IJC Heart & Vasculature 53 (2024) 101457

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: www.sciencedirect.com/journal/ijc-heart-and-vasculature

Development and validation of a nomogram for premature coronary artery disease patients in Guangzhou

Runlu Sun [a,b,1], Qi Guo [a,b,1], Hongwei Li [a,b,1], Xiao Liu [a,b], Yuan Jiang [a,b], Jingfeng Wang [a,b,*], Yuling Zhang [a,b,*]

[a] Department of Cardiology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China
[b] Guangzhou Key Laboratory of Molecular Mechanism and Translation in Major Cardiovascular Disease, Guangzhou, China

ARTICLE INFO

Keywords:
Premature coronary artery disease
Nomogram
Least absolute shrinkage and selection operator
Decision curve analysis
High density lipoprotein 2

ABSTRACT

Background: Data regarding risk factors for premature coronary artery disease (PCAD) is scarce given that few research focus on it. This study aimed to develop and validate a clinical nomogram for PCAD patients in Guangzhou.

Methods: We recruited 108 PCAD patients (female ≤65 years old and male ≤55 years old) and 96 healthy controls from Sun Yat-sen Memorial Hospital of Sun Yat-sen University between 01/01/2021 and 31/12/2022. Twenty potentially relevant indicators of PCAD were extracted. Next, the least absolute shrinkage and selection operator (LASSO) regression analysis was used to optimize variable selection. The nomogram was developed based on the selected variables visually.

Results: Independent risk factors, including body mass index (BMI), history of PCAD, glucose, Apolipoprotein A1 (ApoA1), high density lipoprotein 2-cholesterol (HDL2-C), total cholesterol and triglyceride, were identified by LASSO and logistic regression analysis. The nomogram showed accurate discrimination (area under the receiver operator characteristic curve, ROC, 87.45 %, 95 % CI: 82.58 %-92.32 %). Decision curve analysis (DCA) suggested that the nomogram was clinical beneficial. HDL2, one risk factor, was isolated by a two-step discontinuous density-gradient ultracentrifugation method. And HDL2 from PCAD patients exhibited less ^{3}H-cholesterol efflux (22.17 % vs 26.64 %, $P < 0.05$) and less delivery of NBD-cholesterol detecting by confocal microscope compared with healthy controls.

Conclusions: In conclusion, the seven-factor nomogram can achieve a reasonable relationship with PCAD, and a large cohort were needed to enhance the credibility and effectiveness of our model in future.

1. Introduction

Premature coronary artery disease (PCAD), defined as coronary artery disease (CAD) in patients younger than 65 years for women and 55 years for men [1,2]. Patients with PCAD have a poor long-term outcome and a high likelihood for recurrence after first events [3,4]. Consequences of PCAD can be devastating particularly at a "young" age due to t its greater impact on the patient's psychology, productive life lost and socioeconomic burden [5]. Despite the above, few screening mode have been studied or validated in this population. Thus, doctors often underestimate the prevalence of PCAD and subsequently miss an opportunity for early intervention.

Published studies have demonstrated higher rate of hyper-lipidaemia and lower rates of history of CAD, diabetes mellitus and hypertension in PCAD patients compared to older CAD patients [6–8]. It is speculated that risk factor profile of PCAD is different from traditional CAD risk factors. Moreover, CAD risk is polygenic in nature and addition of single nucleotide polymorphisms (SNP) information to traditional CAD risk factors can improve the predictive performance of PCAD [9,10]. However, the high cost of genetic testing limits its use in clinical settings. So, physicians hope to find more cost-effective and convenient means to predict the risk of PCAD. Our previous studies have showed that HDL-C is beneficial for PCAD and HDL2 is as a superior predictor for CAD than small HDL3 [11,12].

* Corresponding authors at: Department of Cardiology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, No. 107, the West of Yanjiang Road, Yuexiu District, Guangzhou 510120, China.
E-mail addresses: wjingf@mail.sysu.edu.cn (J. Wang), zhyul@mail.sysu.edu.cn (Y. Zhang).
[1] Runlu Sun, Qi Guo and Hongwei Li contributed equally to this work.

https://doi.org/10.1016/j.ijcha.2024.101457
Received 1 April 2024; Received in revised form 29 June 2024; Accepted 1 July 2024
Available online 5 July 2024

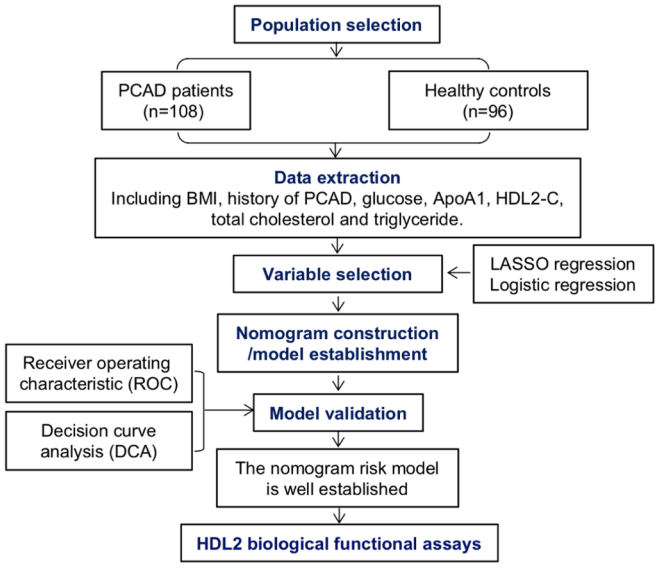

Fig. 1. Workflow and major findings of this study. PCAD, premature coronary artery disease; LASSO, least absolute shrinkage and selection operator; HDL2-C, high density lipoprotein 2 cholesterol; ApoA1, apolipoprotein A1; BMI, body mass index.

Nomogram is a popular tool to predict clinical events by integrating potential risk factors [13]. Thus, this study aims to establish a nomogram model and identify potential factors for PCAD. Hopefully, doctors could easily identify high-risk patients for PCAD through the nomogram model.

2. Materials and methods

2.1. Study design

The overall research workflow is depicted in Fig. 1, including population selection, data extraction, variable selection, nomogram model construction and validation. A brief description is as follows. The PCAD patients diagnosed via coronary arteriography (CAG) and healthy controls were retrospectively selected for this study. Clinical data were then extracted from the two groups, including BMI, history of PCAD, glucose, ApoA1, HDL2-C, total cholesterol, and triglyceride. Next, the LASSO regression analysis was performed to optimize variable selection. The nomogram was developed on the base of the selected variables visually. Finally, receiver operating characteristic curve (ROC) and decision curve analysis (DCA) were performed to validate the model. The nomogram risk model was well established. Additionally, we performed the HDL2 biological functional assays between the two groups.

2.2. Subjects

Based on screening of the electronic medical records system of Sun Yat-sen memorial hospital in Guangzhou, 20 potentially relevant indicators of PCAD. 108 PCAD patients were randomly selected from CAD patients diagnosed via coronary angiography (CAG) in Sun Yat-sen Memorial Hospital, Sun Yat-sen University from 1/2019 to 12/2020. Inclusion criteria were: (1) male patients $\leq$55 years old and female patients $\leq$65 years old; (2) chest pain; (3) At least one major coronary vascular stenosis of the patients was $\geq$50 % according to coronary angiography.

Then 96 healthy controls with no history of CAD (male patients $\leq$55 years old, female patients $\leq$65 years old) in Boji Medical Examination Center were regarded as the control group.

Exclusion criteria: (1) history of rheumatic heart disease, cardiomyopathy, pulmonary heart disease, heart valvular disease or other organic heart diseases; (2) complication with severe infection; (3) severe chronic hepatic insufficiency; (4) hyperthyroidism, hypothyroidism, tumor, autoimmune disease, connective tissue disease, undergoing a major operation or trauma and burn in the previous 2 months; or (5) previous therapy with lipid-regulating drugs.

2.3. Laboratory measurements

The total cholesterol (TC), triglyceride (TG), low-density lipoprotein C (LDL-C), and glucose were tested in the Laboratory Department of Sun Yat-sen Memorial Hospital of Sun Yat-sen University. TC and TG were measured with enzymatic colorimetry. HDL-C and LDL-C were measured with a direct clearance method (homogeneous method). MCP-1 was measured with an enzyme-linked immunosorbent assay (ELISA) kit, the Human-MCP-1 Multisciences Kit (Lianke Biology Company).

2.4. HDL-C, HDL2-C, and HDL3-C measurements

The concentration of HDL-C, HDL2-C and HDL3-C was measured as our previous study. Briefly, HDL-C levels were measured in serum before separation. The serum samples were precipitated with heparin containing $MnCl_2$ and dextran sulfate and separated by centrifugation at 10,000 rpm for 10 min. The levels of HDL3-C in the supernatant were measured using homogeneous HDL-EX HDL-C assays (Denka Seiken, Tokyo, Japan). Levels of HDL2-C were derived from the following formula: HDL2-C = HDL-C-HDL3-C.

2.5. LASSO

LASSO was performed to recognize the variables that were associated with PCAD using the glmnet R package. In LASSO, the lambada (λ) value was selected using 10-fold cross-validation. The number of variables included in the model decreases when λ increases. Lambda.min represents the λ of the minimum mean square error, which means the model best fits at that value of λ. Lambda.1se represents the λ of one standard error away from lambda.min, which means that the model incorporates the least number of variables at that value of λ and achieves acceptable performance. In this study, Lambda.1se was chosen to ensure that the model with fewer variables could be applied more conveniently and quickly.

2.6. Nomogram

A nomogram was created to show a visualization of the logistic regression classification using the rms R package. In the nomogram, each variable corresponds to a point ranging from 0 to 100. After calculating the total point of all variables, the final probability could be checked in the nomogram figure. The receiver operating characteristic (ROC) curve was enrolled to evaluate the discriminatory capacity of nomogram model using the pROC R package. In this study, the entire dataset was used for the ROC analysis. The probability of PCAD was obtained by scoring each sample for the following ROC analysis. The area under the ROC curve (AUC), sensitivity, specificity, and accuracy were calculated to evaluate the classification performance of model. Decision curve analysis was performed to evaluate the clinical usefulness using the rmda R package.

2.7. Isolation of HDL2 and HDL3 subclasses

HDL2 and HDL3 were isolated by a two-step discontinuous density-gradient ultracentrifugation method [14]. Each plasma sample (4 mL) was adjusted to a density of 1.24 g/mL with KBr (0.3816 g/mL) and was added to a 13.2 mL centrifuge tube (Beckman); next, it was slowly overlaid with KBr/phosphate buffer solution (0.0834 g/mL, d = 1.063 g/mL). The samples were centrifuged at 280,000*g* for 4 h at 15 °C in a Beckman XP-100 equipped with a SW41Ti rotor (horizontal; Beckman

Instruments, Fullerton, CA, USA), and HDL subfractions (1.063 < d < 1.21 g/mL) located in the middle of the tube were then collected separately by penetrating the tube with a syringe. Then, HDL fractions were further purified by a second ultracentrifugation that was performed under the same conditions as described above but for 2 h after KBr/phosphate solution (d = 1.24 g/mL) was added to the two fractions. Finally, HDL2 and HDL3 were collected from the top of the tube.

2.8. Tandem efflux-influx assays

For efflux experiments, the gold-standard assay in the field was performed, efflux of tritium-labeled cholesterol (^{3}H-cholesterol) from J774 macrophages. J774 macrophages seeded in collagen-coated 24-well plates were starved for 6 h and labeled with ^{3}H-cholesterol (1 Ci/ml) (PerkinElmer Analytic Sciences, Boston, MA) for 24 h. Cellular cholesterol efflux was initiated by the addition of DMEM containing 0.2 % BSA with 50 μg/ml HDL/HDL2/HDL3 with the presence of cAMP (300 μM, ABCA1 agonist). After incubation for 4 h, the radioactivity of the medium and cells was measured using a liquid scintillation counter. Cholesterol efflux was calculated as the quantity of labeled ^{3}H-cholesterol released into the medium divided by the total amount of label present.

For influx experiments, hepatocytes (HepG2 cells) were cultivated in DMEM, 10 % FBS, 1 % PenStrep in 24 well plates. Immediately after 4 h of efflux, HepG2 cells were washed twice in serum-free MEM, 25 μM HEPES, and then the above efflux media from J774 macrophages was removed and added directly to HepG2 cells. Influx was allowed to proceed for 4 h prior to harvesting. Influx media supernatant was then collected and processed identically as efflux media described above. The radioactivity of the medium and cells was measured using a liquid scintillation counter. Cholesterol influx was calculated as the quantity of labeled ^{3}H-cholesterol influx into the cells divided by the total amount of label present.

2.9. NBD-cholesterol delivery experiments

HepG2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10 %FBS and 1 % PenStrep. One day prior to treatment, cells were plated in 24 well plates on top of glass coverslips. On the day of treatment, cells were washed three times with PBS and fresh media containing 5 μg/mL NBD-cholesterol was then added to each to each well with or without a delivery agent according to treatment regimen. Uptake was allowed to proceed for 30 min when the cells were fixed and prepared for confocal microscopy. Cells were fixed in 4 % PFA for 10 min at rt. Cells were then washed three times in PBS, stained with DAPI (300 nM in PBS) for 5 min, and washed two more times in PBS. Coverslips were then mounted on glass slides and allowed to seal at rt for at least 24 h prior to imaging.

2.10. Statistics

Statistical analysis was performed using SPSS software (V.23.0, IBM, New York, USA) and R software (version 3.6.3; R Foundation for Statistical Computing, Vienna, Austria). All patients included were inpatients and the variables were available in the inpatient data, so there is no missing data in this study. Continuous variables are expressed as the mean ± SD or median (IQR), as appropriate. Categorical data are expressed as numbers (percentages). Continuous variables were analyzed by Student's *t*-test or the rank-sum test, as appropriate. Categorical variables were compared by the $\chi 2$ test. The *glmnet* package of R software was performed to run LASSO regression analysis. Then a multivariable logistic regression analysis was used construct a model by introducing the variables selected in the LASSO regression model. In our study, the data was standard during logistic regression and all the variable selected in above were to develop the nomogram models by the R language package. AUC was used to access the predictive capacity of the model. Ultimately, a decision curve, which quantified the net benefits a threshold ranging from 0 to 1, was plotted to determine the clinical usefulness of nomograms. A two-tailed *P* value < 0·05 was considered statistically significant in this study.

Table 1
Comparison of HDL subclasses and traditional risk factors in PCAD patients and healthy controls.

	PCAD (n = 108)	Healthy controls (n = 96)	*P*
Baseline characteristics/Risk factors			
Age, years	52.53 ± 7.33	51.19 ± 8.09	0.220
Female, n(%)	48 (44.4)	63 (65.6)	0.002*
BMI, kg/m^2	24.91 ± 3.08	23.21 ± 2.78	<0.001*
Smoking, n(%)	30 (27.8)	12 (12.5)	0.009*
Drinking, n(%)	4 (3.7)	4 (4.2)	0.865
Hypertension, n(%)	50 (46.3)	30 (31.3)	0.028*
Diabetes, n(%)	28 (25.9)	11 (11.6)	0.010*
Family history of PCAD, n (%)	21 (19.4)	4 (4.2)	0.001*
HDL subclasses			
HDL-C, mmol/L	1.12 ± 0.34	1.36 ± 0.41	<0.001*
HDL2-C, mmol/L	0.79 ± 0.22	0.96 ± 0.26	<0.001*
HDL3-C, mmol/L	0.39 ± 0.08	0.40 ± 0.11	0.428
Lipid variables			
Total cholesterol, mmol/L	5.41 ± 0.97	4.66 ± 0.80	0.023*
Triglyceride, mmol/L	1.99 ± 0.83	1.44 ± 0.52	<0.001*
LDL-C, mmol/L	3.19 ± 0.93	2.80 ± 0.82	0.002*
ApoA1, g/L	1.01 ± 0.21	1.18 ± 0.23	<0.001*
ApoB, g/L	0.81 ± 0.24	0.76 ± 0.17	0.132
Laboratory variables			
Glucose, mmol/L	5.72 ± 1.74	5.09 ± 1.19	0.003*
Creatinine, μmol/L	101.45 ± 46.53	93.10 ± 29.41	0.133
hs-CRP, mg/L (IQR)	3.86 (1.40–18.69)	1.46 (0.88–4.41)	<0.001*
MCP-1, pg/mL (IQR)	38.98 (28.79–59.65)	34.87 (21.15–48.80)	0.326

Continuous variables are expressed as the means ± SD (for normally distributed variables) or medians with interquartile ranges (for non-normally distributed variables). Categorical variables are expressed as numbers (percentages). PCAD, premature coronary artery disease; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; HDL2-C, high density lipoprotein 2 cholesterol; HDL3-C, high density lipoprotein 3 cholesterol; LDL-C, low density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; hs-CRP, high sensitivity C reactive protein; MCP-1, monocyte chemoattractant protein 1. *$P < 0.05$ as significance.

2.11. Ethics

The previous study and data collection were approved by the Medical Ethical Committee of Sun Yat-sen Memorial Hospital at Sun Yat-sen University (protocol number: SYSEC-KY-KS-2020–083). All patients gave informed consent and signed paper informed consent. performed according to the recommendations of the Declaration of Helsinki. All procedures were carried out following international guidelines and regulations by trained researchers.

3. Results

3.1. Characteristics of the study cohort

The demographic and clinical characteristic of PCAD patients and healthy controls were shown as Table 1. Patients with PCAD had a higher rate of smoking, hypertension, diabetes, history of PCAD than healthy controls ($P < 0.05$). Moreover, compared with the healthy controls, PCAD patients had more men with higher BMI ($P < 0.05$). However, no difference with drinking or age was found between these

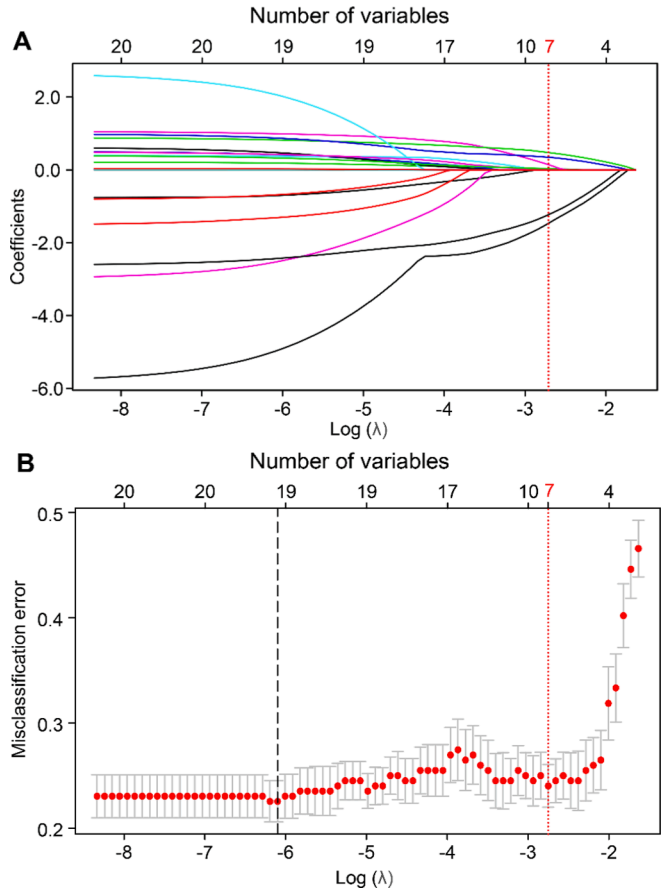

Fig. 2. Variable selection by the LASSO binary logistic regression model. A coefficient profile plot was constructed against the log(λ) sequence. A, Association between coefficient of variables and (λ). Each line corresponded to one distinct variable. Along with the increasing log (λ), the coefficient of variable trended to be close to 0. B, The selection of applicable model. We plotted the misclassification versus Log (λ). Vertical lines were drawn at the optimal values by adopting the minimum criteria (dashed line) and the 1 standard error of the minimum criteria (red dotted line, the 1-SE criteria). In our study, the (λ) value of 0.012832 was chosen according the the 1-SE criteria. Note that 7 variables were selected at last including BMI, Family history of PCAD, Glucose, total cholesterol, Triglyceride, ApoA1, HDL2-C. LASSO, least absolute shrinkage and selection operator; SE, standard error; BMI, body mass index; PCAD, premature coronary artery disease. ApoA1, apolipoprotein 1; HDL2-C, high density lipoprotein 2 cholesterol.

two groups. Compared with the healthy controls, PCAD patients had lower HDL2-C and HDL-C levels (HDL2-C: 0.79 ± 0.22 mmol/L vs 0.96 ± 0.26 mmol/L, $P < 0.001$; HDL-C: 1.12 ± 0.34 mmol/L vs 1.36 ± 0.41 mmol/L, $P < 0.001$). However, HDL3-C was not different between the two groups. Also, PCAD patients had higher total cholesterol (5.41 ± 0.97 mmol/L vs 4.66 ± 0.80 mmol/L, $P = 0.023$) and triglyceride (1.99 ± 0.83 mmol/L vs 1.44 ± 0.52 mmol/L, $P < 0.001$) and lower ApoA1 (1.01 ± 0.21 mmol/L vs 1.18 ± 0.23 mmol/L, $P < 0.001$) than healthy controls. Glucose in PCAD patients was higher than healthy controls (5.72 ± 1.74 mmol/L vs 5.09 ± 1.19 mmol/L, $P = 0.003$). Furthermore, in female, the HDL-C and HDL2-C were significantly higher than that in male as shown in the Supplemental Table 2.

3.2. Results of the risk variable selection

A total of 20 variables, including age, sex, BMI, smoking, drinking, history of hypertension, history of diabetes, family history of PCAD, total cholesterol, triglycerides, HDL, LDL, ApoA1, ApoB, glucose, hypersensitive C-reactive protein, creatinine, HDL3-C, HDL2-C, and MCP-1, were initially enrolled into the LASSO analysis. A total of 7 variables, including HDL2-C, BMI, family history of PCAD, glucose, ApoA1, total cholesterol, and triglyceride, with non-zero coefficients were ultimately obtained (Fig. 2). In our opinion, too many variables might affect the clinical practicality of the model. Therefore, 7 variables corresponding to lambda.1se were chosen to ensure that the model with fewer variables could be applied more conveniently and quickly. Moreover, HDL2-C was an independent protective factor for PCAD by multivariate logistic regression analysis ($\beta = -2.886$, OR = 0.057, 95 %CI: 0.010–0.316, $P = 0.001$) (Table 2). Also, ApoA1 was independent protective factor ($\beta = -3.602$, OR = 0.027, 95 %CI: 0.004–0.198, $P < 0.001$) while total cholesterol was independent risk factor for PCAD ($\beta = 0.948$, OR = 2.580, 95 %CI: 1.637–4.066, $P < 0.001$) (Table 2).

Table 2
Logistic regression analysis of the predictors of the risk of PCAD.

	LASSO regression	Logistic regression		
Variables	β	β	OR (95 %CI)	P
BMI	0.03584189	0.118	1.125 (0.978–1.294)	0.099
Family history of PCAD	0.16905887	1.331	3.785 (0.964–14.865)	0.057
Glucose	0.01946097	0.287	1.333 (0.980–1.814)	0.067
HDL2-C	−1.26677890	−2.886	0.057 (0.010–0.316)	0.001
ApoA1	−1.51411862	−3.602	0.027 (0.004–0.198)	<0.001
Total cholesterol	0.48532292	0.948	2.580 (1.637–4.066)	<0.001
Triglyceride	0.35160666	0.643	1.902 (0.960–3.768)	0.065

BMI, body mass index; PCAD, premature coronary artery disease; HDL2-C, high density lipoprotein 2 cholesterol; ApoA1, apolipoprotein 1; LASSO, least absolute shrinkage and selection operator.

3.3. Nomogram model construction

Combined with HDL2-C and other 6 variables selected, including BMI, history of PCAD, glucose, ApoA1, total cholesterol and triglyceride, a clinical nomogram model was established, as presented in Fig. 3A. For example, using the nomogram model, a male patient with a BMI 28.16 kg/m^2, no family history of PCAD, glucose of 6.0 mmol/L, HDL2-C of 0.80 mmol/L, ApoA1 of 1.08 mmol/L, total cholesterol of 4.0 mmol/L and triglyceride of 2.02 mmol/L has an estimated probability of PCAD of 61 % (Fig. 3B).

3.4. Validation of the nomogram model

The receiver operating characteristic (ROC) curve was used to evaluate the discriminatory capacity of nomogram model. For the model, the pooled area under the ROC of the nomogram was 87.45 % (95 % CI, 82.58 %-92.32 %), which indicates moderately good performance (Fig. 4A). The decision curve analysis (DCA) showed that the net benefits obtained from the application of our nomogram with threshold probabilities of 0.56 (Supplemental Fig. 4B). Additionally, for the accuracy of classification based on the nomogram scoring, Table 3 showed that the AUC (95 %CI) was 0.8745 (0.8258–0.9232), the accuracy (95 % CI) was 0.8235 (0.7642–0.8732), the sensitivity was 0.8056 and the specificity was 0.8438. Therefore, the nomogram model was well established.

3.5. HDL2 subclass biological assays

A critical property for HDL2 subclass is to efflux cholesterol from lipid-laden macrophages using an in vitro radiolabeled cholesterol efflux

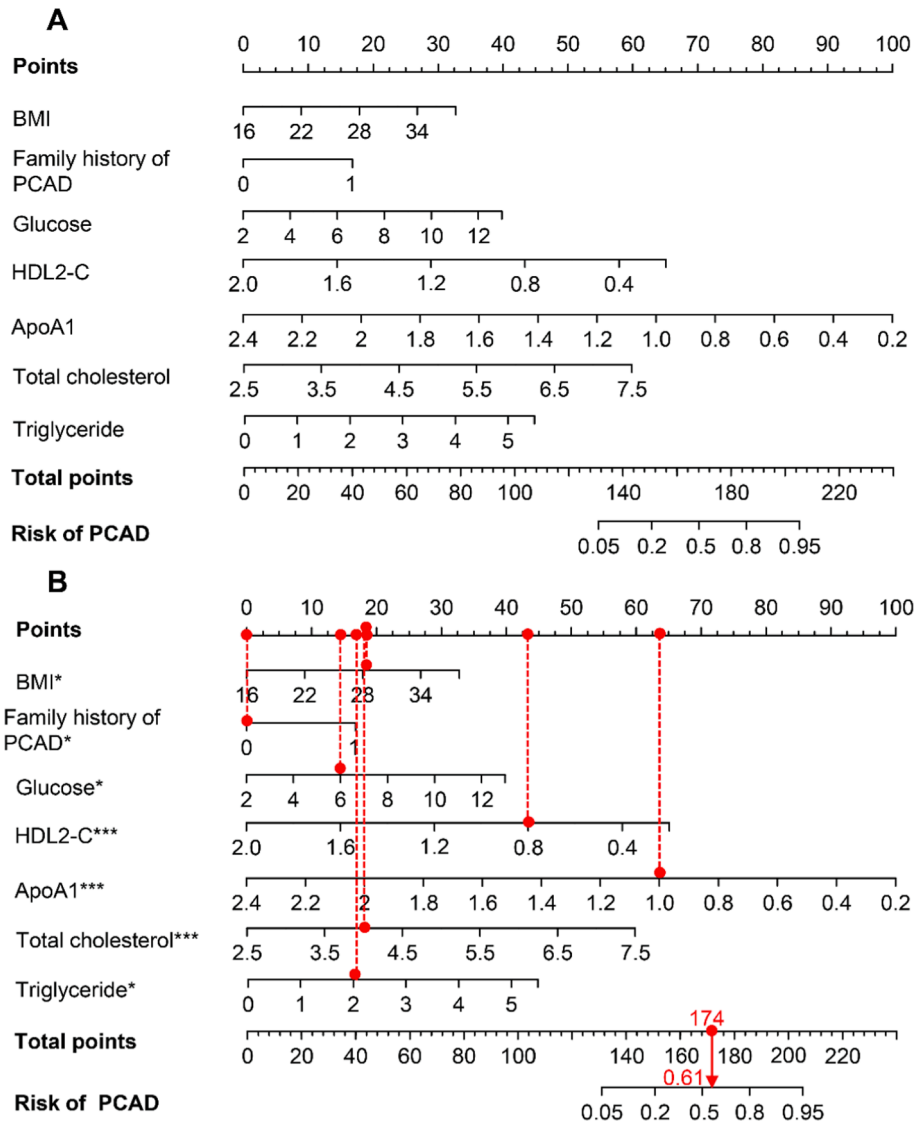

Fig. 3. Nomogram model to calculate risk score of PCAD. A. Risk factors of BMI, family history of CAD, glucose, HDL2-C, ApoA1, total cholesterol and triglyceride for nomogram risk model. Score was assigned for these 7 risk factors by drawing a line upward from the corresponding values to the "Points'' line. The sum of all these points, plotted on the "Total points'' line, corresponds to predictions of PCAD probability. B. Dynamic nomogram used as an example. The significance of the asterisks beside each variable represents importance of all the risk factors. The patient for example has a total point of 174 and a probability of 61% for PCAD. PCAD, premature coronary artery disease; BMI, body mass index; ApoA1, apolipoprotein A1; HDL2-C, high density lipoprotein 2 cholesterol.

assay. We selected 6 PCAD patients and 6 healthy controls (The demographic and clinical characteristic of them were shown as Supplemental Table 2) and got HDL subclass from them. First, J774 macrophages had a basal rate of − cholesterol efflux to BSA at 20.04 %. Also, HDL2 from PCAD patients and healthy controls increased the ^{3}H-cholesterol efflux to 26.64 % and 22.17 %, respectively. However, HDL2 subclass from PCAD patients had a lower rate of cholesterol efflux compared with healthy controls ($P < 0.05$). Interestingly, HDL3 subclass from PCAD patients and healthy controls had no difference in cholesterol efflux (25.98 % vs 26.83 %) (Fig. 5A).

Encouraged by the above results, we next designed an experiment to simulate the entire reverse cholesterol transport process in a single assay. We achieved this by carrying out the radiolabel efflux assay followed by an influx step where conditioned efflux media was introduced to cultured hepatocytes (HepG2) as described in method. Our data revealed that HDL2 subclass from PCAD patients exhibited inferior tend of cholesterol influx compared with healthy controls (16.07 % vs 18.24 %) nevertheless no statistical significance between them. Similarly, HDL3 subclass from PCAD patients and healthy controls had no difference in cholesterol influx to HepG2 cells (Fig. 5B).

Next, we detected the efficacy of HDL2 as cholesterol delivery agents independently of efflux assay. HepG2 cells were cotreated with fluorescent cholesterol (NBD-cholesterol) and HDL2, and then processed for confocal microscopy. Strikingly, HDL2 subclass form PCAD facilitated less delivery of NBD-cholesterol compared to healthy controls (Fig. 5C).

These assays demonstrates that HDL2 subclass from PCAD patients is less capable of cholesterol efflux and delivery sequentially by on- and off-loading cholesterol in a dynamic fashion.

4. Discussion

The three principal findings of this study are summarized as follows: (1) Nomogram model of PCAD includes HDL2-C, ApoA1, total cholesterol, triglyceride, BMI, glucose, and family history of CAD for risk factors. (2) HDL2 subclass from PCAD patients has an inferior cholesterol transport capacity compared to healthy controls.

Patients with PCAD are a concerning population as poor long-term outcomes and a high likelihood for recurrence after first events [4].

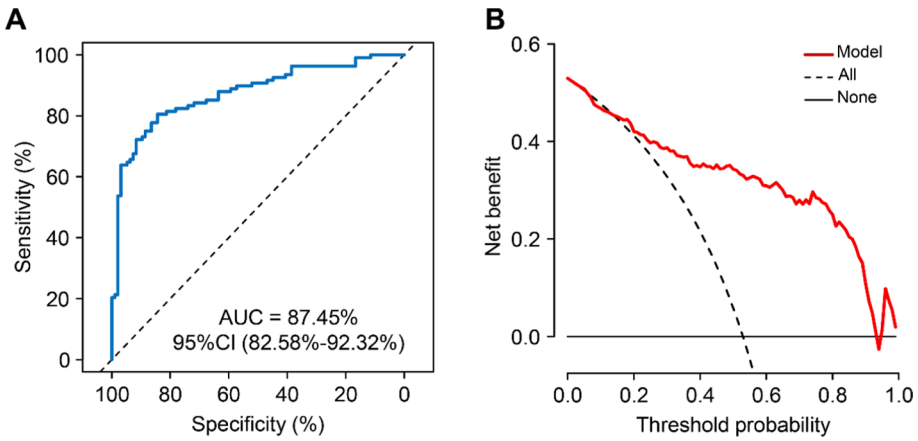

Fig. 4. Performance evaluation of the PCAD nomogram risk model. A. Receiver operating characteristic curve (ROC) analysis of the PCAD nomogram risk model. The y-axis represents the true positive rate of the risk prediction, the x-axis represents the false positive rate of the risk prediction. The thick blue line represents the performance of the nomogram. The AUC of PCAD risk nomogram was 87.45 % (95 % CI, 82.58 %–92.32 %). B. Decision curve analysis (DCA) for the PCAD risk nomogram. The y-axis measures the net benefit. The solid line represents the assumption that all patients have no PCAD, the dotted line represents the assumption that all patients have PCAD, the red line represents the risk nomogram. AUC, area under the receiver operating characteristic curve; CI, confidence interval; PCAD, premature coronary artery disease.

Table 3
Model performance of the nomogram.

	AUC (95 % CI)	Accuracy (95 % CI)	Sensitivity	Specificity
Value	0.8745 (0.8258–0.9232)	0.8235 (0.7642–0.8732)	0.8056	0.8438

AUC, The area under the receiver operating characteristic curve; CI, confidence interval.

Overweight with ST-elevation myocardial infarction (STEMI) was more prevalent in younger than in older patients, young patients were likely to receive early percutaneous coronary interventions [15–17]. PCAD results in significant morbidity and mortality in this demographic [5]. Autopsy studies have identified that more than 50 % of young adults have CAD [18] and almost one-third of all patients with acute coronary syndrome are younger than 55 years old [19]. Also, it results in a substantial societal burden because of limited productivity of a younger demographic in conjunction with unexpected loss of life [3]. However, data regarding risk factors for PCAD is scarce given that few studies focus on CAD in this demographic. Thus, our study established the nomogram risk model for PCAD and found that HDL2-C, ApoA1, total cholesterol, triglyceride, BMI, glucose, and family history of PCAD were potential risk factors for PCAD.

It is well known that a low HDL-C level is a strong established risk factor of CAD [20]. However, to date, some famous large clinical studies, including the AIM-HIGH trial of niacin, the ILLUMINATE trials using the CETP inhibitor torcetrapib and the Dal- OUTCOMES trial with dalcetrapib, demonstrated that elevated HDL-C was not beneficial for CHD events [21–25]. Moreover, Mendelian randomization studies [26,27] have demonstrated that HDL-C level cannot be regarded as a causal factor for CAD. Because plasma HDL consists of various subclasses with distinct structures and functional properties [28], and the structural characteristics and functionality of these subclasses may be diversely changed in CAD patients with low HDL-C level [29]. HDL2 and HDL3 are the main subfractions. And large HDL2 subfraction appears to be more important for cardiovascular protection than the small HDL3 subfraction [30]. Our previous study demonstrated that large HDL2 combined with inflammatory factors as superior predictors for CAD than small HDL3 [31]. Furthermore, impaired HDL2-mediated cholesterol efflux is associated with PCAD, and the phospholipid content of HDL2 was close correlate with the efflux to HDL2 [32]. Our data support the above, and we found HDL2 subclass level was reduced in PCAD patients, which was similar to our previous studies [33,34]. Moreover, HDL2 mediated cholesterol efflux and influx capacity was impaired in PCAD patients. While HDL3 subclass from PCAD patients had no difference in concentration and biological function compared to healthy controls. The underlying mechanism may be that certain phosphatidylcholine (PC) and sphingomyelin (SM) ratios are changed in HDL2 subclass from PCAD patients [35].

Published studies reported elevating trends of PCAD and significantly higher prevalence of traditional cardiovascular (CV) risk factors such as gender, obesity, smoking, family history of PCAD and diabetes mellitus among young adults [36,37]. According to our findings, obesity, family history of PCAD and diabetes mellitus might be also potential risk factors for PCAD. Coincidently, the high-quality data comes from a recent multicenter prospective cohort study for 20 years of data of 880 patients with PCAD (defined before age 45 years) [4]. In this study, these population with events had a history of active smoking (77 %), family history of CAD (40 %), and hypercholesterolemia (50 %). Shockingly, 18.2 % of patients were under 35 years old. The population with PCAD are younger with much more smoking than our study. It is a reminder that smoking should not be ignored in cardiovascular damager for young people.

5. Limitations

However, this study has several limitations. First, this study is imperfect due to its retrospective nature and the source of the data used. Second, the sample size is limited. Thus, it is impending to perform the large sample size, multicenter prospective cohort study focusing on the PCAD patients with modifiable risk factors and non-modifiable risk factors. Third, due to the sample size, we were unable to further analyze the impact of gender differences on this study, which warrants further research in the future.

6. Conclusions

The seven-factor nomogram can achieve a reasonable relationship with PCAD, and a large cohort were needed to enhance the credibility and effectiveness of our model in future.

7. Author's contribution

Conception and design were contributed by RLS, JFW and YLZ. Collection and assembly of data were contributed by QG, HWL and XL.

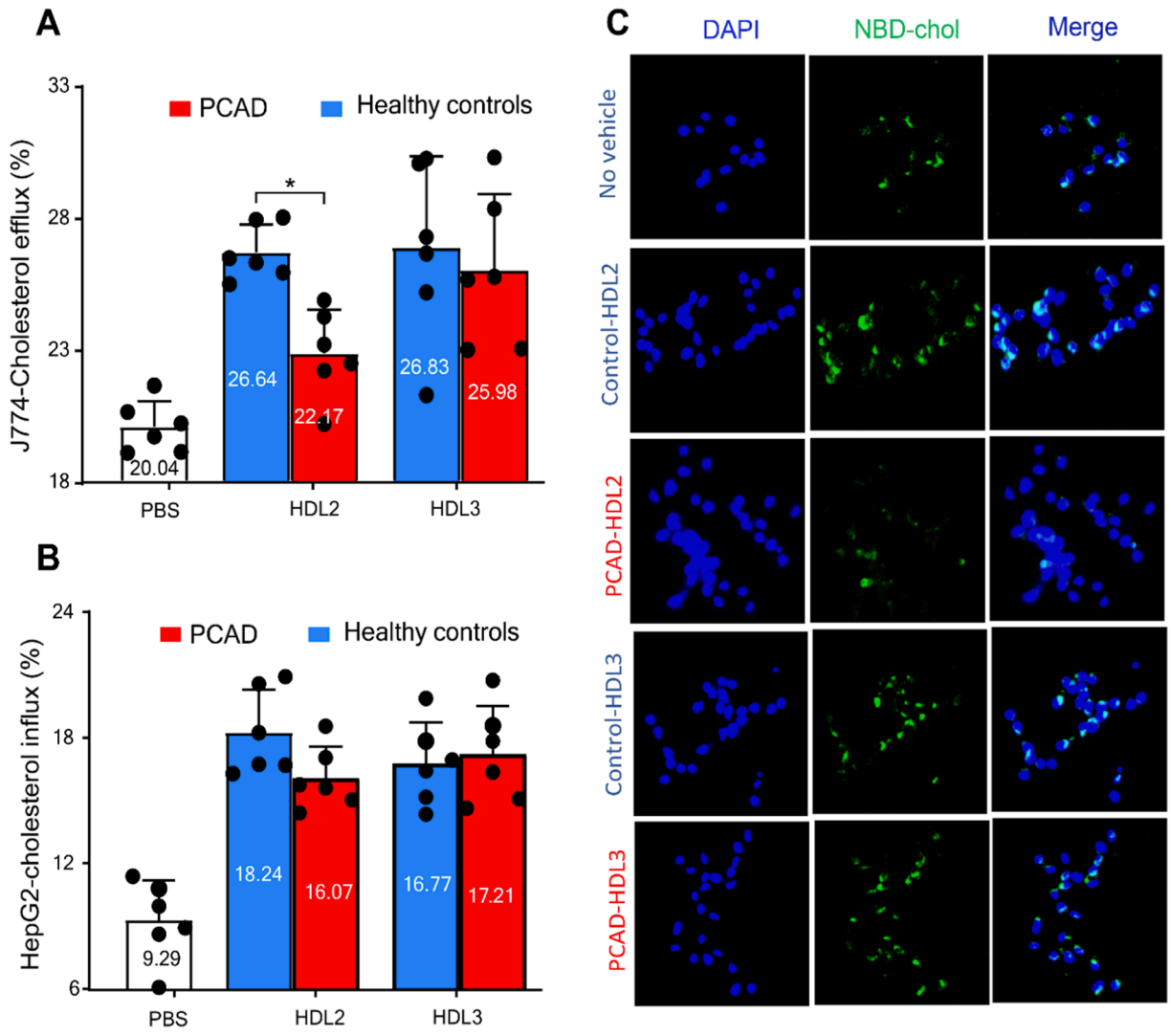

Fig. 5. Cholesterol efflux from J774.1 macrophages and influx from HepG2 cells mediated by HDL2 between patients with PCAD and healthy controls. A. ^{3}H-cholesterol efflux from J774.1 macrophages. B. % influx of ^{3}H-cholesterol to HepG2 cells in a tandem efflux-influx assay. C. HDL2 facilitates delivery of NBD-cholesterol to HepG2 cells in 30 min via confocal microscopy (×20). *$P < 0.05$.

Experiments were performed by HWL, XYW and YJ. Data analysis and interpretation were contributed by RLS and QG. Manuscript writing was contributed by RLS and QG. All authors gave final approval of the manuscript.

CRediT authorship contribution statement

Runlu Sun: Writing – original draft, Data curation, Conceptualization. **Qi Guo:** Writing – original draft, Software, Formal analysis. **Hongwei Li:** Methodology, Formal analysis. **Xiao Liu:** Software, Methodology. **Yuan Jiang:** Validation, Software. **Jingfeng Wang:** Writing – review & editing, Supervision, Conceptualization. **Yuling Zhang:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

None.

Sources of funding

This study was supported by grants from the Natural Science Foundation of China (Grant number: 81700397, 81970388, 82104433), and Guangdong Basic and Applied Basic Research Foundation (Grant number: 2020A1515110313) and Yat-sen Start-up Foundation (Grant number: YXQH202014).

Registration number

ChiCTR2000033297.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2024.101457.

References

[1] K.O. Akosah, A. Schaper, C. Cogbill, P. Schoenfeld, Preventing myocardial infarction in the young adult in the first place: how do the National Cholesterol Education Panel III guidelines perform? J. Am. Coll. Cardiol. 41 (9) (2003) 1475–1479.

[2] J.J. Li, S. Li, C.G. Zhu, N.Q. Wu, Y. Zhang, Y.L. Guo, et al., Familial hypercholesterolemia phenotype in Chinese patients undergoing coronary angiography, Arterioscler. Thromb. Vasc. Biol. 37 (3) (2017) 570–579.

[3] E.M. Bucholz, A.M. Rodday, K. Kolor, M.J. Khoury, S.D. de Ferranti, Prevalence and predictors of cholesterol screening, awareness, and statin treatment among US adults with familial hypercholesterolemia or other forms of severe dyslipidemia (1999–2014), Circulation 137 (21) (2018) 2218–2230.

[4] J.P. Collet, M. Zeitouni, N. Procopi, J.S. Hulot, J. Silvain, M. Kerneis, et al., Long-term evolution of premature coronary artery disease, J. Am. Coll. Cardiol. 74 (15) (2019) 1868–1878.

[5] K.A. Wilmot, M. O'Flaherty, S. Capewell, E.S. Ford, V. Vaccarino, Coronary heart disease mortality declines in the united states from 1979 through 2011: evidence for stagnation in young adults, Especially Women Circulation 132 (11) (2015) 997–1002.

[6] F.H. Zimmerman, A. Cameron, L.D. Fisher, G. Ng, Myocardial infarction in young adults: angiographic characterization, risk factors and prognosis (Coronary Artery Surgery Study Registry), J. Am. Coll. Cardiol. 26 (3) (1995) 654–661.

[7] E. Incalcaterra, M. Caruso, R. Lo Presti, G. Caimi, Myocardial infarction in young adults: risk factors, clinical characteristics and prognosis according to our experience, Clin. Ter. 164 (2) (2013) e77–e82.

[8] L. Chouhan, H.A. Hajar, J.C. Pomposiello, Comparison of thrombolytic therapy for acute myocardial infarction in patients aged < 35 and > 55 years, Am. J. Cardiol. 71 (2) (1993) 157–159.

[9] B. Liu, L. Fang, Y. Xiong, Q. Du, Y. Xiang, X. Chen, et al., A machine learning model based on genetic and traditional cardiovascular risk factors to predict premature coronary artery disease, Front. Biosci. (landmark Ed) 27 (7) (2022) 211.

[10] A.V. Khera, M. Chaffin, K.G. Aragam, M.E. Haas, C. Roselli, S.H. Choi, et al., Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations, Nat. Genet. 50 (9) (2018) 1219–1224.

[11] X. Wu, Z. He, R. Sun, X. Xie, Q. Chen, J. Wang, et al., Large HDL2 combined with inflammatory factors as superior predictors for coronary artery disease than small HDL3, Ann. Transl. Med. 9 (8) (2021) 672.

[12] M. Shahid, R.L. Sun, Y. Liu, J.L. Bao, C.X. Huang, Y. Liao, et al., Is high high-density lipoprotein cholesterol beneficial for premature coronary heart disease? A meta-analysis, Eur. J. Prev. Cardiol. 23 (7) (2016) 704–713.

[13] V.P. Balachandran, M. Gonen, J.J. Smith, R.P. DeMatteo, Nomograms in oncology: more than meets the eye, Lancet Oncol. 16 (4) (2015) e173–e180.

[14] H. Karlsson, P. Leanderson, C. Tagesson, M. Lindahl, Lipoproteomics II: mapping of proteins in high-density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry, Proteomics 5 (5) (2005) 1431–1445.

[15] S.K. Chua, H.F. Hung, K.G. Shyu, J.J. Cheng, C.Z. Chiu, C.M. Chang, et al., Acute ST-elevation myocardial infarction in young patients: 15 years of experience in a single center, Clin. Cardiol. 33 (3) (2010) 140–148.

[16] A.W. Schoenenberger, D. Radovanovic, J.C. Stauffer, S. Windecker, P. Urban, G. Niedermaier, et al., Acute coronary syndromes in young patients: presentation, treatment and outcome, Int. J. Cardiol. 148 (3) (2011) 300–304.

[17] C. Zanchin, S. Ledwoch, S. Bar, Y. Ueki, T. Otsuka, J.D. Haner, et al., Acute coronary syndromes in young patients: Phenotypes, causes and clinical outcomes following percutaneous coronary interventions, Int. J. Cardiol. 350 (2022) 1–8.

[18] J.P. Strong, G.T. Malcom, C.A. McMahan, R.E. Tracy, W.P. Newman 3rd, E. E. Herderick, et al., Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study, J. Am. Med. Assoc. 281 (8) (1999) 727–735.

[19] A. Avezum, M. Makdisse, F. Spencer, J.M. Gore, K.A. Fox, G. Montalescot, et al., Impact of age on management and outcome of acute coronary syndrome: observations from the Global Registry of Acute Coronary Events (GRACE), Am Heart. J. 149 (1) (2005) 67–73.

[20] D.J. Gordon, B.M. Rifkind, High-density lipoprotein–the clinical implications of recent studies, N. Engl. J. Med. 321 (19) (1989) 1311–1316.

[21] C.L. Haase, A. Tybjaerg-Hansen, A.A. Qayyum, J. Schou, B.G. Nordestgaard, R. Frikke-Schmidt, LCAT, HDL cholesterol and ischemic cardiovascular disease: a Mendelian randomization study of HDL cholesterol in 54,500 individuals, J. Clin. Endocrinol. Metab 97 (2) (2012) E248–E256.

[22] P.M. Lavigne, R.H. Karas, The current state of niacin in cardiovascular disease prevention: a systematic review and meta-regression, J. Am. Coll. Cardiol. 61 (4) (2013) 440–446.

[23] P.J. Barter, M. Caulfield, M. Eriksson, S.M. Grundy, J.J. Kastelein, M. Komajda, et al., Effects of torcetrapib in patients at high risk for coronary events, N. Engl. J. Med. 357 (21) (2007) 2109–2122.

[24] S.E. Nissen, J.C. Tardif, S.J. Nicholls, J.H. Revkin, C.L. Shear, W.T. Duggan, et al., Effect of torcetrapib on the progression of coronary atherosclerosis, N. Engl. J. Med. 356 (13) (2007) 1304–1316.

[25] G.G. Schwartz, A.G. Olsson, M. Abt, C.M. Ballantyne, P.J. Barter, J. Brumm, et al., Effects of dalcetrapib in patients with a recent acute coronary syndrome, N. Engl. J. Med. 367 (22) (2012) 2089–2099.

[26] B.F. Voight, G.M. Peloso, M. Orho-Melander, R. Frikke-Schmidt, M. Barbalic, M. K. Jensen, et al., Plasma HDL cholesterol and risk of myocardial infarction: a Mendelian randomisation study, Lancet 380 (9841) (2012) 572–580.

[27] M.V. Holmes, F.W. Asselbergs, T.M. Palmer, F. Drenos, M.B. Lanktree, C.P. Nelson, et al., Mendelian randomization of blood lipids for coronary heart disease, Eur. Heart. J. 36 (9) (2015) 539–550.

[28] A. Kontush, M. Lindahl, M. Lhomme, L. Calabresi, M.J. Chapman, W.S. Davidson, Structure of HDL: particle subclasses and molecular components, Handb. Exp. Pharmacol. 224 (2015) 3–51.

[29] D.J. Rader, G.K. Hovingh, HDL and cardiovascular disease, Lancet 384 (9943) (2014) 618–625.

[30] J. Morgan, C. Carey, A. Lincoff, D. Capuzzi, High-density lipoprotein subfractions and risk of coronary artery disease, Curr. Atheroscler. Rep. 6 (5) (2004) 359–365.

[31] X. Wu, Z. He, R. Sun, X. Xie, Q. Chen, J. Wang, et al., Large HDL(2) combined with inflammatory factors as superior predictors for coronary artery disease than small HDL(3), Ann. Transl. Med. 9 (8) (2021) 672.

[32] T. Paavola, S. Kuusisto, M. Jauhiainen, S. Kakko, T. Kangas-Kontio, J. Metso, et al., Impaired HDL2-mediated cholesterol efflux is associated with metabolic syndrome in families with early onset coronary heart disease and low HDL-cholesterol level, PLoS One 12 (2) (2017) e0171993.

[33] R. Sun, P. Fang, J. Jiang, C. Huang, J. Wang, Q. Guo, et al., Insulin Rescued MCP-1-Suppressed Cholesterol Efflux to Large HDL2 Particles via ABCA1, ABCG1, SR-BI and PI3K/Akt Activation in Adipocytes, Cardiovasc. Drugs Ther. 36 (4) (2022) 665–678.

[34] R.L. Sun, C.X. Huang, J.L. Bao, J.Y. Jiang, B. Zhang, S.X. Zhou, et al., CC-chemokine ligand 2 (CCL2) suppresses high density lipoprotein (HDL) internalization and cholesterol efflux via CC-chemokine receptor 2 (CCR2) induction and p42/44 Mitogen-activated Protein Kinase (MAPK) activation in human endothelial cells, J. Biol. Chem. 291 (37) (2016) 19532–19544.

[35] T. Paavola, U. Bergmann, S. Kuusisto, S. Kakko, M.J. Savolainen, T. Salonurmi, Distinct fatty acid compositions of HDL phospholipids are characteristic of metabolic syndrome and premature coronary heart disease-family study, Int. J. Mol. Sci. 22 (9) (2021).

[36] S. Arora, G.A. Stouffer, A.M. Kucharska-Newton, A. Qamar, M. Vaduganathan, A. Pandey, et al., Twenty year trends and sex differences in young adults hospitalized with acute myocardial infarction, Circulation 139 (8) (2019) 1047–1056.

[37] D.N. Vikulova, M. Grubisic, Y. Zhao, K. Lynch, K.H. Humphries, S.N. Pimstone, et al., Premature atherosclerotic cardiovascular disease: trends in incidence, risk factors, and sex-related differences, 2000 to 2016, J. Am. Heart Assoc. 8 (14) (2019) e012178.