FGF-21, adiponectin, and osteocalcin beyond traditional predictive models in the development of SLD and its subcategories. This study was conducted using a

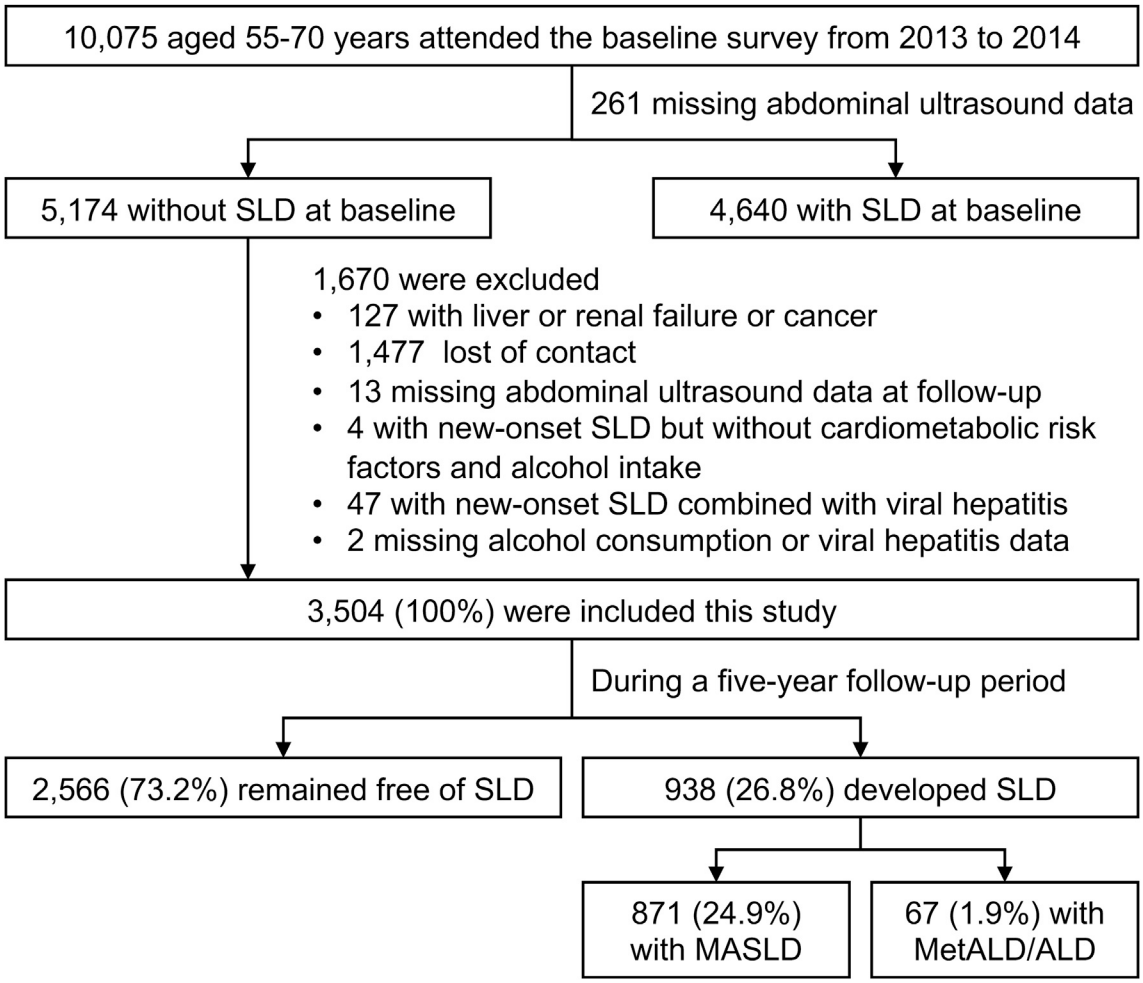


Figure 1. Flowchart of participants

SLD, steatotic liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, metabolic dysfunction and alcohol-associated/related liver disease; ALD, alcohol-associated/related liver disease.

large-scale, community-based cohort. These findings may contribute to the improved application of metabolic biomarkers for the early and accurate identification of individuals at risk for SLD, ultimately aiding in reducing the global disease burden.

RESULTS

Baseline characteristics of participants

A total of 3,504 Chinese participants (1,907 females) from the Shanghai Nicheng cohort study, with a median age of 61.7 years, were included in this analysis. During a 5-year follow-up, 938 participants (26.8%) developed SLD, comprising 871 cases of MASLD (24.9%) and 67 cases of MetALD/ALD (1.9%) (Figure 1). As shown in Table 1, participants with incident SLD were more likely to be female, obese, and diabetic, with higher levels of blood glucose, insulin resistance, and blood pressure, along with unfavorable lipid profiles, including elevated triglycerides (TGs) and reduced high-density lipoprotein cholesterol (HDL-C), compared to those without SLD (all $p \leq 0.032$). Besides, the liver function parameters of patients with new-onset SLD also differed significantly to non-SLD participants, with elevated alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) levels and decreased aspartate transaminase (AST) levels (all $p \leq 0.001$). Additionally, participants with incident SLD, MASLD, and MetALD/ALD exhibited significantly higher levels of RBP-4 and FGF-21 and lower levels of adiponectin compared to those without SLD (all $p \leq 0.016$).

Correlations between metabolic biomarkers and clinical characteristics

As shown in Figure 2, osteocalcin levels were inversely correlated with age in females, while adiponectin levels increased with age in both genders. Additionally, with increasing body mass index (BMI), levels of RBP-4 and FGF-21 significantly rose, whereas adiponectin and osteocalcin levels declined. After adjusting for age and BMI, higher RBP-4 and FGF-21 levels were associated with poorer metabolic profiles, while elevated adiponectin and osteocalcin levels correlated with a healthier metabolic status.

Table 1. Baseline characteristics

Characteristics	Total	No. SLD (1)	SLD					
			All SLD (2)	$p_{(1) \text{ vs. } (2)}$	MASLD (3)	MetALD/ALD (4)	$p_{(1) \text{ vs. } (3)}$	$p_{(1) \text{ vs. } (4)}$
n	3,504	2,566	938	–	871	67	–	–
Age (years)	61.7 (58.7–65.2)	61.6 (58.7–65.2)	61.9 (58.9–65.4)	0.120	61.9 (58.9–65.4)	61.3 (58.8–64.9)	0.118	0.788
Male	1,597 (45.6%)	1,198 (46.7%)	399 (42.5%)	0.032	334 (38.3%)	65 (97.0%)	<0.001	<0.001
Current smoker	806 (23.0%)	609 (23.7%)	197 (21.0%)	0.101	160 (18.4%)	37 (55.2%)	0.001	<0.001
Current drinker	544 (15.5%)	414 (16.1%)	130 (13.9%)	0.111	70 (8.0%)	60 (89.6%)	<0.001	<0.001
BMI (kg/m^2)	23.3 (21.6–25.0)	22.7 (21.1–24.3)	24.9 (23.5–26.7)	<0.001	24.9 (23.6–26.6)	24.9 (23.2–26.8)	<0.001	<0.001
WC (cm)	80.0 (75.0–86.0)	79.0 (74.0–85.0)	85.0 (80.0–90.0)	<0.001	85.0 (80.0–90.0)	87.0 (81.2–92.0)	<0.001	<0.001
FPG (mmol/L)	5.8 (5.4–6.3)	5.8 (5.4–6.2)	5.9 (5.5–6.4)	<0.001	5.9 (5.5–6.4)	6.0 (5.6–6.5)	<0.001	0.002
2hPG (mmol/L)	7.3 (6.2–9.0)	7.2 (6.0–8.8)	7.8 (6.6–9.7)	<0.001	7.8 (6.6–9.7)	7.3 (6.2–9.1)	<0.001	0.639
HbA1c (%)	5.6 (5.4–5.9)	5.6 (5.3–5.8)	5.7 (5.4–6.0)	<0.001	5.7 (5.5–6.0)	5.6 (5.3–5.9)	<0.001	0.668
FINS (μU/mL)	5.4 (4.0–7.4)	5.1 (3.7–6.8)	6.7 (4.9–9.0)	<0.001	6.8 (5.0–9.1)	5.4 (4.0–6.9)	<0.001	0.387
HOMA-IR	1.4 (1.0–2.0)	1.3 (0.9–1.8)	1.8 (1.3–2.4)	<0.001	1.8 (1.3–2.4)	1.4 (1.1–1.8)	<0.001	0.142
Diabetes	558 (15.9%)	377 (14.7%)	181 (19.3%)	0.001	174 (20.0%)	7 (10.4%)	<0.001	0.424
SBP (mmHg)	130.0 (121.0–141.0)	130.0 (120.0–140.0)	132.5 (125.0–143.0)	<0.001	132.5 (125.0–143.0)	133.0 (125.5–145.5)	<0.001	0.026
DBP (mmHg)	81.0 (78.0–87.0)	81.0 (78.0–86.0)	82.0 (79.0–88.0)	<0.001	82.0 (79.0–88.0)	82.0 (80.0–90.8)	<0.001	0.010
TGs (mmol/L)	1.1 (0.8–1.5)	1.0 (0.8–1.4)	1.3 (0.9–1.8)	<0.001	1.3 (0.9–1.8)	1.2 (0.9–1.6)	<0.001	0.063
HDL-C (mmol/L)	1.4 (1.2–1.6)	1.4 (1.2–1.7)	1.3 (1.1–1.5)	<0.001	1.3 (1.1–1.5)	1.4 (1.2–1.6)	<0.001	0.378
ALT (U/L)	15.0 (12.0–19.0)	15.0 (12.0–19.0)	15.0 (12.0–20.0)	0.001	15.0 (12.0–20.0)	16.0 (12.0–19.5)	0.001	0.327
AST (U/L)	22.0 (19.0–26.0)	22.0 (19.0–26.0)	21.0 (19.0–25.0)	<0.001	21.0 (19.0–25.0)	22.0 (20.0–27.0)	<0.001	0.698
GGT (U/L)	20.0 (15.0–28.0)	19.0 (15.0–27.0)	21.0 (16.0–31.0)	<0.001	21.0 (16.0–30.0)	28.0 (21.0–39.0)	<0.001	<0.001
RBP-4 (mg/L)	49.0 (40.0–61.0)	48.0 (39.0–60.0)	52.0 (42.0–63.0)	<0.001	51.0 (42.0–62.5)	62.0 (49.5–73.0)	<0.001	<0.001
FGF-21 (pg/mL)	200.2 (119.0–310.4)	185.2 (110.0–294.7)	239.0 (150.9–354.4)	<0.001	239.6 (150.4–354.1)	225.4 (151.5–364.8)	<0.001	0.016
Adiponectin (μg/mL)	4.6 (3.5–6.0)	4.8 (3.7–6.3)	4.1 (3.2–5.3)	<0.001	4.2 (3.2–5.3)	3.5 (3.0–4.3)	<0.001	<0.001
Osteocalcin (ng/mL)	22.1 (17.4–28.3)	22.3 (17.6–28.5)	21.4 (16.8–27.9)	0.004	21.8 (17.2–28.3)	16.9 (14.1–21.2)	0.108	<0.001

Data are reported as medians (25th–75th percentiles) or frequencies (percentages). Participants were defined as current drinkers if their average alcohol intake exceeded 1 g per week for at least 1 year prior to the survey, and as current smokers if their average cigarette consumption exceeded 1 cigarette per day for at least 1 year prior to the survey.

SLD, steatotic liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, metabolic dysfunction and alcohol-associated/related liver disease; ALD, alcohol-associated/related liver disease; BMI, body mass index; WC, waist circumference; FPG, fasting plasma glucose; 2hPG, 2-h postprandial plasma glucose; HbA1c, glycated hemoglobin; FINS, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TGs, triglycerides; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, γ-glutamyltransferase; RBP-4, retinol-binding protein 4; FGF-21, fibroblast growth factor 21.

Specifically, RBP-4 levels were positively associated with fasting plasma glucose (FPG), the homeostasis model assessment of insulin resistance (HOMA-IR), blood pressure, TGs, HDL-C, and GGT in both genders. FGF-21 levels were positively associated with TGs and GGT and inversely associated with HDL-C. Adiponectin was inversely correlated with waist circumference (WC), FPG, 2-h postprandial plasma glucose (2hPG), HbA1c, fasting insulin (FINS), HOMA-IR, TGs, and GGT, but positively associated with HDL-C and AST. Osteocalcin levels were inversely associated with FPG, 2hPG, HbA1c, HOMA-IR, ALT, and GGT.

Associations between metabolic biomarkers and SLD

The tertile ranges of metabolic biomarkers are listed in Table S1. As shown in Figure 3A, cumulative SLD incidence increased significantly in both males and females, alongside rising RBP-4 and FGF-21 levels and declining adiponectin and osteocalcin levels (all *p for trend* $\leq$ 0.005). To determine if baseline biomarker levels were independently associated with SLD risk and to quantify this association, we developed a multivariable modified Poisson regression model. This model estimates relative risks (RRs) and its 95% confidence intervals (CIs) for the association between biomarker levels and SLD development. As illustrated in Figure 3B, after adjusting for covariables including age, BMI, WC, 2hPG, HbA1c, FINS, systolic blood pressure (SBP), TGs, HDL-C, ALT, AST, and GGT, SLD risk increased significantly with higher RBP-4 levels (per-standard deviation increment RR [95% CI]: 1.17 [1.07–1.27], $p < 0.001$ for males; 1.20 [1.12–1.29], $p < 0.001$ for females) and decreased with higher adiponectin levels (RR [95% CI]: 0.79 [0.73–0.86], $p < 0.001$ for males; 0.87 [0.80–0.93], $p < 0.001$ for females). Additionally, in males,

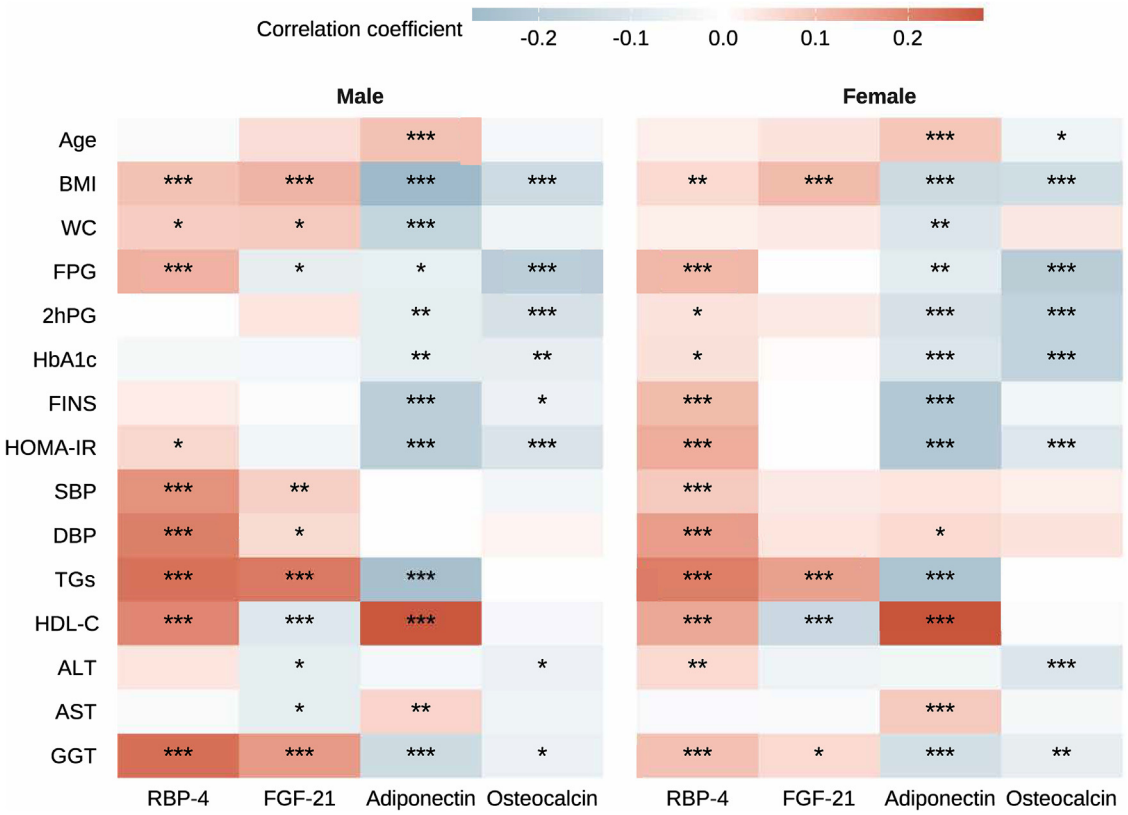


Figure 2. Correlations between metabolic biomarkers and clinical characteristics

The standardized correlation coefficients between metabolic biomarkers and clinical characteristics were calculated by using linear regression. WC, FPG, 2hPG, HbA1c, FINS, HOMA-IR, SBP, DBP, TGs, HDL-C, ALT, AST, and GGT were log-transformed prior to analysis. Partial correlation coefficients between biomarkers with FPG, 2hPG, HbA1c, FINS, HOMA-IR, SBP, DBP, TGs, HDL-C, ALT, AST, and GGT calculated after adjusting for age and BMI. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. BMI, body mass index; WC, waist circumference; FPG, fasting plasma glucose; 2hPG, 2-h postprandial plasma glucose; HbA1c, glycated hemoglobin; FINS, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TGs, triglycerides; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, γ-glutamyltransferase; RBP-4, retinol-binding protein 4; FGF-21, fibroblast growth factor 21.

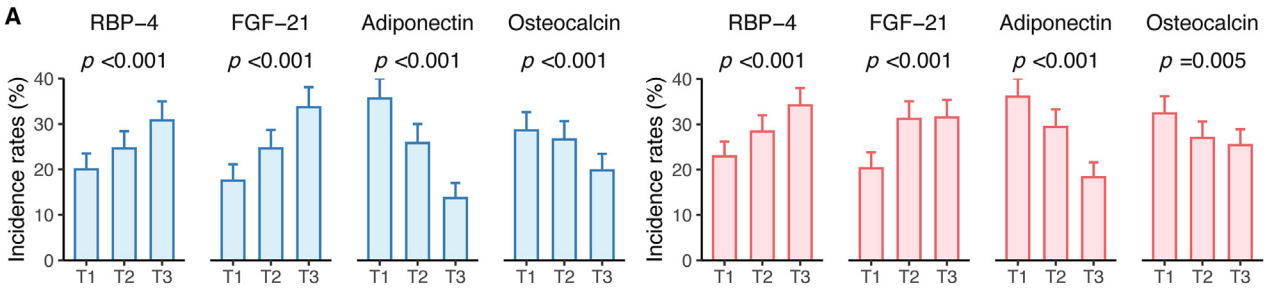
FGF-21 levels were significantly associated with increased SLD risk (RR [95% CI]: 1.15 [1.00–1.32], $p = 0.047$). A sensitivity analysis in participants with complete biomarker data ($n = 3,140$, Figure S2A) yielded consistent results.

Associations between metabolic biomarkers and SLD subcategories

As shown in Figure 4A, the proportions of patients with MASLD and MetALD/ALD varied significantly with levels of RBP-4 ($p = 0.002$). However, the proportions of patients remained stable across the tertiles of FGF-21, adiponectin, and osteocalcin levels in males (all $p \geq 0.689$). Multivariate Poisson regression analyses revealed that, after adjusting for age, BMI, WC, 2hPG, HbA1c, FINS, SBP, TGs, HDL-C, ALT, AST, and GGT, the risk of MASLD and MetALD/ALD significantly increased with higher RBP-4 levels. The relative risks (RRs) for a per-standard deviation increment were 1.12 (95% CI: 1.02–1.23, $p = 0.015$) for MASLD and 1.64 (95% CI: 1.29–2.08, $p < 0.001$) for MetALD/ALD. Furthermore, heterogeneity analyses indicated that the association between RBP-4 levels and MetALD/ALD was stronger than that with MASLD (*p for heterogeneity* = 0.004). Additionally, higher adiponectin levels were negatively associated with the risk of both MASLD and MetALD/ALD, with RRs of 0.81 (95% CI: 0.73–0.89, $p < 0.001$) and 0.65 (95% CI: 0.50–0.83, $p < 0.001$), respectively. Moreover, a positive association was observed between FGF-21 levels and the risk of MASLD, with an RR of 1.20 (95% CI: 1.03–1.40, $p = 0.020$) (Figure 4B). Sensitivity analysis, excluding participants with missing data for any biomarkers, yielded similar results (Figure S2B).

Predictive efficiency of metabolic biomarkers

As shown in Figure 5A, the area under the curve (AUC), net reclassification index (NRI), and integrated discrimination improvement (IDI) demonstrated that, for predicting the development of SLD, baseline adiponectin levels outperformed other biomarkers, as indicated by positive delta AUC, NRI, and/or IDI values. In predicting MASLD, both adiponectin and FGF-21 exhibited better performance compared to baseline levels of RBP-4 and osteocalcin. The optimal cutoff values for diagnosing MASLD were 3.68 μg/mL for males and 5.11 μg/mL for females for adiponectin and 238.44 pg/mL for males and 186.69 pg/mL for females for FGF-21. Furthermore, in males, adiponectin and



B

Biomarkers	Gender	No. of event	RR (95% CI)	p
RBP–4	Male (n=1597)	399	1.17 (1.07–1.27)	<0.001
	Female (n=1970)	539	1.20 (1.12–1.29)	<0.001
FGF–21	Male (n=1425)	360	1.15 (1.00–1.32)	0.047
	Female (n=1717)	475	1.09 (0.97–1.22)	0.140
Adiponectin	Male (n=1450)	364	0.79 (0.73–0.86)	<0.001
	Female (n=1742)	487	0.87 (0.80–0.93)	<0.001
Osteocalcin	Male (n=1596)	399	0.96 (0.89–1.04)	0.347
	Female (n=1906)	539	0.96 (0.90–1.02)	0.202

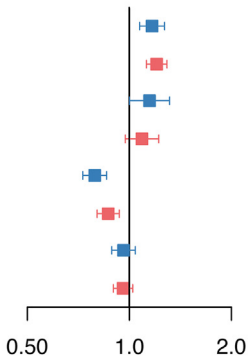


Figure 3. Associations between biomarkers and new-onset SLD

(A) Incidence rate of SLD across tertiles of biomarkers. Data are represented as rate (95% CI).

(B) The RRs for new-onset SLD were calculated after adjusting for age, BMI, WC, 2hPG, HbA1c, FINS, SBP, TGs, HDL-C, ALT, AST, and GGT by using modified Poisson regression models. Data are represented as RR (95% CI). See also Tables S1 and S2; Figures S1 and S2. SLD, steatotic liver disease; RBP-4, retinol-binding protein 4; FGF-21, fibroblast growth factor 21; T1–3, tertiles 1 to 3; RR, relative risk; CI, confidence interval.

RBP-4 showed superior predictive performance for MetALD/ALD compared to FGF-21 and osteocalcin, with optimal cutoff values of 3.62 μg/mL and 59.00 mg/L, respectively (Figure S3).

Additionally, we developed biomarker-based models by combining these biomarkers with BMI and WC, which are simple and non-invasive indicators commonly used in traditional SLD prediction models. As illustrated in Figures 5B and 5C, models utilizing each of the four biomarkers significantly improved the prediction of SLD, MASLD, and MetALD/ALD compared to traditional SLD prediction models, including the fatty liver index, hepatic steatosis index, and NAFLD-liver fat score. Moreover, predictive models that combined multiple biomarkers further enhanced the predictive performance compared to any single biomarker model (Table S3).

Mediative roles of HOMA-IR and TGs in the associations between metabolic biomarkers and MASLD

As shown in Figure 6, structural equation modeling analyses indicated that potential mediators, HOMA-IR and TGs, were positively associated with the development of MASLD. The standardized path coefficients were 0.16 and 0.10 for males and 0.19 and 0.07 for females, respectively (all $p < 0.01$).

Furthermore, HOMA-IR levels were significantly correlated with the baseline levels of RBP-4, adiponectin, and osteocalcin, exhibiting path coefficients of 0.08, −0.27, and −0.09 in males and 0.12, −0.25, and −0.08 in females (all $p < 0.01$), respectively. Similarly, TG levels were significantly correlated with the baseline levels of RBP-4, FGF-21, and adiponectin, with path coefficients of 0.18, 0.23, and −0.28 in males and 0.20, 0.20, and −0.22 in females (all $p < 0.001$), respectively.

Thus, based on the mediation analysis hypothesis, HOMA-IR accounted for 30.8%, 21.3%, and 33.4% of the effects of RBP-4, adiponectin, and osteocalcin on MASLD in males, respectively. In females, HOMA-IR mediated 20.9%, 32.1%, and 23.9% of the effects of RBP-4, adiponectin, and osteocalcin, respectively. Similarly, TGs mediated 42.9%, 24.4%, and 13.1% of the effects of RBP-4, FGF-21, and adiponectin on MASLD in males and 12.6%, 13.7%, and 10.0% of these effects in females, respectively.

DISCUSSION

This study evaluates the performance of various metabolic biomarkers in predicting SLD and its subcategories and explores the potential mediators involved in their associations. Our findings indicate that RBP-4, FGF-21, and adiponectin can independently predict the development of SLD and MASLD, with their associations partially mediated by HOMA-IR and TGs. Furthermore, all models based on metabolic biomarkers show significant improvements in predicting SLD and its subcategories

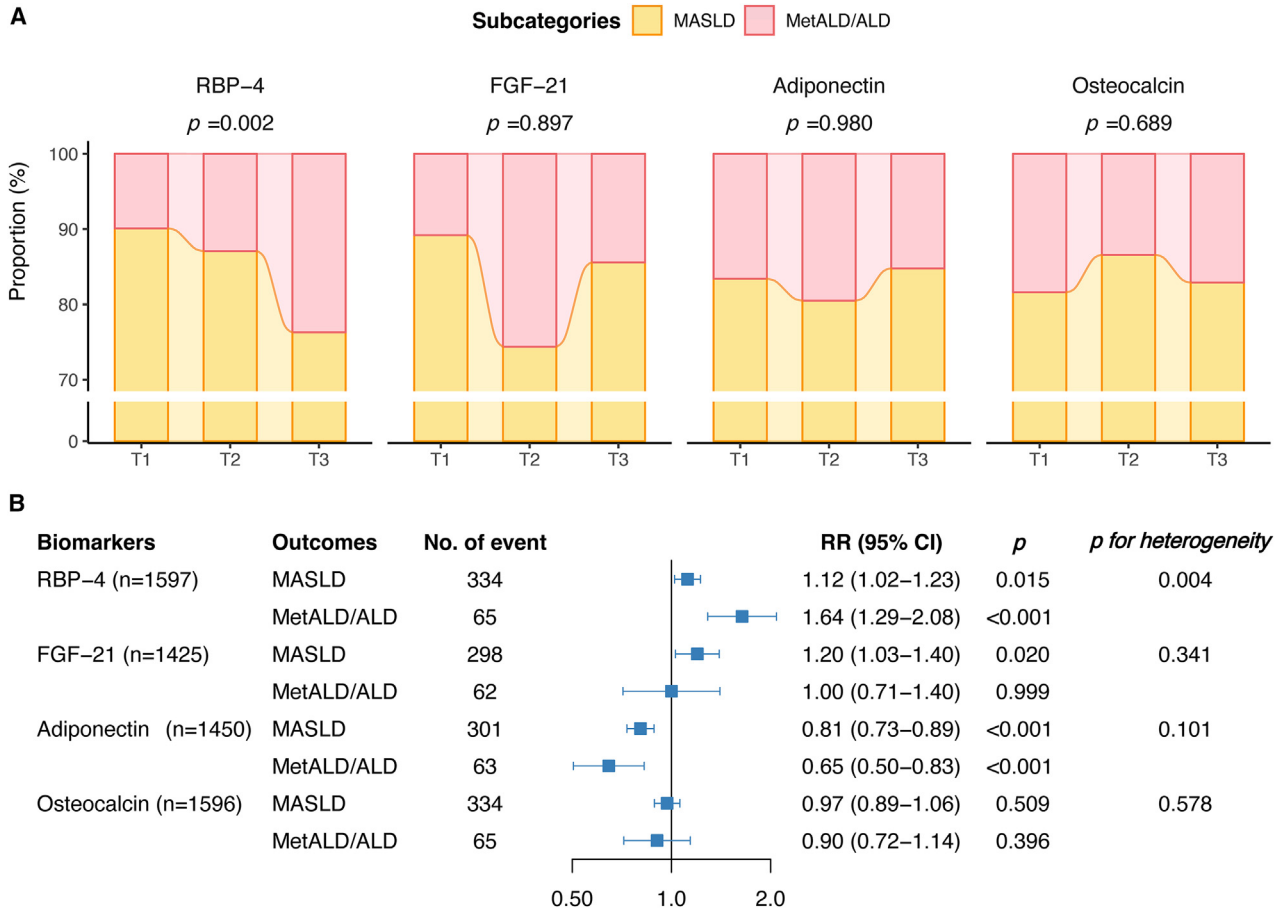


Figure 4. Associations between biomarkers and new-onset SLD subcategories in males

(A) The proportion of patients with MASLD and MetALD/ALD in all SLD patients.

(B) The RRs for new-onset SLD subcategories were calculated after adjusting for age, BMI, WC, 2hPG, HbA1c, FINS, SBP, TGs, HDL-C, ALT, AST, and GGT by using modified Poisson regression models. Data are represented as RR (95% CI). Heterogeneity between the RRs of biomarkers for different subcategories of SLD was examined using the *Q* test. See also Tables S1 and S2; Figures S1 and S2. SLD, steatotic liver disease; RBP-4, retinol-binding protein 4; FGF-21, fibroblast growth factor 21; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, metabolic dysfunction and alcohol-associated/related liver disease; ALD, alcohol-associated/related liver disease; RR, relative risk; CI, confidence interval.

compared to traditional predictive models. Among these biomarkers, adiponectin emerges as a robust predictor across all SLD subcategories, suggesting its potential as a primary biomarker for SLD. Additionally, FGF-21 is proved more suitable for predicting MASLD, while RBP-4 demonstrates superior performance in predicting MetALD/ALD compared to other biomarkers. These findings provide real-world evidence for the application of metabolic biomarkers in SLD screening and monitoring.

Although the term "NAFLD" is widely used, it has long been recognized that "non-alcoholic" fails to accurately reflect the disease's etiology. Notably, individuals with risk factors for NAFLD, such as excessive alcohol consumption, have historically been overlooked by existing nomenclature and excluded from clinical trials and therapeutic discussions.[2,23,24] It is now understood that overlapping biological processes may contribute to both NAFLD and ALD.[25] All of these factors drove a redefinition of NAFLD. In 2020, Eslam et al. proposed the term "metabolic dysfunction-associated fatty liver disease (MAFLD)" as a more appropriate overarching term.[24] Based on this, Rinella et al. introduced the updated nomenclature of MASLD and MetALD.[2] While these new terms avoid stigmatizing language such as "non-alcoholic" and "fatty" and include individuals previously excluded due to excessive alcohol consumption and other combined etiologies, a particular concern remains regarding the validity of established NAFLD biomarkers for these new terms.[2,24,26]

RBP-4 is a specific transport protein for retinol in circulation, mainly secreted by the liver and adipose tissue and excreted through the kidneys.[27,28] In 2008, we investigated the association between serum RBP-4 levels and NAFLD in 102 diabetic patients, finding that NAFLD patients had significantly higher levels of RBP-4 than those without NAFLD, even after adjusting for confounders.[7] These findings were consistent with results from other studies.[16–18,29] In the present study, we found that baseline RBP-4 is an independent predictor of MASLD. Interestingly, the

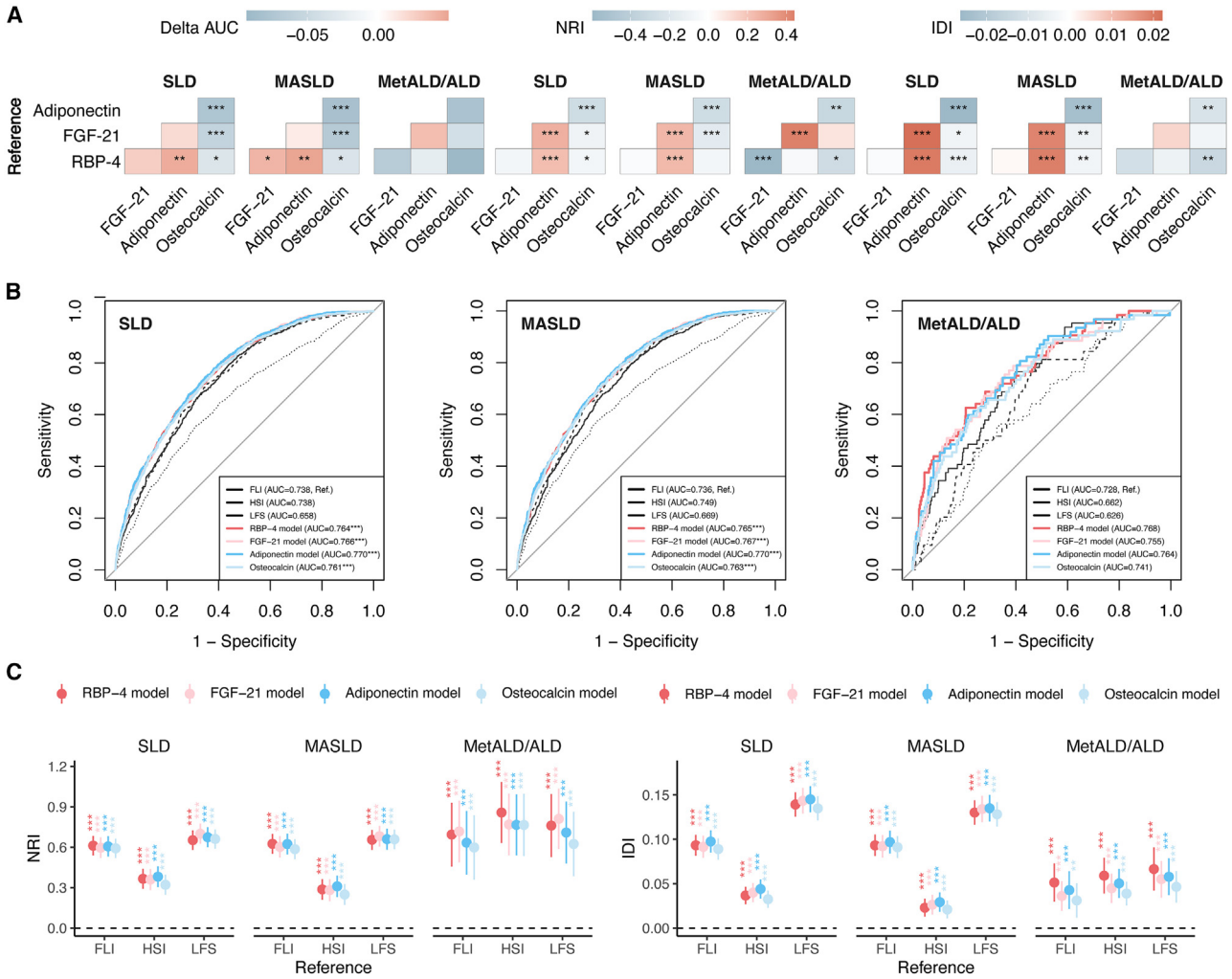


Figure 5. Predictive performance of biomarker-based models

The predictive performance of each of the four biomarkers was evaluated by comparing the differences in their AUC (delta AUC), NRI, and IDI (A). Delta AUC, NRI, and IDI > 0, = 0, or < 0 indicate the biomarkers had a better, similar, or worse performance than the reference in predicting the development of SLD, MASLD, or MetALD/ALD, respectively. Further, the RBP-4/FGF-21/adiponectin/osteocalcin models were constructed by including the RBP-4/FGF-21/adiponectin/osteocalcin combined with body mass index and waist circumference. The predictive performance of these biomarker-based models compared with traditional SLD predictive models, including FLI, HSI, and LFS, was evaluated by AUC (B), NRI (C), and IDI (C). Data are represented as NRI (95% CI) and IDI (95% CI) (C). Predictive performance for MetALD/ALD was evaluated exclusively in males due to the limited case number in females. See also Figure S3. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. AUC, area under the curve; NRI, net reclassification index; IDI, integrated discrimination improvement; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, metabolic dysfunction and alcohol-associated/related liver disease; ALD, alcohol-associated/related liver disease; RBP-4, retinol-binding protein 4; FGF-21, fibroblast growth factor 21; FLI, fatty liver index; HSI, hepatic steatosis index; LFS, liver fat score; CI, confidence interval.

predictive role of circulating RBP-4 was even stronger for MetALD/ALD than for MASLD. This may be due to RBP-4's dual roles as both an adipokine and a hepatokine.[30,31] On one hand, *in vivo* and *in vitro* studies suggest that fat-derived RBP-4 contributes to MASLD development by increasing insulin resistance, a shared trigger for both MASLD and MetALD. RBP-4 promotes insulin resistance through multiple pathways,[28,32–39] such as promoting basal glucose production, reducing insulin-induced suppression of glucose production in hepatocytes, inhibiting the phosphorylation of the insulin receptor substrate 1, and impairing insulin signaling in adipocytes through the activation of macrophages and induction of proinflammatory cytokine production.[35] Our mediation analysis indicated that over one-third of RBP-4's effect on MASLD operates through increased insulin resistance. On the other hand, the liver is the main site of RBP-4 synthesis, and alcohol consumption might stimulate liver hyperfunction, leading to elevated RBP-4 levels.[40]

FGF-21 was initially identified as a liver-expressed fibroblast growth factor and has recently been recognized as an endocrine hormone regulating insulin sensitivity, energy expenditure, and hepatic TGs.[41] In our previous studies involving 808 Chinese adults, FGF-21 was identified as a predictor of NAFLD. Results

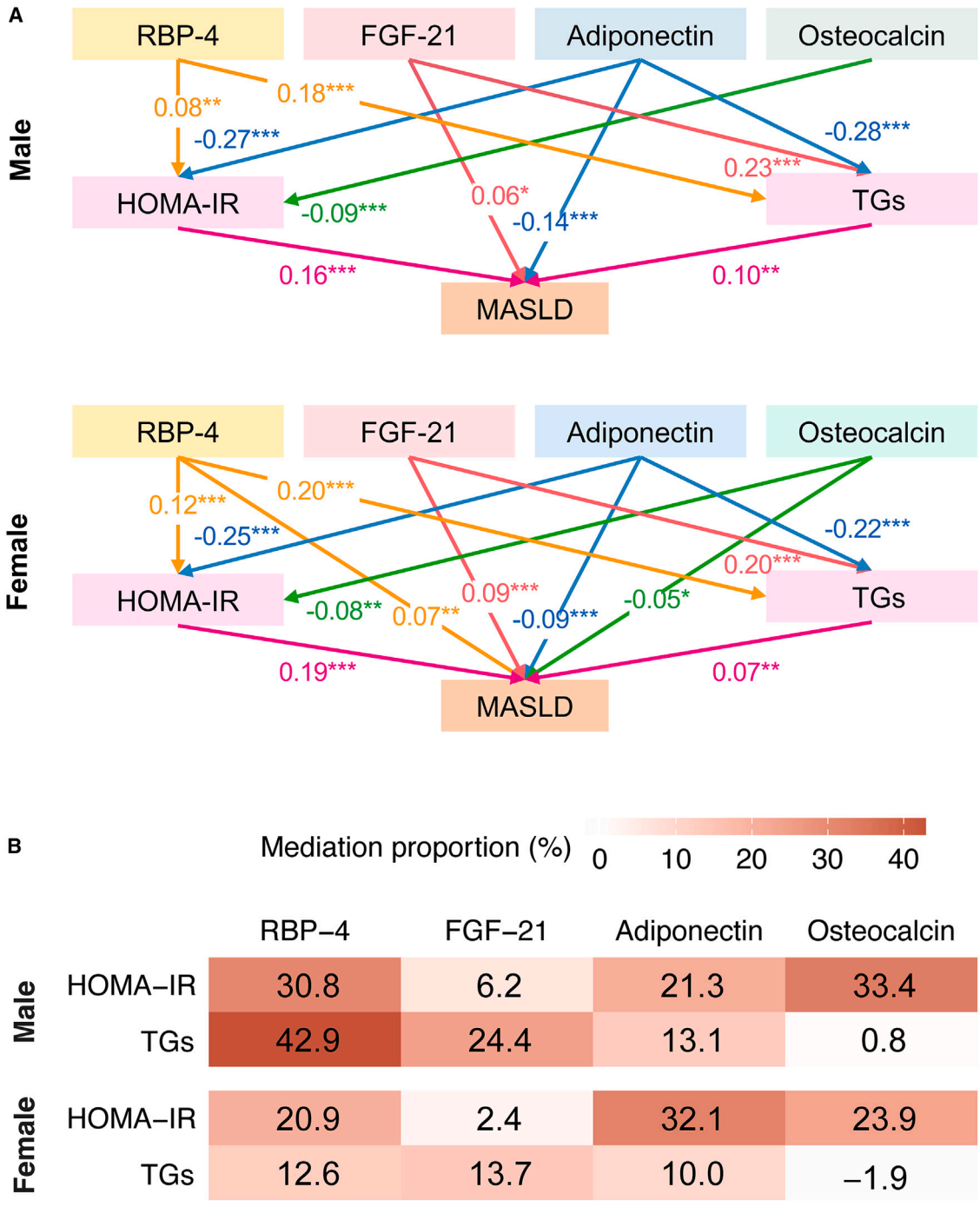


Figure 6. Mediation analyses for MASLD

(A) The structural equation model with significant standardized path coefficients. Coefficients were calculated with adjustments for age. Potential mediators that are significantly associated with both biomarker and outcome were considered to mediate the role of the biomarker in the development of outcome.

(B) The mediation proportion of HOMA-IR and TGs was calculated using the following formula: 100% × (coefficient$_{\text{biomarkers-mediator}}$ × coefficient$_{\text{mediator-outcome}}$)/(coefficient$_{\text{biomarkers-outcome}}$ + coefficient$_{\text{biomarkers-mediator}}$ × coefficient$_{\text{mediator-outcome}}$). ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. MASLD, metabolic dysfunction-associated steatotic liver disease; RBP-4, retinol-binding protein 4; FGF-21, fibroblast growth factor 21; HOMA-IR, homeostasis model assessment of insulin resistance; TGs, triglycerides.

indicated that higher baseline levels of FGF-21 were significantly associated with an increased risk of NAFLD over a 3-year follow-up period.[9] Pharmacologically, FGF-21 or its analogs promote lipid-lowering effects by speeding up lipoprotein catabolism, suppressing postprandial lipolysis in adipose tissues, reducing hepatic endoplasmic reticulum stress and oxidative stress, and enhancing lipid beta-oxidation in the liver.[41] For instance, pegozafermin, an analog of FGF-21, is shown to reduce hepatic fibrosis in patients with non-alcoholic steatohepatitis in a phase 2b, multicenter, double-blind, 24-week randomized placebo-controlled trial.[21] Although both FGF-21 and RBP-4 are primarily secreted by the liver and adipose tissue,[42,43] our study finds no significant heterogeneity in FGF-21's role as a predictor for MASLD and MetALD/ALD. These findings suggest that FGF-21 could serve as a specific biomarker for MASLD, a condition primarily driven by metabolic disorders rather than mixed etiologies, such as excessive alcohol consumption.

In contrast to RBP-4 and FGF-21 findings, our study suggests that adiponectin is significantly negatively associated with MASLD and MetALD/ALD development. Adiponectin, a 244-amino acid adipokine, acts as an insulin sensitizer, modulating glucose uptake and lipid metabolism by reducing gluconeogenesis and enhancing glycolysis and fatty acid oxidation in the liver through the AMPK pathway and PPAR-α cascade.[44] Our results indicate that approximately a quarter of adiponectin's effect on MASLD is mediated by HOMA-IR, with about a tenth mediated by TGs. Previous studies have shown adiponectin's beneficial role in NAFLD,[45] and receptor agonists like AdipoRon and ADP355 have shown efficacy in ameliorating NAFLD and non-alcoholic steatohepatitis *in vitro* and *in vivo*.[20] These findings affirm that adiponectin is a reliable predictor not only for MASLD but also for SLD involving alcohol consumption.

Additionally, research on bone-liver crosstalk has opened up new perspectives on NAFLD biomarkers.[46] Among these, osteocalcin, a 49-amino acid bone matrix protein secreted by osteoblasts, has been found significantly and negatively associated with NAFLD development.[47,48] In 2015, we reported an inverse correlation between circulating osteocalcin and NAFLD among 733 postmenopausal Chinese women,[14] consistent with a 4.2-year prospective study of 2,055 participants.[19] Our current study further demonstrates that baseline osteocalcin levels are significantly negatively associated with the incidence rate of MASLD.

Limitations of the study

This study has several limitations. First, ultrasonography was used to detect fatty liver, which, though commonly employed in large-scale population studies due to its acceptable accuracy, is not sensitive enough to detect low-level steatosis (i.e., <20%). More advanced imaging techniques, such as MRI-based fat quantification, could enhance sensitivity in future studies. Second, the limited sample sizes for MetALD and ALD groups necessitated their combination, which restricted our ability to explore differences between these two conditions. Future research should aim to include larger cohorts of each subgroup to better assess potential heterogeneity. Third, the study population consisted solely of middle-aged and elderly Chinese individuals. This demographic limitation may reduce the generalizability of our findings to other age groups or racial/ethnic groups. Replicating the study across diverse populations would help determine the broader applicability of our conclusions. Fourth, while we made efforts to control confounding variables, residual confounding factors, such as genetic predispositions, medication effects, and lifestyle variations, may still influence the results. Further stratified analyses or genetic profiling could help clarify these impacts. Lastly, the conclusions drawn here would benefit from additional validation studies. Although our findings provide initial insights, further longitudinal studies and experimental validations are necessary to confirm these associations and establish causality.

Conclusions

In conclusion, metabolic biomarkers, including RBP-4, FGF-21, adiponectin, and osteocalcin, are applicable for predicting the occurrence of SLD and its subcategories with additional value beyond the traditional model, and they are involved in the early stages of the disease, such as insulin resistance and lipid metabolism. Moreover, among the four candidates, adiponectin is a robust predictor across all SLD subcategories. Based on this, elevated baseline levels of FGF-21 or RBP-4 emphasize the importance of metabolic control or alcohol restriction in their prevention strategies from a precision medicine perspective, respectively.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Weiping Jia (wpjia@sjtu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The export of human-related data is governed by the Ministry of Science and Technology of China (MOST) and must adhere to the Regulations of the People's Republic of China on Administration of Human Genetic Resources (State Council No. 717). This dataset is intended solely for non-profit academic research purposes. Requests for data access should be sent via email to the corresponding author, Weiping Jia, and will receive a response within 10 business days. Upon initial agreement, the data requester, in conjunction with the corresponding author, must submit a joint application for data sharing to MOST. Upon approval from MOST, the data will then be provided to the requester.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.C., S.C., X.H., and W.J.; methodology, H.C., S.C., Y.L., and W.J.; investigation, D.L., H.L., Y.B., Z.Z., K.D., W.L., L.F., D.C., F.J., and L.W.; writing – original draft, H.C. and S.C.; writing – review and editing, H.C., S.C., and W.J.; funding acquisition, X.H. and W.J.; resources, Y.B., Z.Z., K.D., and W.J.; supervision, X.H. and W.J.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Blood	Participants in this study	N/A
Critical commercial assays		
Human FGF-21 Immunoassay Kit	Antibody and Immunoassay Services, University of Hong Kong	Cat#31180
Software and algorithms		
R version 4.0.3	R Project	https://www.r-project.org

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study period and population

The Shanghai Nicheng Cohort Study is a community-based cohort designed to investigate the epidemiology of cardiometabolic diseases among adults in Nicheng Town, a suburb of Shanghai, China.[49] The baseline survey was conducted from April 2013 to August 2014, targeting 23,375 residents aged 45–70 years who had lived in Nicheng Town for at least five years. A total of 17,212 participants completed the baseline survey (median age: 56.9 years; 45.3% male). In 2018, we invited 10,075 participants aged 55–70 years at baseline to participate in the follow-up survey, with 7,069 completing it (median age: 61.7 years; 42.7% male).

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki and Istanbul and was approved by the Ethics Committee of Shanghai Sixth People's Hospital. Before data collection, all participants completed an informed consent form.

METHOD DETAILS

Inclusion and exclusion criteria

Participants without SLD at baseline were eligible for this study. Furthermore, participants with the following conditions were excluded from this analysis: (1) liver failure (ALT or AST >2 upper limits of normal), kidney failure (serum creatinine >1.5 upper limits of normal), or cancer at baseline; (2) loss to contact at follow-up; (3) missing abdominal ultrasound data at follow-up; (4) new-onset SLD patients without any cardiometabolic risk factors and alcohol intake; (5) new-onset SLD patients with viral hepatitis; and (6) missing alcohol consumption or viral hepatitis data. Ultimately, 3,504 participants (1,907 females) were included in the study (Figure 1).

Outcome definition

SLD was diagnosed by ultrasonography (Z.One Ultra, Zonare, CA, USA) based on known standard criteria, including the presence of a diffuse increase in fine echoes in the liver parenchyma compared with the kidney or spleen parenchyma, deep beam attenuation, and bright vessel walls.[50] The images were assessed by qualified ultrasonographers who were blinded to the participants' conditions.

SLD patients with at least one of five cardiometabolic risk factors were divided into two subcategories according to the latest multisociety Delphi consensus statement[2]: MASLD (without any known causes of steatosis) and MetALD/ALD (with an alcohol intake greater than 140 g/week and 210 g/week for females and males, respectively). Five cardiometabolic risk factors involved in the diagnostic criteria including: (1) BMI $\geq$23 kg/m^2 or WC > 90 cm (male)/80 cm (female); (2) FPG $\geq$5.6 mmol/L or 2hPG $\geq$7.8 mmol/L or HbA1c $\geq$ 5.7% or diabetes; (3) blood pressure $\geq$130/85 mmHg or with history of hypertension; (4) TGs $\geq$ 1.7 mmol/L or lipids lowing treatment; and (5) HDL-c $\leq$ 1.0 mmol/L (male)/1.3 mmol/L (female).

Biomarkers and covariates collection

Blood samples were collected from the individuals after they had fasted for more than 10 h to measure the concentrations of RBP-4, FGF-21, adiponectin, osteocalcin, FPG, 2hPG, HbA1c, FINS, TGs, HDL-C, ALT, AST, GGT, hepatitis B surface antigen, and hepatitis C virus antibody. The RBP-4 concentration was determined using a commercial turbidimetric immunoassay system (Beijia, Shanghai, China) with a Hitachi 7600 automated analyzer. FGF-21 concentrations were quantified using ELISA kits (Antibody and Immunoassay Services, University of Hong Kong, Hong Kong).[11] Serum adiponectin levels were quantified using a latex

particle-enhanced immunoturbidimetric assay (Antibody and Immunoassay Services, University of Hong Kong, Hong Kong).[11] Total osteocalcin levels were detected using an electrochemiluminescence immunoassay (Cobas e601; Roche Diagnostics GmbH, Mannheim, Germany).[13] All intra- and inter-assay coefficients of variance were less than 10% and the investigators responsible for laboratory testing were blinded to the clinical diagnosis of participants. Other laboratory measurement methods are described in Table S4. Data on age, gender, smoking, alcohol consumption, and medication history were collected using a standardized questionnaire. The weekly alcohol intake of all participants was evaluated based on interview data about their drinking habits over the past year, using the formula: weekly alcohol intake (g/week) = drinking volume (ml/occasion) × drinking frequency (times/week) × alcohol content (%) × 0.789 (g/cm^3). BMI was calculated by dividing the weight (kg) by the height squared (m^2). WC was measured along the midline between the lower margin of the costal arch and the upper margin of the iliac crest on the midaxillary line. Blood pressure was measured twice using a mercury sphygmomanometer and the average was calculated. The degree of insulin resistance was determined using HOMA-IR, which was calculated as FINS (μU/ml) × FPG (mmol/L)/22.5.[51]

Traditional SLD predictive models

This study compared the predictive performance of metabolic biomarkers with the following three traditional SLD models: (1) the fatty liver index (FLI) = $(e^{0.953\times\log_e(TGs)+0.139\times BMI+0.718\times\log_e(GGT)+0.053\times WC-15.745})/(1+e^{0.953\times\log_e(TGs)+0.139\times BMI+0.718\times\log_e(GGT)+0.053\times WC-15.745})\times 100$ (TGs in mg/dL; BMI in kg/m^2; GGT in U/L; WC in cm); (2) the hepatic steatosis index (HSI) = 8 × (ALT/AST ratio) + BMI (+2, if female; +2, if diabetes mellitus) (ALT and AST in U/L; BMI in kg/m^2); (3) the NAFLD-liver fat score (LFS) = - 2.89 + 1.18 × Metabolic Syndrome (Yes: 1, No: 0) + 0.45 × Type 2 Diabetes (Yes: 2, No: 0) + 0.15 × FINS +0.04 × AST – 0.94 × AST/ALT (FINS in mU/L; ALT and AST in U/L).

QUANTIFICATION AND STATISTICAL ANALYSIS

Continuous and categorical variables are reported as medians (25th–75th percentiles) and frequencies (percentages), respectively. Comparisons between the two groups were conducted using the Kruskal-Wallis rank-sum test (for continuous variables) and the χ^2 test (for categorical variables). *p* values were corrected for multiple hypothesis testing using the Benjamini–Hochberg procedure. The *p for trend* across groups was calculated using the Mantel-Haenszel test for trend. The correlations between the levels of metabolic biomarkers and clinical characteristics were evaluated by using linear regression models. Modified Poisson regression models were used to calculate the RR (95% CI) of biomarker levels for the development of SLD and its subcategories.[52] Covariates in multivariate analyses were selected from a set of previously identified demographic and clinical-related factors of SLD (variables listed in Table 1) using the least absolute shrinkage and selection operator method (Table S2; Figure S1). Heterogeneity between the RR of biomarkers for different subcategories of SLD was examined using the *Q* test. The performance of biomarkers and traditional SLD predictive models including FLI, HSI, LFS, for predicting the development of SLD subcategories was evaluated using the AUC, NRI, IDI. The optimal cutoff values of biomarkers for SLD subcategory diagnosis were determined based on the Youden Index which maximizes the sum of sensitivity and specificity. The mediation role of HOMA-IR and TGs, the indicators for insulin resistance and lipids metabolism, were evaluated using the structural equation modeling method. Sensitive analyses were conducted among participants without missing values for any of the four biomarkers.

Statistical significance was defined as two-tailed $p < 0.05$. All analyses were performed using R version 4.0.4 (R Foundation for Statistical Computing).