ORIGINAL ARTICLE

WILEY

Prognostic implication of lipidomics in patients with coronary total occlusion undergoing PCI

You Zhou[1] | Jinxiang Chen[1] | Su Li[1] | Ao Chen[1] | Chunfeng Dai[1] | Muyin Liu[1] | Danbo Lu[1] | Zhangwei Chen[1] | Xiangdong Wang[2] | Juying Qian[1] | Junbo Ge[1]

[1]Department of Cardiology, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University; National Clinical Research Center for Interventional Medicine; Shanghai Clinical Research Center for Interventional Medicine, Shanghai, People's Republic of China

[2]Shanghai Institute of Clinical Bioinformatics, Fudan University Center of Clinical Bioinformatics; Shanghai Respiratory Research Institute, Shanghai, People's Republic of China

Correspondence
Zhangwei Chen and Juying Qian, Department of Cardiology, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University; National Clinical Research Center for Interventional Medicine; Shanghai Clinical Research Center for Interventional Medicine 180 Fenglin Road, Shanghai 200032, People's Republic of China.
Emails: chen.zhangwei@zs-hospital.sh.cn (Z.C.); qian.juying@zs-hospital.sh.cn (J.Q.)

Xiangdong Wang, Shanghai Institute of Clinical Bioinformatics; Fudan University Center of Clinical Bioinformatics; Shanghai Respiratory Research Institute 180 Fenglin Road, Shanghai 20032, People's Republic of China.
Email: xdwang@fuccb.com

Abstract

Background: Predictors of prognosis in patients with coronary chronic total occlusion (CTO) undergoing elective percutaneous coronary intervention (PCI) have remained lacking. Lipidomic profiling enables researchers to associate lipid species with disease progression and may improve the prediction of cardiovascular events.

Methods: In the present study, 781 lipids were measured by targeted lipidomic profiling in 350 individuals (50 healthy controls, 50 patients with coronary artery disease and 250 patients with CTO). L1-regularized logistic regression was used to identify lipid species associated with adverse cardiovascular events and create predicting models, which were verified by 10-fold cross-validation (200 repeats). Comparisons were made between a traditional model constructed with clinical characteristics alone and a combined model built with both lipidomic data and traditional factors.

Results: Twenty-four lipid species were dysregulated exclusively in patients with CTO, most of which belonged to sphingomyelin (SM) and triacylglycerol (TAG). Compared with traditional risk factors, new model combining lipids and traditional factors had significantly improved performance in predicting adverse cardiovascular events in CTO patients after PCI (area under the curve, 0.870 vs. 0.726, $p < .05$; Akaike information criterion, 129 versus 156; net reclassification improvement, 0.312, $p < .001$; integrated discrimination improvement, 0.244, $p < .001$). Nomogram was built based on the incorporated model and proved efficient by Kaplan–Meier method.

You Zhou, Jinxiang Chen and Su Li contributed equally to the article.

Conclusions: Lipidomic profiling revealed lipid species which may participate in the formation of CTO and could contribute to the risk stratification in CTO patients undergoing PCI.

KEYWORDS

biomarker, chronic total occlusion, coronary artery disease, lipidomics, risk prediction

1 | BACKGROUND

Coronary artery chronic total occlusion (CTO) is most technically challenging for percutaneous coronary intervention (PCI). The underlying mechanisms of coronary artery to go beyond stenosis to total occlusion have not been fully explained by traditional risk factors. Innovations of techniques and equipment have improved the success rate of revascularization; however, patients are still at undisregardable risk of adverse cardiovascular events after PCI.[1,2] Identification of biomarkers predictive of long-term outcomes in CTO patients following PCI has drawn intense interest.

Lipid metabolism plays a major role in the aetiology and progression of coronary plaques. Well-known lipid-associated parameters, such as low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG), explain only a small portion of cardiometabolic risk. In recent decades, the advent of lipidomics provides detailed information of lipid species, which could not be revealed by traditional lipid tests.[3–5] Lipidomic analysis proves to be a promising method to identify and quantify potential biomarkers that could help to understand the disease process and develop models assisting in diagnosis and risk stratification.[6–9]

Herein, we measured lipidome changes in 350 individuals including 50 healthy controls, 50 patients with coronary artery disease (CAD) and 250 patients with CTO. All patients with CTO underwent elective percutaneous revascularization of at least one totally occluded lesion and were followed up with a median period of 19 months. Given lipidomic analysis provided more detailed lipid profiles, we hypothesized that specific lipid species would be revealed participating in the aetiology of CTO. Combination of lipidomic information and clinical outcomes would identify lipids associated with cardiovascular events and develop lipidomic-based clinical models to stratify long-term risks in CTO patients after PCI.

key points

1. This study revealed lipid species exclusively dysregulated in patients with coronary chronic total occlusion, which mainly belongs to sphingomyelin and triacylglycerol.
2. This is the pioneer research to investigate the prognostic implications of lipidomic profiles in patients with coronary chronic total occlusion and identified prognostically significant lipid species.
3. A clinical-lipidomic predicting model was built with substantially improved performance in stratifying high-risk patients with coronary total occlusion after percutaneous coronary intervention.

2 | METHODS AND MATERIALS

2.1 | Population

A total of 350 participants were recruited in Zhongshan Hospital, Fudan University, from 2017 to 2019, including 50 healthy controls without coronary stenosis confirmed by coronary computed tomography angiography or angiogram, 50 patients with at least one coronary stenosis >50% but no CTO, and 250 patients had at least one CTO successfully revascularized through PCI during the index hospitalization. This study was proved by the Ethical Committee of Zhongshan Hospital (ethical code B2016-018). Informed consent was obtained from all subjects, and the study was conducted in accordance with the Declaration of Helsinki.

2.2 | Study definitions and endpoints

CTO is defined as a coronary occlusion with absent antegrade flow through the lesion (TIMI [Thrombolysis in

Myocardial Infarction] grade 0 flow) with a presumed or documented duration of ≥3 months.[10] The primary endpoint is a composite of all-cause death, myocardial infarction and unplanned revascularization [major adverse cardiovascular events (MACEs)]. Patients with CTO undergoing PCI were followed up by telephone review or clinic visit with a median period of 19 months [interquartile range (IQR), 16–22].

2.3 | Sample collection and lipid measurement

Venous blood samples were routinely taken on admission prior to the angiography, stored at 4°C and then transferred to the laboratory for further analysis within 4 h after collection. Plasma was prepared by centrifugation (1500 g, 15 min, 4°C) and stored at −80°C. Group information was blinded to researchers carrying out lipid extraction and analysis. 20 μl plasma was spiked with 9 μl internal standard cocktails (Avanti Lipids Polar, coefficient of variation <10%, details were provided in Supplementary Materials) and 350 μl pre-cooled isopropanol (−20°C). The mixture was vortexed, let on stand at room temperature for 10 min and then incubated at −20°C overnight. After vigorous shaking and centrifuged at 12,000 rpm for 20 min, supernatant in the upper layer was extracted to a new tube and centrifuged at 10,500 g for 10 min. Supernatant was transferred to a glass tube for further analysis.

The normal-phase liquid chromatography coupled Triple-Quadrupole mass spectrometer (QTRAP 5500, SCIEX) was used for lipid extraction. Both negative and positive electrospray ionization modes were used. The Q-Trap was operated to scan precursor/product ion pairs. Each test was repeated thrice. Peak area of each pair was processed with MultiQuant™ software (AB Sciex) for further quantification. Additional parameters, as reported previously,[11] are provided in Supplementary Materials. 781 lipid species were measured in the following classes: cholesterol (Cho), cholesterol ester (CE), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), alkylphosphatidylethanolamine [PE(O)], alkenylphosphatidylethanolamine [PE(P)], lysophosphatidylethanolamine (LPE), phosphatidylglycerol (PG), phosphatidylinositol (PI), lysophosphatidylinositol (LPI), phosphatidylserine (PS), ceramide (Cer), G_{M3} ganglioside, sphingosine (Sph), sphingomyelin (SM), diacylglycerol (DAG) and triacylglycerol (TAG).

In order to validate the methods and ensure stability, quality control (QC) procedures were employed, that is, 20 μl aliquots of QC samples were prepared by pooling identical volumes of the individual plasma samples. Eight pooled QC samples were evenly distributed and measured during each analysis to evaluate the relative standard deviation (RSD). Variables with RSD over 20% were excluded. The peak height for internal standards was monitored to certify stability.

2.4 | Statistical analysis

Lipid species with over 50% missing values were excluded, and 699 lipids were left for further analysis. We conducted analysis using both normalized and original lipid concentrations. Since the results were almost the same, we presented the results derived from the original data, which would be easier to understand. Categorical variables were summarized as numbers and percentages per group, and continuous variables as mean $\pm$ SD or median with IQRs. Comparisons between categorical variables were made by the Chi-squared test, whereas continuous variables were compared by t test (normally distributed) or Mann–Whitney U test (non-normally distributed), accordingly. The p values (two-sided) were corrected for multiple comparisons using the Benjamini–Hochberg method.[12] Statistical significance was defined as a corrected p value of <.05. Volcano map showed the specifically elevated/decreased lipids in CTO patients.

The Least Absolute Shrinkage and Selection Operator (LASSO) approach[13] was used to identify lipid species associated with clinical endpoints and create predicting models, which were verified by 10-fold cross-validation (200 repeats). Lipid species were then ranked by the frequency of inclusion. Top 15 lipids and putative traditional risk factors were further filtrated by stepwise approach in logistic regression. Predictive performance of each model was assessed by area under the receiver operating characteristic curve (AUC), Akaike information criterion (AIC), net reclassification improvement (NRI), integrated discrimination improvement (IDI) and Brier Score. The model's calibration was evaluated by calibration plots predicting the probability of MACEs versus observed probability. AUC and calibration plots were internally validated by bootstrap process (1000 resampling). Decision curve analysis (DCA) was conducted evaluating the net clinical benefits of predicting models. Nomogram was built from the model including both lipid species and traditional factors. Individual risk scores were obtained by applying the nomogram in the cohort. The median risk score as well as the cut-off score calculated using X-tile software (version 3.6.1; Yale University),[14] respectively, was set as the threshold stratifying patients into low-risk and high-risk groups. Kaplan–Meier survival analysis was used to assess the risk of MACEs in different groups. All data were analysed with R software (version 4.0.2).

3 | RESULTS

3.1 | Baseline characteristics

The demographics and clinical characteristics of the study population are shown in Table 1. Compared with healthy controls, in brief, patients with CAD/CTO were older, more frequently encountered with hypertension, diabetes, smoking and dyslipidaemia and had higher levels of body mass index (BMI), high-sensitivity C-reactive protein (hs-CRP), uric acid and decreased ejection fraction. In patients with CTO, 43.6% were male with a median age of 61 years. 18.4% had old myocardial infarction and 36.0% had prior PCI. The long-term clinical endpoints in CTO patients after PCI are displayed in Table 2. In median

duration of 19-month follow-up, the incidence of MACEs (composed of death, MI and ischemia-driven revascularization) was 12%.

3.2 | Lipidomic profiles of patients with CTO

Total 781 lipid elements in the plasma were measured, and 699 were left for analysis excluding those with >50% missing values. Compared with healthy controls and patients with CAD, 24 lipid species specifically changed in CTO patients. Six of 12 (50.0%) SM and one CE (0.5%) was declined. The elevated lipids included one PE(O) (0.5%), one Cer (50.0%), two DAG (5.6%) and 13 TAG (3.1%). The

TABLE 1 Baseline demographics and clinical characteristics

	Healthy controls (*n* = 50)	CAD (*n* = 50)	CTO (*n* = 250)	*p* value
Age (years)	50.0 (39.0–68.7)	67.5 (62.0–74.8)	61.0 (52.3–70.0)	<.001
Male	21 (42.0%)	37 (74.0%)	218 (87.2%)	<.001
Hypertension	18 (36.0%)	30 (60.0%)	172 (68.8%)	<.001
Diabetes mellitus	11 (22.0%)	17 (34.0%)	85 (34.0%)	<.001
Smoking history	6 (12.0%)	20 (40.0%)	112 (22.4%)	<.001
OMI	0 (0%)	8 (16.0%)	46 (18.4%)	.004
Prior PCI	0 (0%)	11 (22.0%)	90 (36.0%)	<.001
BMI (kg/㎡)	22.4 (20.2–25.0)	24.8 (22.0–27.4)	25.0 (23.2–26.9)	<.001
Laboratory and auxiliary examinations				
Creatinine (mg/dl)	67 (61–76)	84 (74–95)	80 (70–91)	<.001
hs-CRP (mg/L)	0.6 (0.2–0.9)	1.2 (0.5–3.1)	1.2 (0.5–3.8)	<.01
White Blood Cell Count ($\times 10^9$/L)	6.33 (5.08–7.22)	5.96 (5.18–7.58)	6.54 (5.46–7.64)	.160
Platelet ($\times 10^9$/L)	231 (185–258)	201 (176–232)	205 (168–248)	.054
Haemoglobin (g/L)	131 (124–148)	136 (129–145)	138 (128–148)	.310
NT-proBNP (pg/ml)	36.2 (23.1–70.3)	152.0 (56.9–309.8)	151.8 (69.7–414.0)	<.001
Cardiac troponin T (ng/ml)	0.003 (0.002–0.005)	0.016 (0.014–0.021)	0.012 (0.008–0.021)	<.001
Total cholesterol (mmol/L)	4.35 (3.67–4.78)	3.52 (3.04–3.91)	3.41 (2.96–3.98)	<.001
Low-density lipoprotein (mmol/L)	1.19 (1.00–1.49)	1.47 (1.15–2.24)	1.55 (1.22–2.09)	<.001
Triglyceride (mmol/L)	1.09 (0.88–1.71)	1.57 (1.21–2.29)	1.58 (1.09–2.49)	.001
High-density lipoprotein (mmol/L)	2.44 (1.88–2.96)	1.08 (0.91–1.24)	0.98 (0.85–1.18)	<.001
Lp(a) (mg/L)	79 (30–115)	139 (75–278)	147 (60–472)	<.001
HbA1c (%)	5.4 (5.1–5.7)	6.1 (5.7–6.8)	6.0 (5.5–6.8)	<.001
Uric acid (μmol/L)	306 (252–355)	371 (304–430)	356 (289–419)	<.001
Ejection fraction (%)	68 (65–70)	64 (60–67)	61 (53–66)	<.001
Use of statins	7 (14.0%)	48 (96.0%)	244 (97.6%)	<.001

Note: Data are shown as median(interquartile range), *n* (%) or mean ± SD.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CTO, chronic total occlusion; hs-CRP, high-sensitivity C-reactive protein; Lp(a), lipoprotein (a); NT-proBNP, N-terminal pro-B-type natriuretic peptide; OMI, old myocardial infarction; PCI, percutaneous coronary intervention.

concentrations of these lipids among three groups were displayed in heatmap (Figure 1), and fold change among groups is shown in Figure 2.

We compared lipidomic profiles in subgroups to assess the association between lipid elements and clinical characteristics (age, sex, diabetes and hypertension) (Online Table 1 to Online Table 4). We found that compared with healthy controls, SM(16:0) and SM(24:1) were universally declined in each subgroup of CTO patients. In patients ≤65 years, 11 lipid species in TAG were significantly elevated, while in patients older than 65 years, no relationship was found between TAG and CTO. Similar result was revealed regarding sex, that is, TAG56:9-FA18:3, TAG56:9-FA20:4 and TAG56:9-FA20:5 were elevated in male CTO patients but TAG did not significantly change in female patients. Compared with CTO patients without diabetes, diabetic patients had slightly more kinds of dysregulated lipids [PC(18:0/18:0), CE(22:5) and SM(26:0)]. However, patients with hypertension had 7 lipid species substantially changed in contrast to 3 in nonhypertensive patients, among which Cer(14:0), SM(26:0), SM(26:1), TAG56:9-FA18:3 and TAG56:9-FA20:5 were specifically elevated or declined in the hypertensive group.

3.3 | Association of lipids with clinical outcomes

In order to identify lipids correlated with cardiovascular events, we conducted 10-fold-validation verified L1-regularized logistic regression analysis of each individual species for 200 times. The lipid species were ranked by the frequency of inclusion into the models (shown in Online Table 5). The proportion of lipid classes/subclasses included in regression models were as follows: TAG (21.4%), DAG (19.0%), PC (14.2%), PE(P) (9.5%), CE (9.5%), PE(O) (7.1%), PE (4.8%). These lipid species were fitted into Kyoto Encyclopedia of Genes and Genomes (KEGG) database, which revealed that glycerophospholipid metabolism was the key altered metabolic pathways (impact = 0.11, p = .044).

TABLE 2 Clinical outcomes in patients with CTO

	n = 250 patients
Death	1 (0.4%)
MI	2 (0.8%)
Unplanned revascularization	27 (10.8%)
MACEs[a]	30 (12.0%)

Note: Data are shown as n (%).

Abbreviations: CTO, chronic total occlusion; MACEs, major adverse cardiovascular events; MI, myocardial infraction.

[a]MACEs were a composite of death, MI and unplanned revascularization.

We also investigated whether lipid patterns, that is, numbers of carbon atom or double bonds in lipid classes/subclasses, were associated with MACEs (Figure 3). The colour of each circle depicts the strength and direction (positive or negative) of the given associations. The size of the circle indicates the level of statistical significance. It seemed that most LPC tended to correlate with higher risk of cardiovascular events. As for TAG, only those with lower carbon atom numbers and double-bond numbers tended to be significantly associated with elevated risk.

3.4 | Model development to predict long-term cardiovascular risks

Clinical features between CTO patients with and without MACEs are shown in Table 3. There was no difference regarding procedural characteristics including Syntax score, number of treated vessels and stent length. Based on previously published results[15] in our centre, 20 candidates of traditional risk factors were filtrated by a stepwise approach in logistic regression. The covariates used in the analysis were continuous measures of age (per 5 years increase), BMI, LDL, HDL, TG, Lp(a), creatine, white blood cell count, haemoglobin, platelet count, stent length, target vessel numbers, ejection fraction, and the categorical measures of PCI or MI history, hypertension, diabetes, smoking, sex and in-stent CTO. Only hypertension and in-stent CTO were independently related to MACEs and built as the traditional model. Top 15 most frequently included lipid species shown in Online Table 5 were added to the traditional model, and a stepwise approach was conducted again for filtrating lipids independently associated with MACEs. Finally, the combined model was constructed consisting of hypertension, in-stent CTO and 6 lipid species: LPC(20:2), PE(P-18:0/16:0), DAG(18:1/18:1), TAG44:3-FA18:2, PE(O-18:0/16:1) and PC(20:0/20:4).

In order to confirm whether lipidomic information would help to improve the performance of predicting models, we calculated AUC, AIC, NRI, IDI and Brier score (Table 4). The addition of the 6 lipid species to the traditional model led to an increase in AUC from 0.726 to 0.870, declined AIC from 156 to 129, Brier score from 10.5% to 7.6% with a NRI of 0.312 and IDI of 0.244. Receiver operating characteristic curve is shown in Figure 4A, which confirmed that the combined model had better performance in discrimination. On the calibration plot (Figure 4B), predicted probabilities were similar between models at low risks. The combined model was closer to the observed probabilities at intermediate risk, but deviated when higher probabilities were predicted. DCA was performed to compare the net benefit between the traditional and combined models for predicting prognosis (Figure 4C), which confirmed better clinical benefit of the combined model.
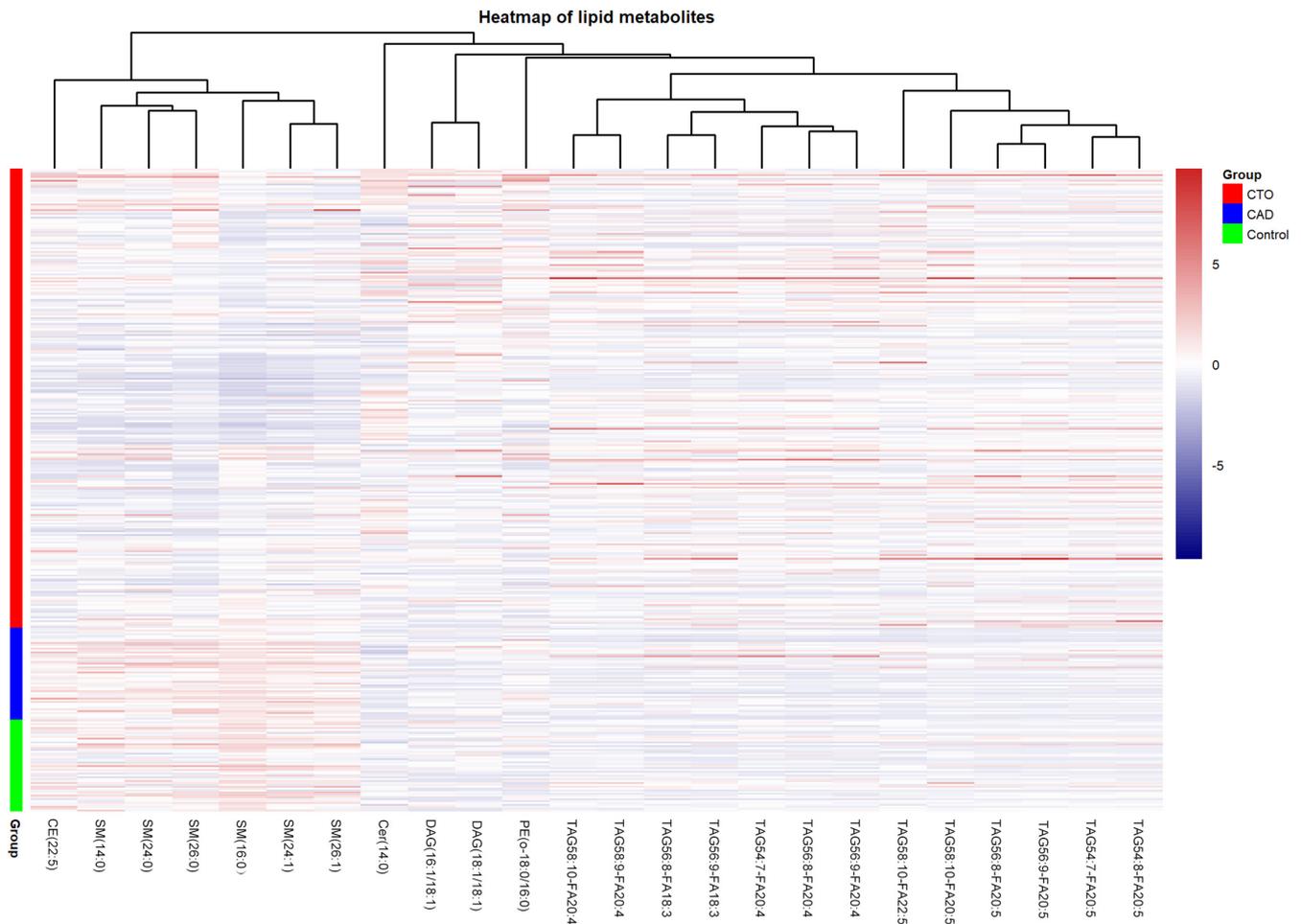
Heatmap of lipid metabolites

FIGURE 1 Heatmap of exclusively changed lipid species in CTO patients. The heatmap describes the 24 lipid elements exclusively changed in CTO patients. The concentration of lipids increased from blue (low) to red (high). CTO, chronic total occlusion

3.5 | Development of the nomogram

A nomogram was computed based on the combined model we constructed in the former context (Figure 5). Patients were divided into high/low-risk groups based on the total risk scores calculated by the nomogram model. The median risk score and the optimal cut-off score derived from X-tile software were used as the threshold of classification, respectively. Survival curves were plotted to compare the incidence of MACEs in different risk groups using the Kaplan–Meier method, and the results showed the nomogram had satisfactory discriminatory ability for cardiovascular risks in CTO patients ($p < .01$, Figure 6).

4 | DISCUSSION

While the development of CAD has been associated with traditional risk factors, including LDL and TG, the role of lipid elements in the aetiology of CTO remains less understood. The advent of lipidomic analysis provides detailed information of lipid metabolism with an unprecedented coverage, which enhances our understanding and management of cardiovascular diseases.[6,16,17] However, most studies have focused on stable CAD or acute coronary syndrome, lipid metabolic information of CTO patients is lacking. Besides, there has not been a satisfactory model to predict the prognosis of CTO patients after PCI. Since the recanalization of a totally occluded vessel requires great consumption of time and finance, it would be necessary to find a method effectively stratify cardiovascular risks and guide clinical practice.

In the present study, we qualitatively and quantitatively measured plasma lipidomic profiles and identified lipid species exclusively dysregulated in patients with CTO, which could be involved in the process of total occlusion. We also integrated clinical outcomes and lipidomic data to build a combined model consisting of both traditional risk factors and lipid species, which demonstrated better performance in predicting long-term cardiovascular risks in CTO patients accepting PCI. Moreover, a nomogram was developed and proved to be efficient for clinical application.

CTO and CAD share common pathological process; nevertheless, the underlying mechanism of coronary
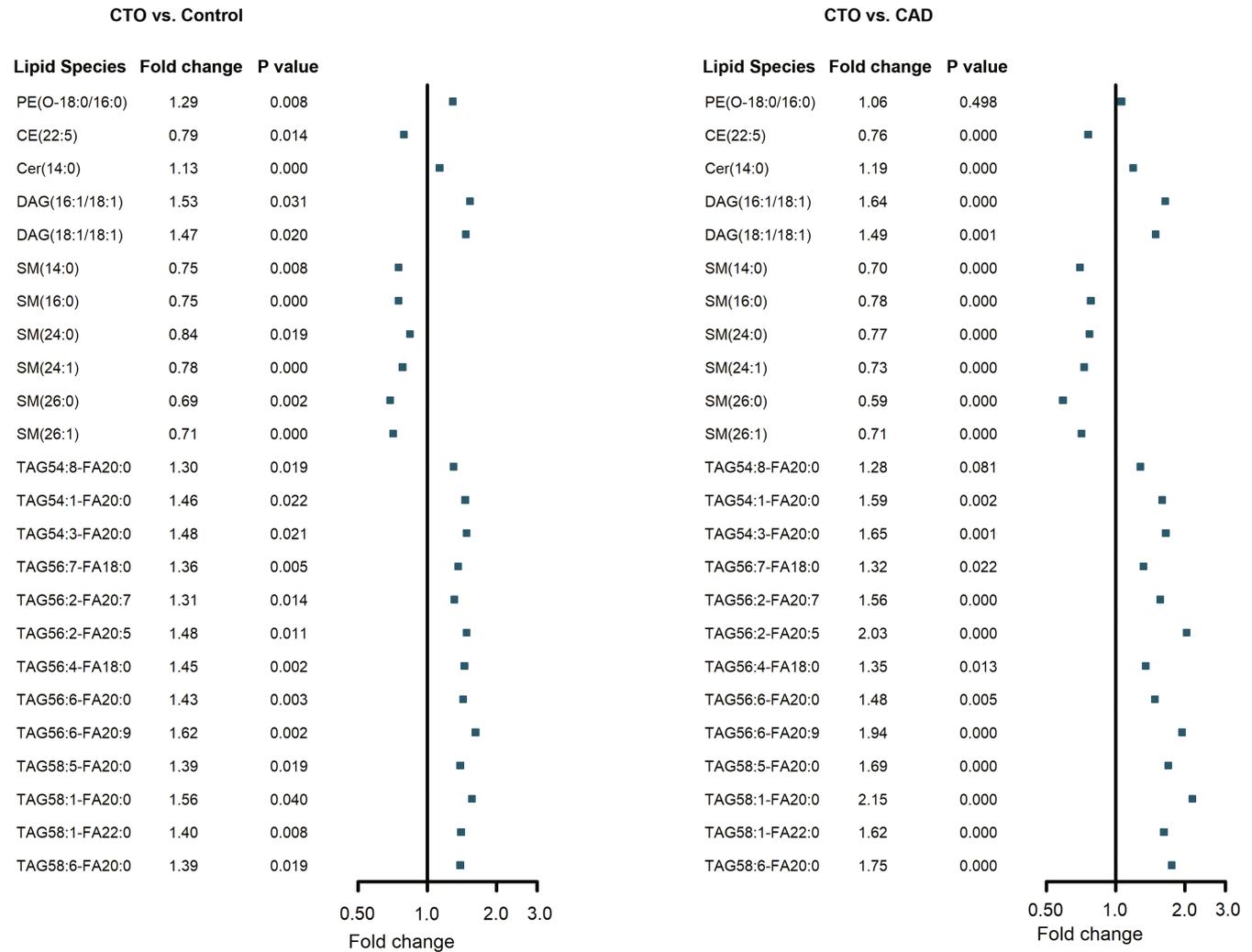
CTO vs. Control

Lipid Species	Fold change	P value
PE(O-18:0/16:0)	1.29	0.008
CE(22:5)	0.79	0.014
Cer(14:0)	1.13	0.000
DAG(16:1/18:1)	1.53	0.031
DAG(18:1/18:1)	1.47	0.020
SM(14:0)	0.75	0.008
SM(16:0)	0.75	0.000
SM(24:0)	0.84	0.019
SM(24:1)	0.78	0.000
SM(26:0)	0.69	0.002
SM(26:1)	0.71	0.000
TAG54:8-FA20:0	1.30	0.019
TAG54:1-FA20:0	1.46	0.022
TAG54:3-FA20:0	1.48	0.021
TAG56:7-FA18:0	1.36	0.005
TAG56:2-FA20:7	1.31	0.014
TAG56:2-FA20:5	1.48	0.011
TAG56:4-FA18:0	1.45	0.002
TAG56:6-FA20:0	1.43	0.003
TAG56:6-FA20:9	1.62	0.002
TAG58:5-FA20:0	1.39	0.019
TAG58:1-FA20:0	1.56	0.040
TAG58:1-FA22:0	1.40	0.008
TAG58:6-FA20:0	1.39	0.019

CTO vs. CAD

Lipid Species	Fold change	P value
PE(O-18:0/16:0)	1.06	0.498
CE(22:5)	0.76	0.000
Cer(14:0)	1.19	0.000
DAG(16:1/18:1)	1.64	0.000
DAG(18:1/18:1)	1.49	0.001
SM(14:0)	0.70	0.000
SM(16:0)	0.78	0.000
SM(24:0)	0.77	0.000
SM(24:1)	0.73	0.000
SM(26:0)	0.59	0.000
SM(26:1)	0.71	0.000
TAG54:8-FA20:0	1.28	0.081
TAG54:1-FA20:0	1.59	0.002
TAG54:3-FA20:0	1.65	0.001
TAG56:7-FA18:0	1.32	0.022
TAG56:2-FA20:7	1.56	0.000
TAG56:2-FA20:5	2.03	0.000
TAG56:4-FA18:0	1.35	0.013
TAG56:6-FA20:0	1.48	0.005
TAG56:6-FA20:9	1.94	0.000
TAG58:5-FA20:0	1.69	0.000
TAG58:1-FA20:0	2.15	0.000
TAG58:1-FA22:0	1.62	0.000
TAG58:6-FA20:0	1.75	0.000

Fold change (x-axis: 0.50, 1.0, 2.0, 3.0)

FIGURE 2 Fold change of exclusively altered lipid species in CTO. Fold change and statistical significance of lipid species exclusively altered in CTO patients were displayed. P values were corrected using the Benjamini–Hochberg method. CAD, coronary artery disease; CTO, chronic total occlusion

artery progressing from stenosis to complete obstruction has not been fully clarified. Therefore, we recruited healthy controls, CAD and CTO patients so that we could find out lipid elements which were uniquely altered in CTO. Compared with healthy controls, clinical risk factors were markedly more prevalent in patients from both diseased groups, but demographic characteristics between CAD and CTO patients were pretty much comparable. In some ways, this confirmed the lipids exclusively changed in CTO patients were not solely attributed to the discrepancy in baseline features, but independent pathogenic factors on their own.

There were 24 lipid species significantly and specifically dysregulated in CTO patients, with SM being the most profoundly declined lipid and TAG the most notably elevated classes. It was reported that low levels of SM were associated with the incidence of coronary atherosclerosis and the degree of decreased SM was related to the severity

of CAD.[18] Besides, SM was a substantial component of HDL. SM in HDL was demonstrated to have the strongest association with CAD among all HDL-related parameters and the only HDL-related parameter independently correlated with the number of coronary stenoses.[19] It has been proposed that the development of CAD extends beyond the routinely measured levels of HDL and the disturbance with particles was fundamental to the development of disease.[20,21] The reduced SM in HDL could impair the stability of HDL particles resulting in the disruption of reverse cholesterol transport and in part, at least, enhance the progression of disease.[22] In this study, TAG was the most remarkably elevated lipid class in CTO patients. TAG has been implied to be associated with a high risk of CAD.[23] In conform to previous publications, a subgroup of TAG with lower carbon atom and double-bond numbers (i.e. short-chained, saturated or monosaturated) was more significantly related to increased cardiovascular risks.[24,25]
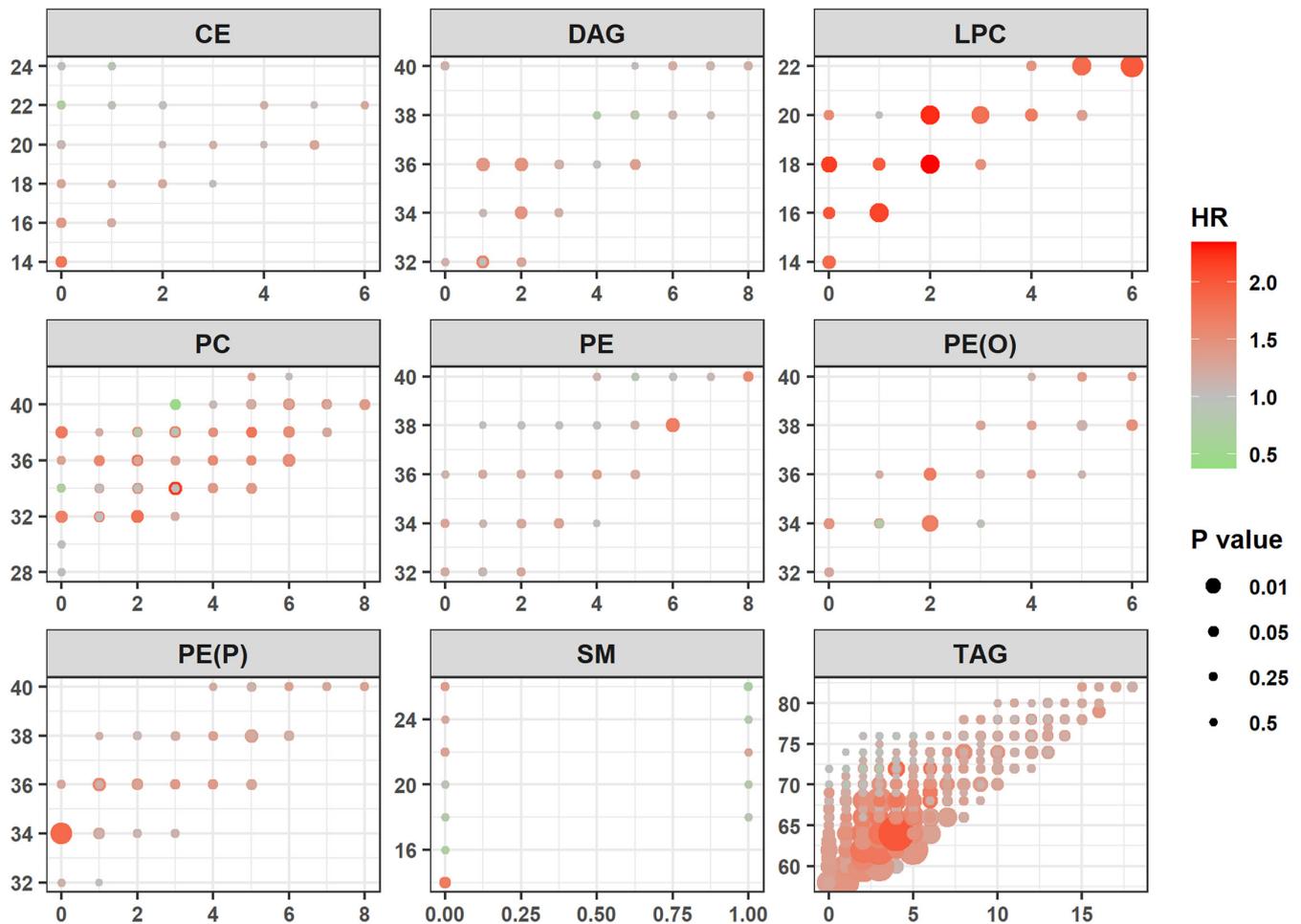
FIGURE 3 Association of cardiovascular risks and total number of carbon atoms and double bonds of various lipid classes. Individual lipid species are depicted by filled circles and arranged by lipid class in 9 panels according to the number of total carbon atoms (*x* axes) and number of double bonds (*y* axes). Circle colour indicates the magnitude of odds ratio (OR), and circle size corresponds to the significance level. The endpoint is a composite of death, MI and unplanned revascularization. The logistic regression model is adjusted for hypertension and in-stent CTO

TAG was most abundant in very-low-density lipoprotein (VLDL) at fasting condition and in chylomicron postprandially.[26] Rather than elevated LDL, triglyceride-rich lipoproteins (TGRL) now become a ubiquitous pattern of lipid abnormality in many patients with atherosclerosis. Accumulation of remnants from chylomicron and VLDL was demonstrated to be involved in plaque formation and progression.[27] Our results provided more evidence for the cardiovascular risks attributed to TGRL and justify the development of TGRL-lowering therapies to manage atherosclerotic disease.[28,29]

We also compared the lipid profiles in subgroups of CTO patients with healthy controls. As shown in Online Tables 1–4, we found out some clinical phenotype-related lipidomic patterns. In this cohort, patients ≤65 years and males were more likely to have dysregulated TAG. In general, younger patients were at relatively low risk of CAD. As mentioned above, the elevation of certain TAG could cause superimposed risk of atherosclerosis in addition

to traditional detrimental factors. In the meanwhile, the higher risk of cardiovascular events in male patients may be at least partially due to the harmful role of TAG. Patients with hypertension and diabetes tend to have more types of dysregulated lipid species. We could not verify whether these alternations occurred as the cause or consequence of clinical features, which requires further research.

Lipidomic analysis provides detailed information of lipid metabolites, most of which are end products and more closely related to clinical conditions.[6,30] In this study, it is implied 6 lipid species, that is, LPC(20:2), PE(P-18:0/16:0), DAG(18:1/18:1), TAG44:3-FA18:2, PE(O-18:0/16:1) and PC(20:0/20:4), were independent predictors of MACEs after adjustment to traditional factors. We put these lipids into KEGG database and revealed that the most profoundly dysregulated pathway was glycerophospholipid metabolism. Most glycerophosphates with long-chain saturated fatty acids tend to be positively associated increased risk of atherosclerosis,[31] which could result in

TABLE 3 Clinical characteristics in CTO patients

	Patients with MACEs ($n = 27$)	Patients without MACEs ($n = 223$)	p value
Age (years)	62.0 (52.8–69.2)	61.3 (53.0–68.8)	.256
Male	24 (88.9%)	194 (87.0%)	.781
Hypertension	22 (81.5%)	150 (67.2%)	.112
Diabetes mellitus	4 (14.8%)	81 (36.3%)	.011
Smoking history	10 (37.0%)	102 (45.7%)	.390
OMI	6 (22.2%)	40 (17.9%)	.587
Prior PCI	12 (44.4%)	78 (35.0%)	.333
Syntax score	21.5 (14.1–33.1)	20.5 (15.0–25.5)	.917
Stent length (mm)	75.0 (57.5–109.3)	76.0 (58.8–108.0)	.445
Number of treated vessels			
1	15 (55.6)	129 (57.8)	.820
2	12 (44.4)	73 (32.7)	.225
3	0 (0.0)	21 (9.4)	<.001
Creatinine (mg/dl)	80 (71–96)	83 (70–97)	.213
hs-CRP (mg/L)	0.9 (0.3–2.5)	1.2 (0.6–3.85)	.534
White blood cell count ($\times 10^9$/L)	6.09 (4.80–7.75)	6.45 (5.51–7.60)	.369
Platelet ($\times 10^9$/L)	198 (149–255)	216 (168–254)	.547
Haemoglobin (g/L)	141 (136–145)	135 (121–144)	.541
NT-proBNP (pg/ml)	95.5 (60.0–184.5)	184.5 (88.4–477.8)	.045
Cardiac troponin T (ng/ml)	0.009 (0.007–0.014)	0.011 (0.007–0.018)	.007
Total cholesterol (mmol/L)	3.45 (3.17–4.18)	3.23 (2.87–3.96)	.726
Low-density lipoprotein (mmol/L)	1.50 (0.86–2.26)	1.51 (1.13–2.06)	.487
Triglyceride (mmol/L)	2.13 (1.45–3.19)	1.47 (1.05–2.01)	.167
High-density lipoprotein (mmol/L)	0.96 (0.85–1.05)	0.96 (0.84–1.23)	.817
Lp(a) (mg/L)	241 (95–605)	183 (73–594)	.586
Ejection fraction (%)	60 (51–66)	61 (54–67)	.947

Note: Data are shown as median(interquartile range), n (%) or mean $\pm$ SD.

Abbreviations: CTO, chronic total occlusion; hs-CRP, high-sensitivity C-reactive protein; Lp(a), lipoprotein (a); NT-proBNP, N-terminal pro-B-type natriuretic peptide; OMI, old myocardial infarction; PCI, percutaneous coronary intervention.

TABLE 4 Performance of models predicting MACEs

	AUC	AIC	NRI	IDI	Brier score
Traditional model[a]	0.726 (0.623–0.782)	156	—	—	10.5% (7.5–13.6%)
Combined model[b]	0.870 (0.773–0.945)	129	0.312 (0.098–0.634)	0.244 (0.152–0.337)	7.6% (5.1–10.1%)
p value	<.05	—	<.001	<.001	<.01

Abbreviations: AIC, akaike information criterion; AUC, area under the curve; IDI, integrated discrimination improvement; NRI, net reclassification improvement.

[a]Comprised of hypertension and intra-stent total occlusion.

[b]Comprised of hypertension, intra-stent total occlusion, LPC(20:2), PE(P-18:0/16:0), DAG(18:1/18:1), TAG44:3-FA18:2, PE(O-18:0/16:1) and PC(20:0/20:4).

HDL surface rigidity and affect the uptake of cholesterol from peripheral tissues.[32] It has been implied that glycerophospholipids were associated with immunometabolism and chronic low-grade inflammation,[33,34] which were predisposing factors of endothelial dysfunction and plaque disruption. Hence, lipid species could be the link among
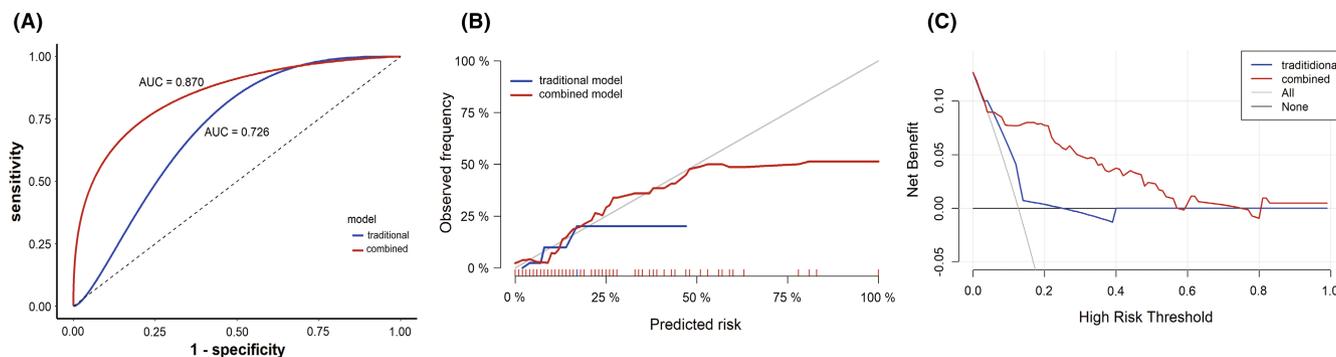
FIGURE 4 Performance of the predicting models. (A) The receiver operating characteristic curve of the traditional and combined models. AUC was significantly improved in the combined model (from 0.726 to 0.870, $p < .05$). (B) The calibration curve of the traditional and combined models. Perfect prediction is shown by the diagonal black dashed line. The combined model was closer to the observed probabilities indicating better calibration. (C) The decision curve analysis of the traditional and combined models. The grey is the presumption that all patients suffered from MACEs, while the black represents the presumption that none of the patients suffered from MACEs. The combined model brought better clinical benefit over most of the risk thresholds. AUC, area under the receiver operating characteristic curve; MACEs, major adverse cardiovascular events
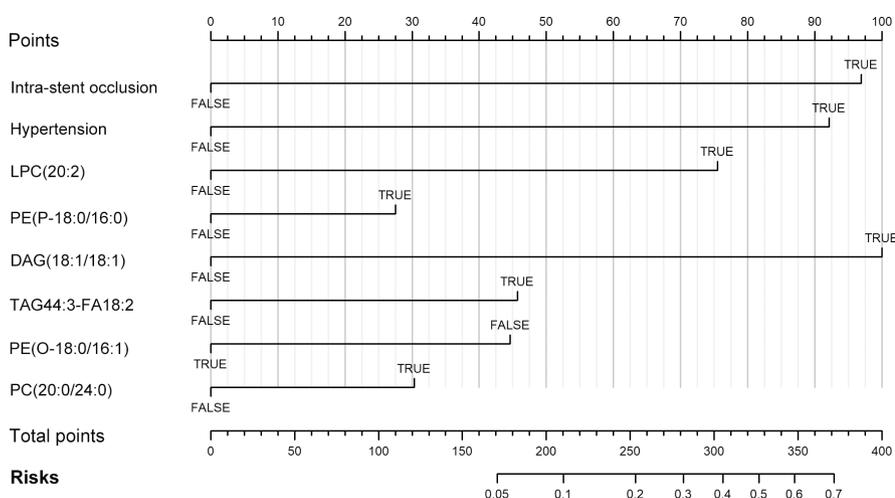
FIGURE 5 Nomogram for predicting the risk of MACEs. Nomogram for predicting the risk of MACEs is developed with the incorporation of traditional risk factors and lipid species. Each score was calculated to assess the variable. The total points were summed to predict the probability of MACEs. MACEs, major adverse cardiovascular events

atherosclerosis, inflammation and acute cardiovascular events. Lipidomic analysis identified lipid elements related to increased risk of MACEs, implying structural features within lipid species, independent of traditional lipid-associated parameters, played an important role in the homeostasis of artery plaques.

The success rate of recanalizing a totally occluded coronary artery has been dramatically improved in the past decades. Yet, the incidence of MACEs in the long term after PCI could not be overlooked. There has not been a generally accepted model to identify CTO patients at high risk of MACEs and ameliorate treatments accordingly. Lipidomic profiling has been proven a novel and effective method to discover biomarkers and build predicting models.[17,25,35,36] We used L1-regularized logistic regression to identify prognostically relevant lipids and a stepwise approach to filtrate presumed traditional factors. Incorporating lipidomic statistics and clinical data,

we constructed a combined model with notably improved ability of discrimination, better calibration and more net clinical benefits. Furthermore, we computed a nomogram on the basis of the combined model. Confirmed by Kaplan–Meier survival analysis, our nomogram performed well in identifying CTO patients at high risk of MACEs after PCI and had the potential to assist in risk stratification and clinical decision-making.

This study has several strengths. First, to our knowledge, this is the pioneer research to investigate the prognostic implications of lipidomic profiles in CTO patients. Second, we included not only controls and CTO patients, but also participants with CAD, which permitted us to identify risk factors that were exclusively changed in CTO. Besides, we revealed lipid species possibly involved in the aetiology of CTO and those correlated with long-term cardiovascular risks, which extended our understanding of the onset and progress of the disease. Some
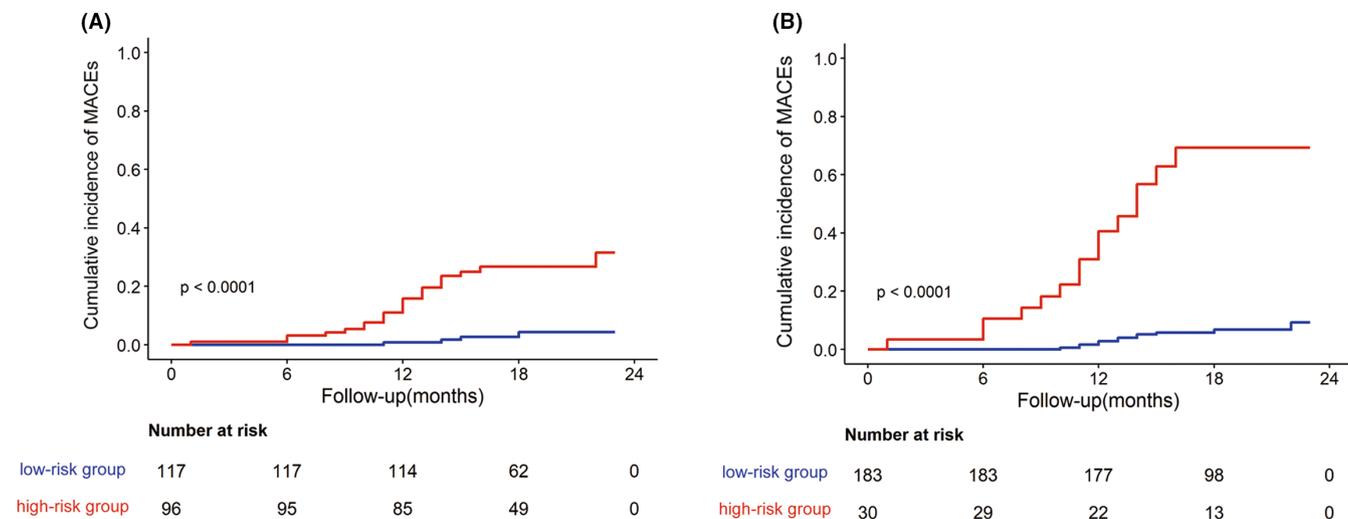
FIGURE 6 Time-to-event curves for clinical endpoints. (A) The cumulative incidence of MACEs in patients grouped by the median risk score. computed by the nomogram. (B) The cumulative incidence of MACEs in patients grouped by the optimal cut-off risk score[†] computed by the nomogram. [†]Derived from X-tile software (version 3.6.1; Yale University). MACEs, major adverse cardiovascular events. *Note*: All figures should be coloured in publication

limitations of this study deserve to be acknowledged. This is a single-centred retrospective study with relatively a small sample size. Although we implemented internal validation, multi-centred and externally validated researches are needed to draw more persuasive conclusions. In this study, we used a targeted approach and measured 781 lipids. Although the range of detection was already relatively wide among analogous studies, this was not a complete coverage of lipidome which could miss some of the potential biomarkers. At last, the median interval of follow-up was 19 months and the incidence of MACEs was comparatively low in the cohort. This limits the utility of our model in patients with remarkably high risks (e.g. above 60%). Studies in larger scale with higher cardiovascular risks and longer duration are pended to complement the predicting model and prognostic implications.

5 | CONCLUSION

Our study highlights the value of lipidomic profiling in revealing novel biomarkers for the pathogenesis and prognosis of CTO. Circulating lipid elements could be used to improve risk stratification in CTO patients following PCI and also be promising targets for the treatment of atherosclerotic disease.

CONFLICT OF INTEREST
There are no conflicts of interest pertained to this submission. All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

ORCID
You Zhou https://orcid.org/0000-0001-9250-0982
Chunfeng Dai https://orcid.org/0000-0003-4318-931X

REFERENCES
1. Strauss BH, Knudtson ML, Cheema AN, et al. Canadian multicenter chronic total occlusion registry: ten-year follow-up results of chronic total occlusion revascularization. *Circ Cardiovasc Interv*. 2021;14:e10546.
2. Megaly M, Ali A, Saad M, et al. Outcomes with retrograde versus antegrade chronic total occlusion revascularization. *Catheter Cardiovasc Interv*. 2020;96:1037-1043.
3. Stock J. The emerging role of lipidomics. *Atherosclerosis*. 2012;221:38-40.
4. Han X. Lipidomics for studying metabolism. *Nat Rev Endocrinol*. 2016;12:668-679.

5. Wang R, Li B, Lam SM, Shui G. Integration of lipidomics and metabolomics for in-depth understanding of cellular mechanism and disease progression. *J Genet Genomics*. 2020;47:69-83.

6. Hoefer IE, Steffens S, Ala-Korpela M, et al. Novel methodologies for biomarker discovery in atherosclerosis. *Eur Heart J*. 2015;36:2635-2642.

7. Laaksonen R. Identifying new risk markers and potential targets for coronary artery disease: the value of the lipidome and metabolome. *Cardiovasc Drugs Ther*. 2016;30:19-32.

8. Anroedh S, Hilvo M, Akkerhuis KM, et al. Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients. *J Lipid Res*. 2018;59:1729-1737.

9. Greenhill C. Lipidomics reveals early markers. *Nat Rev Endocrinol*. 2021;17:706.

10. Ybarra LF, Rinfret S, Brilakis ES, et al. Definitions and clinical trial design principles for coronary artery chronic total occlusion therapies: CTO-ARC consensus recommendations. *Circulation*. 2021;143:479-500.

11. Gao D, Zhang L, Song D, et al. Values of integration between lipidomics and clinical phenomes in patients with acute lung infection, pulmonary embolism, or acute exacerbation of chronic pulmonary diseases: a preliminary study. *J Transl Med*. 2019;17:162.

12. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. *Stat Med*. 1990;9:811-818.

13. Tibshirani RJ. Regression Shrinkage and Selection via the LASSO. *J R Stat Soc B Methodol*. 1996;73:273-282.

14. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bioinformatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res*. 2004;10:7252-7259.

15. Zhou Y, Chen Z, Chen A, et al. Association between the magnitude of periprocedural myocardial injury and prognosis in patients undergoing elective percutaneous coronary intervention. *Eur Heart J Qual Care Clin Outcomes*. 2021. doi:10.1093/ehjqcco/qcab103. Online ahead of print.

16. Shah SH, Kraus WE, Newgard CB. Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. *Circulation*. 2012;126:1110-1120.

17. Ellims AH, Wong G, Weir JM, Lew P, Meikle PJ, Taylor AJ. Plasma lipidomic analysis predicts non-calcified coronary artery plaque in asymptomatic patients at intermediate risk of coronary artery disease. *European Heart Journal - Cardiovascular Imaging*. 2014;15:908-916.

18. Ding M, Rexrode KM. A review of lipidomics of cardiovascular disease highlights the importance of isolating lipoproteins. *Metabolites*. 2020;10:163.

19. Horter MJ, Sondermann S, Reinecke H, et al. Associations of HDL phospholipids and paraoxonase activity with coronary heart disease in postmenopausal women. *Acta Physiol Scand*. 2002;176:123-130.

20. Darabi M, Guillas-Baudouin I, Le Goff W, Chapman MJ, Kontush A. Therapeutic applications of reconstituted HDL: when structure meets function. *Pharmacol Ther*. 2016;157:28-42.

21. Kostara CE, Papathanasiou A, Psychogios N, et al. NMR-based lipidomic analysis of blood lipoproteins differentiates the progression of coronary heart disease. *J Proteome Res*. 2014;13:2585-2598.

22. Rye KA, Hime NJ, Barter PJ. The influence of sphingomyelin on the structure and function of reconstituted high density lipoproteins. *J Biol Chem*. 1996;271:4243-4250.

23. Razquin C, Liang L, Toledo E, et al. Plasma lipidome patterns associated with cardiovascular risk in the PREDIMED trial: a case-cohort study. *Int J Cardiol*. 2018;253:126-132.

24. Lu J, Lam SM, Wan Q, et al. High-coverage targeted lipidomics reveals novel serum lipid predictors and lipid pathway dysregulation antecedent to type 2 diabetes onset in normoglycemic chinese adults. *Diabetes Care*. 2019;42:2117-2126.

25. Stegemann C, Pechlaner R, Willeit P, et al. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. *Circulation*. 2014;129:1821-1831.

26. Christinat N, Masoodi M. Comprehensive lipoprotein characterization using lipidomics analysis of human plasma. *J Proteome Res*. 2017;16:2947-2953.

27. Ginsberg HN. New perspectives on atherogenesis: role of abnormal triglyceride-rich lipoprotein metabolism. *Circulation*. 2002;106:2137-2142.

28. Ginsberg HN, Packard CJ, Chapman MJ, et al. Triglyceride-rich lipoproteins and their remnants: metabolic insights, role in atherosclerotic cardiovascular disease, and emerging therapeutic strategies-a consensus statement from the European Atherosclerosis Society. *Eur Heart J*. 2021;42:4791-4806.

29. Libby P. The changing landscape of atherosclerosis *NATURE* 2021;592:524–33, The changing landscape of atherosclerosis.

30. Meikle TG, Huynh K, Giles C, Meikle PJ. Clinical lipidomics: realizing the potential of lipid profiling. *J Lipid Res*. 2021;62:100127.

31. Chai JC, Deik AA, Hua S, et al. Association of lipidomic profiles with progression of carotid artery atherosclerosis in HIV infection. *JAMA Cardiol*. 2019;4:1239-1249.

32. Kontush A, Lhomme M, Chapman MJ. Unraveling the complexities of the HDL lipidome. *J Lipid Res*. 2013;54:2950-2963.

33. Petkevicius K, Virtue S, Bidault G, et al. Accelerated phosphatidylcholine turnover in macrophages promotes adipose tissue inflammation in obesity. *eLife*. 2019;8. doi:10.7554/eLife.47990

34. Zhu Q, Wu Y, Mai J, et al. Comprehensive metabolic profiling of inflammation indicated key roles of glycerophospholipid and arginine metabolism in coronary artery disease. *Front Immunol*. 2022;13:829425.

35. Meikle PJ, Wong G, Tsorotes D, et al. Plasma lipidomic analysis of stable and unstable coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2011;31:2723-2732.

36. Alshehry ZH, Mundra PA, Barlow CK, et al. Plasma lipidomic profiles improve on traditional risk factors for the prediction of cardiovascular events in type 2 diabetes mellitus. *Circulation*. 2016;134:1637-1650.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.